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ABSTRACTS

FREE COMMUNICATIONS

BIOLOGY

Preliminary characterization of mucins of *Catostylus tagi*

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Introduction: Osteoarthritis is the third most frequent rheumatic disease in Portugal, affecting 50% of the population aged over 50 years. Current treatments have several adverse effects, which leads to the need to find alternatives. Recent studies by Takagaki et al. [1] on jellyfish mucin presented encouraging results, in particular by raising the hypothesis of joint cartilage regeneration. Previous research of Masuda et al. [2] showed a similarity between the human mucin type MUC5AC and a jellyfish mucin named quiumucin, in the tandem repeat of peptide chain and also in water solubility. The present project aims to characterize the mucins of the scyphozoa *Catostylus tagi*, native of Tagus and Sado estuaries [3], in order to initiate the studies about its possible use in the treatment of osteoarthritis.
Materials and methods: Exemplars of *C. tagi* were collected at Tagus estuary, brought to Egas Moniz laboratory, homogenised and dialised against water type II in a membrane pore <1 kDa, water changed 3×24h. Desalted sample was lyophilised. A polyacrylamide gel with 1 mm spacer and 4–12% crosslinking was homemade with reagents and Mini-PROTEAN® Tetra Handcast Systems from BioRad. Sample preparation for electrophoresis included suspension of 4 mg in 1 mL Laemmli buffer/β-mercaptoethanol (95/5), heating for 5 min at 95 °C, cooling in ice for 10 min and centrifugation at 1000 rpm for 10 min. Application volumes of supernatant were 5, 10, 15 and 20 μL. The applied voltage was 150 V. The gel was stained with Coomassie Brilliant Blue R250.
Results: At least 12 bands from 250 to 10 kDa were found in *C. tagi* sample, the ones near 50, 15 and 75 kDa being the most intense (Figure 1), which is in line with the possible occurrence of quiumucin [2]. The bands near 50 and 75 were selected for PMF analysis at *Instituto de Investigação e Inovação em Saúde da Universidade do Porto*.
Discussion and conclusions: The results showed no significant similarity between the sequence found in the *C. tagi* mucin and the one found in qniumucin. However, the band near 50 had the same five amino acids with similar molecular mass as the one of the mucins found in *C. mosaicus* [4].

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Acknowledgements

This study was funded by Egas Moniz Coop Ensino Superior CRL, project EM-ZM 03/04.

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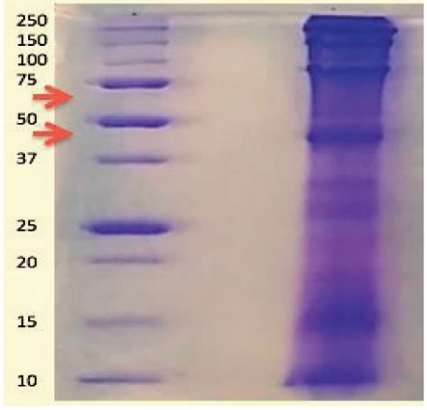

Figure 1. Left: Protein standards. Right: *C. tagi* whole medusa. Arrows highlight the possible region for a band of qniumucin.

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The statoliths of *Catostylus tagi*, chemical characterization by energy dispersive X-ray spectroscopy

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Introduction: In recent years, marine organisms of the phyla Chordata, Mollusca and Cnidaria have been proposed as models for the study of drug ototoxicity. McAfee et al. [1] successfully used live ephyras of *Aurelia aurita*, raised in artificial sea water at 24 °C, to look for a relationship between the antibiotic gentamycin and changes in mobility patterns due the destruction of hair cells. The scyphozoan *Catostylus tagi*, native to the estuaries of the Tagus and Sado, has been studied for health application purposes [2]. In order to start the studies of *C. tagi* as a model in ototoxicity tests, the chemical nature of its statoliths was determined as described for other cnidarians [3].
Materials and methods: *C. tagi* specimens were collected at Tagus estuary in September 2016. At Egas Moniz laboratory, the bell margin was separated and stored at –80 °C (Figure 1). Before spectroscopy, the crude and solvent-free sample was spread on a glass slide and allowed to air dry at −20 °C (Figure 1). Samples were analysed on a FEG-SEM, JEOL 7001F with Oxford INCA light elements EDS detector and EBSD detector.
Results and discussion: Statolith crystals of *C. tagi* were unequivocally identified a calcium sulphate compound (background spectra confirmed the absence of calcium and sulphur) (Figure 2). As far as we know, this is the first characterization for a specimen of Catostylidae family. Untill now, all the crystals of sensory equilibrium have been found to be calcium compounds, such as calcium carbonate (humans, most fish, molluscs), calcium phosphate & carbonate (Lamprey fish), calcium & magnesium phosphate (hidrozoans), calcium sulphate (scyphozoans) [4].
Conclusions: Our results confirmed the trend for $CaSO_4$ statoliths showed by scyphozoans. Further studies on the crystalline arrangement and hydration degree of *C. tagi* statoliths are now been planned.

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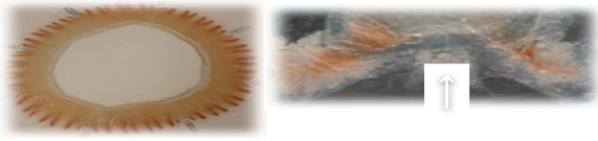

Figure 1. Left: whole bell margin. Up: zoom to rhopalia in bell margin; arrow points to statoliths.

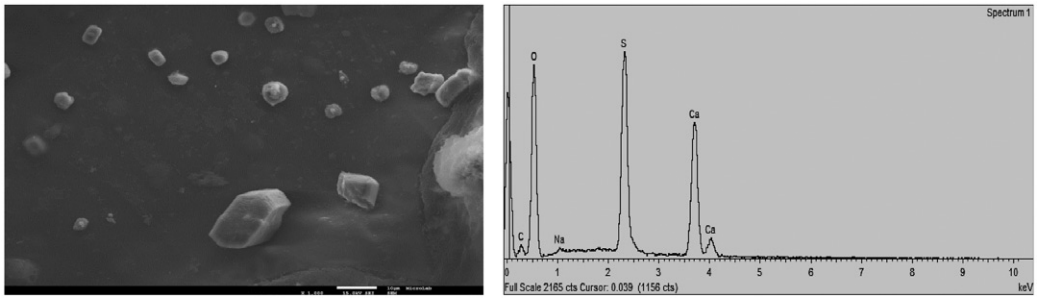

Figure. 2. Left: SEM of *C. tagi* statoliths crystals, scale bar 10 μm. Right: EDS spectrum of the largest crystal.

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Preliminary studies on the lifecycle of *Catostylus tagi*: differentiation between female and male at morphological level

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Introduction: Recent studies have proposed jellyfish as a source in drug development to treat diseases such as arthritis and hypertension [1]. A sustainable use of a marine resource in health sciences requires appropriate studies on its biology and lifecycle. The majority of scyphozoans seem to reproduce both sexually and asexually, according to pelagic or benthic stages of lifecycle [2]. *Catostylus tagi* is an edible Scyphozoa which occurs in summer at Tagus and Sado estuaries. In view of its application in health sciences, some studies have already been started [3]. This assay aimed to investigate the morphological characteristics of its sexual differentiation (female, F; male, M).
Materials and methods: Forty specimens were randomly collected at Tagus estuary in September 2016 and brought to Egas Moniz laboratory. The macroscopic aspects of the gonads were compared with microscopic observation of fresh preparations and histological slides, stained by hematoxylin-eosin (H&E).
Results and discussion: Of the studied specimens, 28 were M and 12 F. An unequivocal relation between the colour of the gonad and the density of matter with the sex was not found. F presented brown gonads more frequently than greenish (8/12) and lower density of matter more frequently than higher (9/12). M were more frequently greenish (15/28) with higher density of matter (20/28).
Ovocytes had a regular spherical shape with a well-defined cell wall, and a mean diameter of 31 μm ($n = 400$), in different development stages. The spermatocytes were formed in a follicle with an irregular, smaller elongated shape (24 μm, $n = 400$) (Figure 1). These results are in agreement with other authors who found microscopy to be the safest way to distinguish sex in similar species [2].

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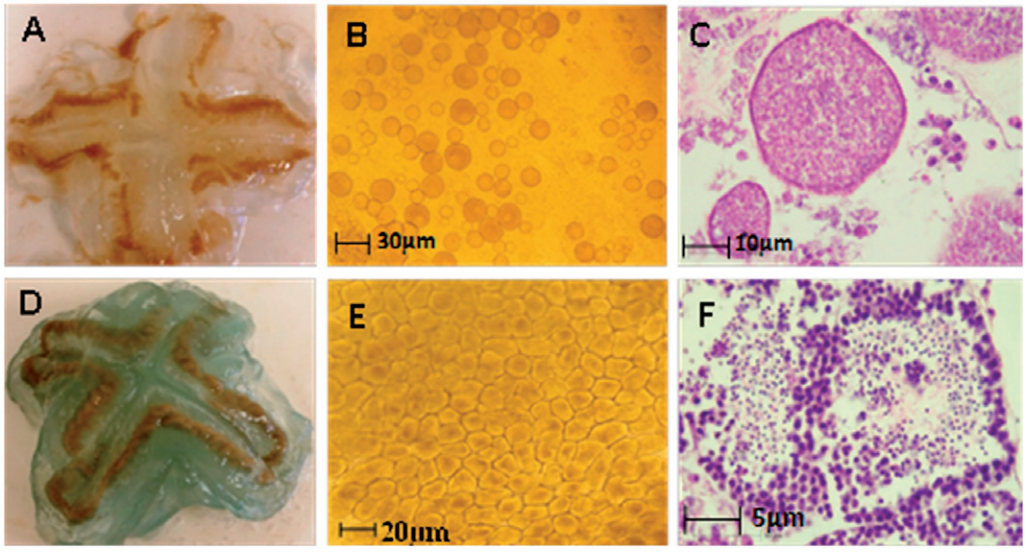

Figure 1. Representative specimens of F and M characteristics. (A) Photograph of F gonad from a just captured live specimen; (B) microphotograph of F gonad fresh sample (100x); (C): microphotograph of F gonad after H&E staining (1000x). D, E and F: M gonad, same procedures.

Acknowledgements

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Microscopy as a tool in drug-discovery marine biotechnology: a marine annelid as case study

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Introduction: There is a growing interest in searching biosubstances as alternatives to synthetic compounds to be used, e.g. in pharmacology and as marine anti-foulants, whose exploitation may be more cost-effective and more environmental friendly. There are recent indications that marine invertebrates, albeit understudied, may produce a wider range of biotoxins than expected, in parallel with their biodiversity. Polychaeta annelids, in particular, are beginning to yield new biotoxins and other secretions of interest, benefiting from their high ecological relevance. The present work aims at localising and describing organs and cells responsible for the secretion of special mucotoxins and pigments in the polychaete *Eulalia viridis*, which has been showing very promising results for biotechnological applications [1,2].
Materials and methods: Specimens of *E. viridis* collected from the W Portuguese coast, were processed for several microscopy analyses in order to obtain a comprehensive set of information for these little studied organisms. Scanning electron microscopy (SEM) was used to describe external anatomy, and histological and histochemical techniques (light microscopy), allied to transmission electronic microscopy (TEM), for integument microanatomy description.
Results: SEM analyses revealed a segmented body with a characteristic retractile organ, the proboscis, covered with sensorial papillae, which the animal uses to feed on soft-bodied prey, like mussels. Histological analyses revealed a complex microanatomy of the skin, showing several cell types, each bearing different functions such as structural support, pigmentation, sensory and mucotoxin secretion related to defense and feeding. The cytology of the integument showed novel subcellular features that related, as examples, to pigment granule organization and specialized synthesis of mucous substances and peptidic toxins.
Discussion and conclusions: The combination of different microscopy techniques identified organs and cells that synthesise novel substances whose biotechnological applications are being tested, including the value of toxins and pigments as biocidals and anti-tumour compounds [2]. This permits, among other factors, to target specific organs for substance harvesting and to correlate the substances' function and adaptative value. This information, on its own, helps understanding how these frail marine animals evolved to cope with environmental challenges, indicating that there is likely a wide variability of interesting biological "chemical warfare" within the Polychaeta, under the biotechnological point-of-view.

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Long-term responses to increasing temperatures in Scleractinia corals: HSP70 and ubiquitin induction

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Introduction: The increase in gas emissions (e.g. CO_2) results in temperature increase on a global scale. Models projections predict a sea surface temperature to increase by 1–4 °C [1]. Thus, the main objective of this study is to evaluate the vulnerability of the tropical coral species *Galaxea fascicularis* and *Montipora capricornis* to long-term temperature increase, by measuring the synthesis of heat shock protein (HSP70) and Ubiquitin.
Materials and methods: The specimens ($n = 30$) were exposed for 60 d to 26 °C (control) and potential stress temperatures (30 and 32 °C). Samples were assessed for HSP70 and Ubiquitin as described by Madeira et al. [2,3]. Statistics used the Mann–Whitney *U*-test.
Results: All *M. capricornis* exposed to 32 °C, died. The highest levels of HSP70 and Ubiquitin were found in *G. fascicularis* (Figure 1). Significant differences were found only in exposed *M. capricornis,* compared with controls, for both biomarkers analysed.
Discussion and conclusions: This study indicates that *M. capricornis* are more sensitive to rising temperature than *G. fascicularis* as shown by the elevated mortality at 32 °C. The results suggest that *G. fascicularis* can cope better with thermal stress than *M. capricornis* as shown by the HSP70 levels induced to protect cells. While the increase in ubiquitin levels may indicate that they are more vulnerable to thermal stress. This study demonstrates that different tropical coral species present different sensitivities to thermal stress.

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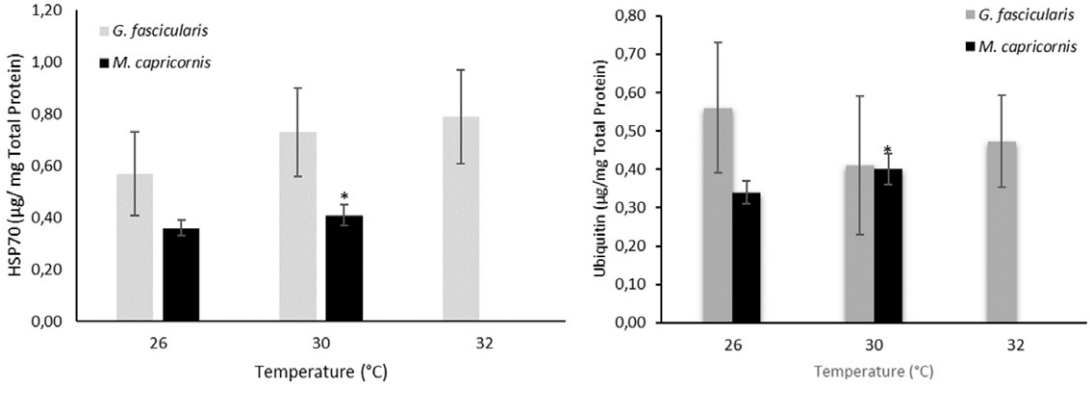

Figure 1. HSP70 and Ubiquitin levels in exposed corals. *Significant differences from controls ($p < .05$).

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MEDICINE

The rapid maxillary expander efficacy in the obstructive sleep apnea syndrome treatment – systematic review

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Introduction: The syndrome of obstructive sleep apnea (OSA) is a breathing disorder characterized by a reduction or absence of airflow in the nasal cavity or mouth [1–3]. Orthodontic treatment has been considered an important treatment option, particularly with the use of rapid maxillary expansion (RME) devices. This study aims to conduct a systematic review to assess the effectiveness of rapid maxillary expansion in the treatment of children with OSAS, based on existing scientific evidence [4,5].
Methods: A research was developed using the following primary and secondary electronic databases MEDLINE/PubMed, Embase, Web of Science, LILACS and Cochrane. A search strategy was made using the English terms: "Palatal Expansion Technique" [MeSH] AND "Sleep Apnea, Obstructive" [MeSH] limited to randomized controlled trials, cohort studies, systematic reviews and meta-analysis, in Portuguese and English, performed in humans under the age of 18 and regarding articles published between January 2000 and January 2016.
Results: In this review, a total of 84 articles were identified, 16 of which were considered potentially relevant. After applying the inclusion and exclusion criteria, six publications were accepted. Among these, only five had a positive result after the CASP quality assessment and, therefore, were included in this study.
Discussion and conclusions: The RME is effective in treating children with OSAS who have inadequate respiratory capacity and transverse maxillary deficiency, resulting in the improvement of polysomnographic variables, especially in the apnea-hypopnea index (AHI).

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Investigation of type 1 Diabetes Mellitus as risk factor for secondary osteoporosis

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Introduction: Clinical risk factors for use in fracture risk assessment through WHO validated models include secondary osteoporosis due to specific clinical disorders, such as type 1 diabetes mellitus (T1DM). The models are suitable for use in men and women of ages between 40 and 90 years [1], but fail to provide evidence linking T1DM to low bone mass and fractures in adolescents and young adults whose onset of T1DM occurs before the peak bone mass is achieved [2]. Nearly no longitudinal data exist on the relationship between changes in bone mineral density (BMD) and the duration of T1DM, in the first years of the disease and onset of insulin treatment. Very few longitudinal studies investigated the relationship between changes in BMD and the metabolic control of diabetes or the biochemical markers of calcium metabolism and bone turnover [1,2]. Not a single study investigated the major and trace elemental composition of T1DM bone, an important dimension of bone strength [3].
Materials and methods: Three animal groups (control, uncontrolled T1DM, controlled T1DM) were followed for 6 months, after T1DM was induced in adult (3 months) male Wistar rats by single intraperitoneal injection of 60 mg/kg Streptozotocin (STZ). The aim of the study was to assess the relationship between duration, treatment and metabolic control of T1DM, on one side, and bone composition and biochemical markers of calcium metabolism and turnover, on the other side, from the onset of the disease and insulin treatment to later life.
Results: ANCOVA models have shown that Calcium (Ca) content in compact bone of uncontrolled T1DM animals was significantly lower than in control ($p = .001$) and controlled T1DM animals ($p = .041$), whereas Ca content in trabecular bone of uncontrolled T1DM animals was significantly higher than in controlled T1DM animals ($p = .027$). A similar trend between groups was observed for the Phosphorus (P) content in both bone tissues. Concomitantly, bone resorption was significantly higher in uncontrolled T1DM animals than in control ($p < .001$) and controlled T1DM animals ($p < .001$), whereas bone formation was significantly decreased in uncontrolled T1DM animals, compared with control ($p < .001$) and controlled T1DM animals ($p = .011$).
Discussion and conclusions: These results show that uncontrolled T1DM has the potential to decrease bone mass, and thus, induce osteoporosis, in young adulthood, since the onset of the disease.

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Effects of altered bone turnover on energy metabolism

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Introduction: Emerging evidence suggests that bone can be considered as a true endocrine organ, a reliable tool in a whole-organism approach to physiology, such as regulation of bone mass, energy metabolism and fertility [1]. These findings take bone beyond its function of body support and organ protection, performing an important role in regulating intermediary metabolism and possibly being both a target of diabetic complications and a potential pathophysiologic

factor in the disease itself [2]. Previous research by our group has shown that altered bone turnover induces changes in hepatic metabolism [3]. Thus, long-term effects of altered bone turnover on other physiological pathways, in which liver plays a key role, namely glucose metabolism, are conceivable. The main goal of the current project is to assess the long-term effects of altered bone turnover on glucose metabolism, using an animal model.
Materials and methods: An animal study is being conducted at Faculdade de Medicina Veterinária, according to the Portuguese law and EU Directive 2010/63/EU on animal research, in order to assess the long-term effects of altered bone turnover on glucose metabolism. At baseline, two groups of 25 adult (3 month) female Wistar rats were formed: Group 1 was taken as control and Group 2 included animals who had undergone ovarioctomy (OVX) 1 month prior to baseline, to induce osteopenia [4]. At baseline and every two months thereafter, during 12 months, serum or blood levels of biomarkers of bone turnover and diabetes control are determined by current immunoassay protocols (ELISA).
Results: Data obtained so far (6 month) show that OVX animals have increased bone resorption and unaltered bone formation compared with controls, as expected in this animal model of osteopenia. Moreover, current results show that the time variations of blood glucose and insulin levels occur differently between groups: at month 6, blood glucose and insulin levels in OVX animals are higher than those of control animals.
Discussion and conclusions: Since this is a pioneer and exploratory work and the animal experiments are not concluded, it is premature to anticipate further results. However, higher levels of glucose and insulin found in OVX animals are suggestions of insulin resistance or an early-stage of type 2 diabetes. Also, increased levels of insulin promoted animal weight gain, which strengthen the hypothesis that altered bone turnover might influence glucose metabolism at long-term.

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Evaluation of the prevalence of musculoskeletal diseases in physicians, dentists and stomatologists

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Introduction: Today, the musculoskeletal disorders affect millions of workers around the world, belonging to all business sectors and are one of the most serious problems of occupational health. The importance of this issue it is significant in several health areas, such as dentistry. The choice of this theme has as objective to evaluate if the practice of Dental Medicine is subject to musculoskeletal professional diseases and of which type.
Materials and methods: Questionnaire consisting of 10 multiple choice questions and two open questions to answer. The target population was composed of dentists of Dental Medicine University Clinic ISCSEM, University Clinic Egas Moniz and two private offices, who agreed to participate and signed the respective informed consents. A descriptive statistical analysis was performed to obtain the data.
Results: The average age of dentists is 32.81 years, average year of activity 9.0880, weekly working time is over 40. Relatively to the pauses more than half of the doctors dentists and stomatologists (61.0%), pauses at least 5 min during the normal day of work. Of the dentists and stomatologists who take breaks, we find that 28.8% take three breaks or more during the work day. As for a work position adopted by dentists and stomatologists, most report that they work in a seated position (96.6%), while only 15.3% report standing positions. Mean intensity in the cervical spine, shoulders and

dorsal vertebral vertebral, luminous intensity in the lumbar spine and forearm and minimum in the hands, wrists, knees, feet and ankles, more frequent in females.
Discussion and conclusions: The results found are mostly similar to those of other authors, such as age, sex, years of profession, type of specific acts more practiced and preferential location of the symptomatology [1–3]. However, it can be verified from the results of our study that the complaints of dentists for the lesions were smaller compared with other studies. In summary, the study group showed to know and adopt ergonomic recommendations about position and equipment and it can be affirmed that these professionals did not present these pathologies in an intense way.

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Main reasons for rejection of deep brain stimulation surgery in candidates with Parkinson Disease

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Introduction: Stimulation at high frequency (>130 Hz) of basal ganglia has been shown to improve motor signs in patients with advanced Parkinson Disease (PD), namely bradykinesia, muscular rigidity and tremor. Deep brain stimulation (DBS) is particularly indicated for PD patients when optimised medical treatment is no longer efficient [1,2]. Still, when verifying conditions for surgery, many patients are refused the procedure. The main reasons that lead to rejection of referral patients for DBS need to be studied. We reviewed the reasons for exclusion of PD patients for the surgery in Centro Hospitalar Lisboa Norte, (Hospital de Santa Maria), between 2006 and 2016 where roughly 250 patients were implanted during this period.
Material and methods: We performed a retrospective collation of data of PD patients not implanted after being fully evaluated by a multidisciplinary team. The reasons for exclusion were classified in seven categories: age, behavioural/psychiatric dysfunction, cognitive dysfunction, PD with predominant axial symptoms (gait and/or balance impairment where DBS is not efficient), pharmacological treatment not optimized, refusal of surgery by the patient, and unrealistic goals. Anonymised patients data was analysed after study approval by the Ethics Commissions of Cooperativa de Ensino Superior Egas Moniz CRL and Centro Académico de Medicina de Lisboa.
Results: A total of 48 PD patients were identified as candidate for DBS but have not been implanted: 39.6% women (64.6 ± 5.9 years old) and 60.4% men (67.2 ± 7.2 years old) with a mean duration of the disease of 15.3 (±7.7) years. The main reason for DBS exclusion was related to poor axial motor function: 56.2% had a Movement Disorders Society Unified Parkinson Disease Rating Scale – (MDS-UPDRS) part III score >2 in "On" time in items of gait, freezing or postural instability. Behavioural/psychiatric disorders came second (39.6%), followed by dementia (22.9%), and age above 70 years old (20.8%). In 14.5% of the cases, patients gave up surgery. Refusal was related to poor response to levodopa in 12.5% of the cases and to unrealistic goals in 8.3%. Some patients had more than one reason to be rejected.
Discussion and conclusion: Our results show that the main reason for rejection in our patients is the presence of axial motor symptoms, contrary to the results from the study of Abboud et al. (2014) that identified significant cognitive decline as the main reason for exclusion [3]. This may be related to the external referral to specialized centers. A significant proportion of patients who show interest in this intervention are identified as not suitable. It is important to carry out a rigorous multidisciplinary evaluation to improve the efficiency of the intervention, reduce adverse events and meet patient and health care provider expectations.

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Evaluation of the relation between xerostomia and chronic systemic diseases

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Introduction: Xerostomia is defined as a subjective complaint of feeling of dry mouth and is usually related to reduction of salivary secretion but sometimes it can also occur due to changes in the properties of the components of saliva [1]. It can be referred by individuals of any age; however, the prevalence of this symptomatology is more associated with middle-aged and elderly individuals [2]. It is a serious condition, often neglected by medical practitioners and oral health professionals, and often goes unnoticed to the dentist or hygienist during a routine dental examination, despite contributing to a strong disturbance in the quality of life of these patients [3]. The xerostomia may have other etiologies and its higher incidence is not confined only to the elderly but is often referred by individuals with systemic chronic diseases, such as hypertension, diabetes, kidney insufficiency, cardiovascular diseases, among others [4].
Materials and methods: The target population consisted of adults of both sexes and of different ages who were patients that frequented general medicine consultations in the Social Services of Lisbon's City Hall. The target population proceeded to fill in a questionnaire. This questionnaire was anonymous and optional. Subsequently the results of the questionnaires were analysed using the SPSS software, where the sample of 270 patients, and the possible relationship between the complaint of xerostomia and the presence of diagnosis of chronic systemic diseases was studied. The statistical significance was measured using the Qui-Square test, where a p value lower or equal to .05 was considered as significant.
Results: Statistical associations were found between diabetes, hypertension, cardiovascular diseases, rheumatic diseases, depression and anxiety with complaints of xerostomia.
Discussion and conclusion: Our study allowed us to observe that there is a statistical association between xerostomia and certain chronic systemic diseases, such as diabetes, hypertension, cardiovascular diseases, rheumatic diseases, depression and anxiety. The association between systemic diseases and chronic xerostomia is referenced by various authors and explained as the result of the mechanisms of diseases or due to drug interaction [5]. Our results also come in agreement with the findings of previous published works that associated these previous diseases individually with xerostomia. This work helps reenforce the need of a better bond between medical practitioners and dentists to assure a better understanding of the patient well being.

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The South Region Cancer Registry: an evaluation of its exhaustiveness in a cohort of lung cancer patients

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Introduction: The increasing incidence of cancer in Portugal is worrisome and may be explained by lifestyle changes, growing life expectancy and improved early detection. The regional cancer registry for the south region exists since 1996. It was initially set up to observe and monitor the disease incidence, prevalence and survival, i.e. register all information about new cancer cases [1]. More recently, since adaptive pathways have been proposed [2], together with the creation of Sinats [3], led to an emerging area of interest in ROR-Sul, therapeutic effectiveness monitoring. This area includes collecting high quality data to further refine the known benefit–risk ratio and to judge the therapeutic added value [4]. It is particularly important for the Portuguese health service (PHS) to evaluate the effectiveness of new high-priced medicines [5]. This study aimed to evaluate the exhaustiveness of the *Registo Oncológico Regional Sul*.

Materials and methods: To assess the exhaustiveness of cancer registry in a cohort of lung cancer patients diagnosed during the year 2014 (01/01/2014 to 01/01/2015). Cases were selected based on the topographical codes using the 10th International Classification of Disease for Oncology: C34.0 Main bronchus; C34.1 Upper lobe, lung; C34.2 Middle lobe, lung; C34.3 Lower lobe, lung; C34.8 Overlapping lesion of lung; C34.9 Lung, NOS. Data was analyzed using IBM SPSS software, v.24, comprising descriptive analysis of missing data. Data was anonymized and no patient was identifiable.

Results: The selected cohort included 3457 patients, the majority of male gender ($n = 2540$; 73.5%). Variables considered to have a high exhaustiveness (defined as missing $<1\%$) of missing values were: gender (0%), date of diagnosis (0%), date of first medical appointment (0%), district where diagnosis was made (0%), stage of disease at first diagnosis (Unknown – 8.2%); cancer morphology (0%) and topography (0%), cancer differentiation (Unknown – 63.8%; non-applicable), vital state (0%), and date of last contact (0%). Medium exhaustiveness (defined as missing 1–15%) was found for mutations (ALK mutation – 12.8%, KRAS mutation – 7.5%, EGFR mutation – 2.5%) and immunotherapy (7.8%). Low exhaustiveness (defined as missing $>15\%$) was found for: performance status (66.3%) treatment received (36% for chemotherapy regimen) and chemotherapy treatment response (44.9%).

Discussion and conclusions: Cancer registry is organised to foresee compulsory variables and optional ones. As expected, the exhaustiveness of compulsory variables was near 100%. So far, treatment and mutation variables are not compulsory. However, if the cancer registry is intended to support effectiveness studies in the future, this option should be carefully considered. The low level of detail in clinical files should also be considered, in regards to treatment, where perhaps an educational investment must be made.

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BIOCHEMISTRY

Caspases and base excision repair genes polymorphisms and therapeutic response to hydroxyurea in Philadelphia-negative myeloproliferative neoplasms: a study in a Portuguese population

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Introduction: Several single nucleotide polymorphisms (SNPs), influencing DNA repair capacity and apoptotic status, may confer genetic predisposition to Philadelphia-chromosome negative chronic myeloproliferative neoplasms (PN-MPNs), and determine therapeutic response and clinical course [1–5]. Moreover, despite the development of more efficient drugs in the last years, some patients with PN-MPNs still progress to conditions more aggressive and difficult to treat such as myelodysplasia, myelofibrosis and acute leukaemia [6,7]. We intend to evaluate the role of SNPs in genes involved in apoptosis and base excision repair (BER) pathway in PN-MPNs therapeutic response to hydroxyurea, the more common drug used, in terms of disease progression and predisposition to new non-myeloid neoplasms and thrombotic events.

Materials and methods: Case–control study of 133 Caucasian Portuguese patients diagnosed with PN-MPNs, during the period 1992–2016, with a total of 17 cases with progression to myelofibrosis/leukemia, 11 who developed new non-myeloid neoplasms and 22 who presented thrombotic events. All apoptosis and BER pathways SNPs included in this study were genotyped using real-time PCR (RT-PCR 7300 Applied Biosystem), through TaqMan® SNP genotyping assays (Life Technology), according to manufacturer instructions, and were evaluated using regression analysis by SPSS 22.0 (SPSS Inc.). The informed consent of the subjects and acceptance of the study protocol by a local ethics committee has been obtained.

Results: BER polymorphisms in heterozygosity showed a decreased risk for APEX1 Asp148Glu (OR = 0.19; 95%CI, 0.04–0.99) and an increased risk for *XRCC*1 Arg194Trp OR = 6.58; 95%CI, 1.60–27.06) for progression to myelofibrosis/leukemia in ET patients. Globally, *CASP8* Asp270His variant is associated with an increased risk for development of nonmyeloid malignancies (OR = 5.700; 95%CI, 1.366–23.783). Concerning thrombotic events, and also globally, there is an increased risk for the *MUTYH* Gln335His variant (OR = 5.073; 95%CI, 1.13–22.80) and a border-libne effect for *XRCC1* Gln399Arg variant presence (OR = 0.13; 95%CI, 0.02–1.04). Evaluation of other possible predictive factors (ex.: exposure to citoredutive agents) is still ongoing.

Discussion and conclusions: Although larger studies are required to confirm these results and to provide conclusive evidence of association between these and other caspases/BER variants and PN-MPNs therapeutic response/clinical course, these new data may contribute to a best knowledge of the pathogenesis of these disorders and, in the future, to a more rational and efficient choice of therapeutic strategies to be adopted in PN-MPNs treatment.

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Epigenetic alterations in sepsis

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Introduction: Sepsis is a life-threatening complication of infection. Typically, a localized infection triggers a systemic inflammatory cascade resulting in widespread organ damage, organ failure, and often death. Of the 31.5 million cases of sepsis worldwide annually, it is estimated that there are more than 5 million deaths [1]. The pathophysiology of sepsis involves an extensive reprogramming of gene expression between the phase of immune activation and immunosuppression. We hypothesize that septic and non-septic critically ill patients have different and specific patterns of genomic DNA methylation.
Material and methods: We created the first cohort (72 septic patients and 69 non-septic critical ill patients) to evaluate differences in methylation. These patients were admitted to an intensive care unit (ICU). Whole blood samples were extracted on the first day of the admission (DNA PaxGene Tubes or Buffy Coat) to compare the % of gDNA methylation, through Illumina 450k arrays. Bioinformatic approaches were used to correlate changes in epigenetic and gene expression. Epigenetic mechanisms, including histone modifications, DNA methylation and microRNA expression are master regulators of gene expression in both normal and pathological states.
Results: We constructed two sets of genes, one from the literature, that were directly correlated with sepsis and different metabolic consequences (coagulopathy, apoptosis, endothelial dysfunction, immunoactivation, immunosuppression). And another set of genes from genes that have been reported to be differentially expressed in sepsis. We filtered the set genes of the literature with a $\Delta\beta \leq 0.02$ and *p*-value $<.05$ and applied a pipeline of 1.5-fold-change and a *p*-value $<.05$ in the set of transcriptomics genes. We fused both lists to create a list of genes that were differentially methylated and expressed when comparing patients with and without sepsis and we obtained 10 genes (including HLA-C, HLA-DQB1, FADD, C3AR1).
Discussion and conclusion: Although there is limited data on epigenetic regulation of sepsis, we could localize plausible differences either in the methylome and genome between septic and non-septic critically ill patients. This extensive analysis constitutes the basis for future studies disease-specific methylation and gene expression and for the potential development of epigenetic biomarkers for sepsis. For our knowledge, this was the first time that a group did a study in septic patients methylome and also it was the first time that a pipeline of genes that were simultaneously differentially methylated and expressed was established, when we compared septic and non-septic critical ill patients.

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Epigenetic alterations of the PI3K/Akt signaling pathway as potential pancreatic cancer biomarkers

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Introduction: Pancreatic cancer remains one of the most fatal cancers worldwide due to a late diagnosis and lack of therapeutic strategies, with a 5-year survival rate of approximately 5% worldwide [1,2]. Both genetic and epigenetic events contribute to its development and progression by affecting gene expression regulation [3]. Aberrant activation of the PI3K/Akt pathway occurs in more than half of pancreatic cancers and triggers a cascade of signals that promote cell survival and proliferation and is associated with tumor undifferentiation and a poor prognosis [4]. The present work assessed the epigenetic regulation of the genes related with PI3K/Akt pathway and its clinical impact, which has not been yet fully scrutinized.

Materials and methods: We selected the genes related with this pathway that presented differential expression between normal and malignant tissue using the pancreatic expression database (fold-change $\geq$1.5, *p*-value $<$.05) [5]. We assessed the methylation status of the selected genes using The Cancer Genome Atlas (TCGA) data on the cohort of pancreatic adenocarcinoma [6]. Only data from patients with no history of neoadjuvant therapy were included. We evaluated the impact of DNA methylation alterations on patient's outcome through the analysis of Kaplan–Meier curves for overall and recurrence-free survival. Analysis of the receiver operator characteristic (ROC) curve was used to determine the optimal cut-off value for maximal sensitivity/specificity. For this analysis only the methylation probes with *p*-value $<$.05 and delta-beta value $>$0.2 were considered.

Results: Methylation levels of the selected genes allowed the distinction between normal and malignant tissue ($p < .0001$) and the methylation levels of the *ITGA4*, *ITGA2*, *PPP2R5C*, *SFN* and *PIK3R1* genes could act as a prognostic indicator of patients' survival with high sensitivity and specificity (Figure 1). Also, the methylation levels of *SFN* and *PIK3R1* could also predict recurrence of the patients (Figure 2).

Discussion and conclusions: Despite the promising results in this area none epigenetic biomarker has reached the clinic yet. Our results reveal that the methylation levels of genes related with the PI3K/Akt pathway could be used to predict the outcome of pancreatic cancer patients with high sensitivity and specificity. These results provide new evidences of the potential of epigenetic alterations as pancreatic cancer biomarkers for disease screening and management.

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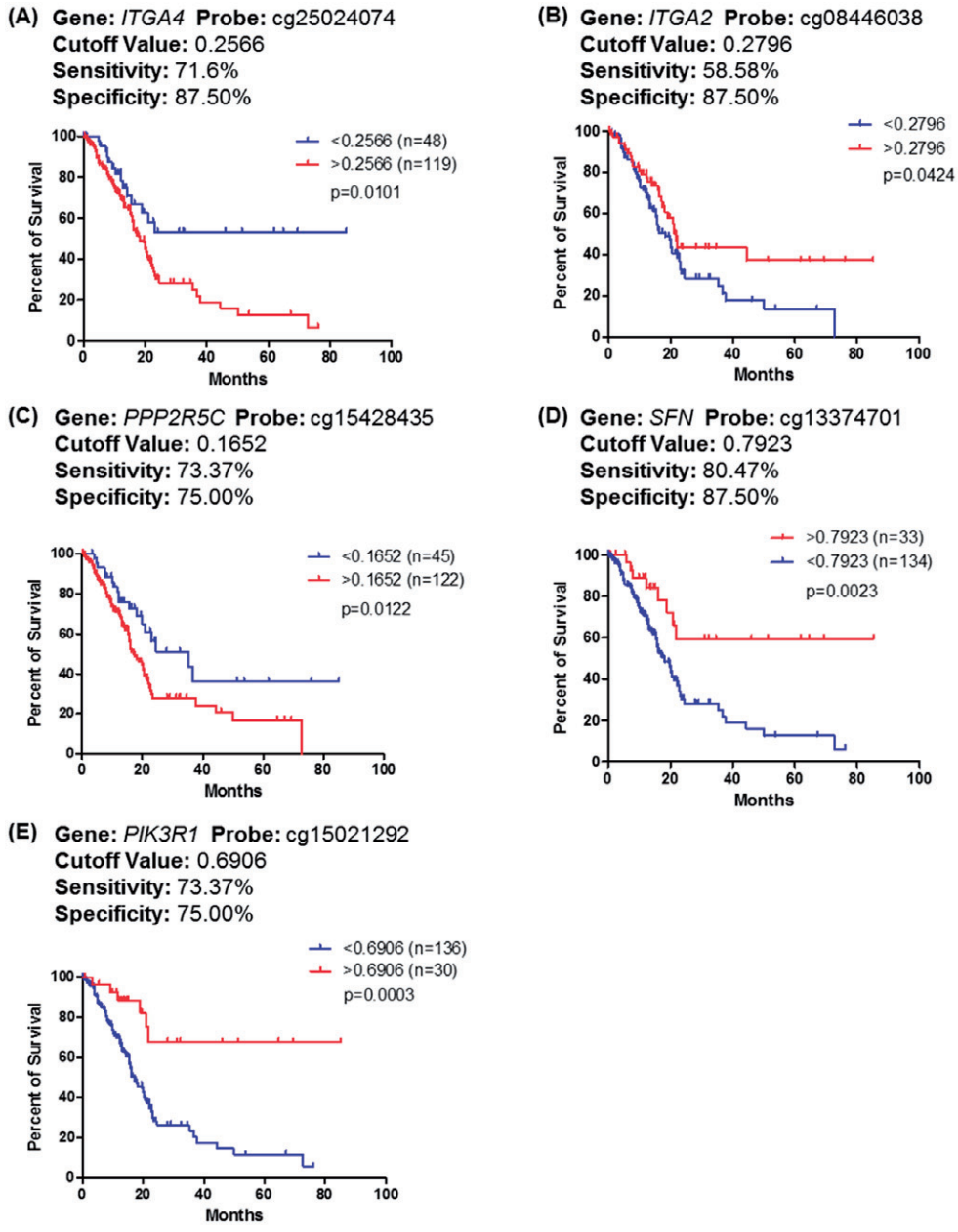

Figure 1. Methylation levels of PIK3/Akt-related genes can predict survival in pancreatic cancer. Kaplan–Meier curve for overall survival of pancreatic cancer patients considering the methylation of (A) *ITGA4*, (B) *ITGA2*, (C) *PPP2R5C*, (D) *SFN* and (E) *PIK3R1* genes. The cut-off value for each gene was selected through the analysis of the receiver operator characteristic (ROC) curve for maximal sensitivity/specificity. Patients with methylation levels inferior to the cutoff value are considered as hypomethylated (represented in blue) and patients with methylation levels superior to the cutoff value are considered hypermethylated (represented in red).

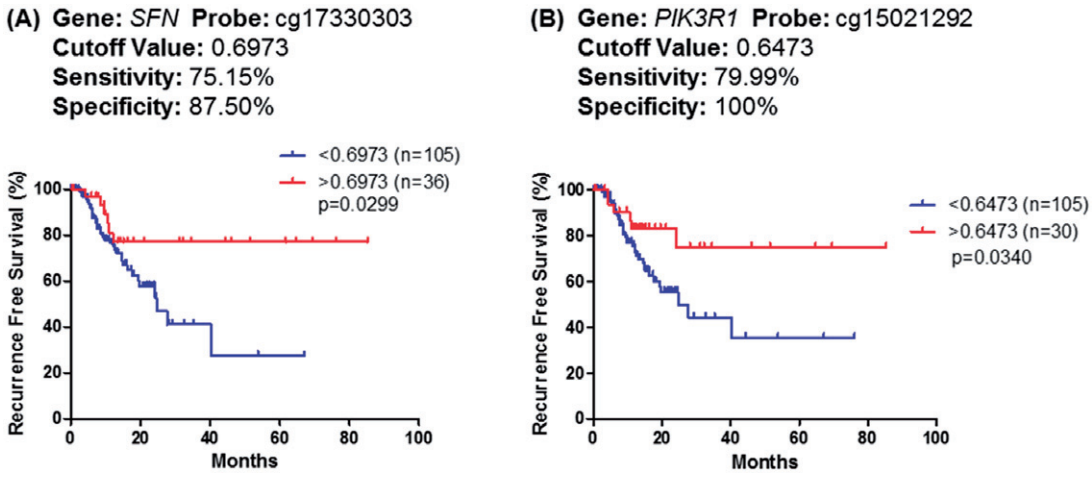

Figure 2. Methylation levels of PIK3/Akt related genes can predict recurrence in pancreatic cancer. Kaplan-Meier curve for recurrence free survival of pancreatic cancer patients considering the methylation of (A). *SFN* and (B) *PIK3R1* genes. The cut-off value for each gene was selected through the analysis of the receiver operator characteristic (ROC) curve for maximal sensitivity/specificity. Patients with methylation levels inferior to the cutoff value are considered as hypomethylated (represented in blue) and patients with methylation levels superior to the cutoff value are considered hypermethylated (represented in red).

Characterization of APOBEC3 expression in breast cancer cell lines

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Introduction: All cancers are caused by driver somatic mutations that confer growth advantage to the cell in which they occur. Each mutational process generates combinations of mutations producing specific "signatures" [1] and some of these have been associated with APOBEC3 deaminase activity because of the high incidence of C to T mutations [2]. Physiologically, C deamination plays an important part in innate and adaptive cell immunity but when inappropriately targeted may induce carcinogenesis. Our goal is to characterize APOBEC3 expression in different tumour breast cell lines, for further use as *in vitro* systems to study the relationship between APOBEC3 activity and increase of somatic cell mutations, particularly in target genes, such as *TP53* gene.
Materials and methods: RNA was extracted from breast cancer cell lines (MCF7, BT20, SK-BR3, HCC1806 and HCC70), non-tumour breast cells (MCF10A) and from embryonic kidney cells (HEK-293T) using High Pure RNA Kit (Roche). cDNA synthesis was performed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). APOBEC3 expression levels were analyzed by qRT-PCR using TaqMan Technology on a Rotor Gene RG-3000 Corbett Research instrument. APOBEC3 amplifications were made with specific qPCR primers and probes [3] and the housekeeping gene TBP was used as a reference gene. Quantification of different transcripts was done according to published methods [3].
Results: Breast cancer cell line HCC1806 overexpressed APOBEC3B, 3C and 3G. BT20 overexpressed APOBEC3B and 3C and MCF7 only expressed APOBEC3B. Non-tumour cell line MCF10A expressed all these three proteins, mainly APOBEC3C. The remaining cell lines have no significant expression of APOBEC3.
Discussion and conclusions: Our results show variation in APOBEC3 expression in different breast cancer cell lines. With these cell models we will be able to test the hypothesis that inhibition of APOBEC3 prevents TP53 inactivation and may influence chemotherapy resistance.

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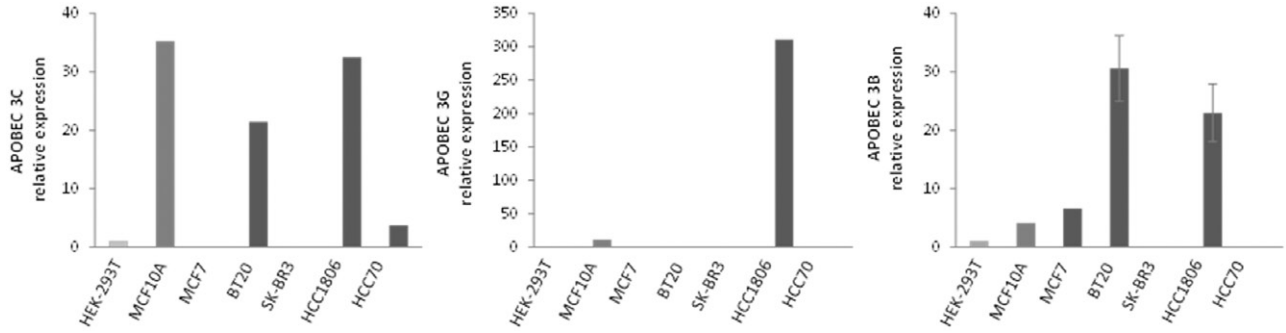

Figure 1. Expression profiles for APOBEC3C, A3G and A3B in a panel of representative breast cell models.

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Identification of protein markers for bioactivity in *Streptomyces aculeolatus*: a proteomic approach

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Introduction: Actinomycetes *Streptomyces aculeolatus* belong to MAR4 streptomycete lineage and are known to produce hybrid isoprenoids [1]. These compounds have great bioactive potential as antibacterial, antifungal, and anticancer agents making them potentially attractive to the pharmaceutical industry [1,2]. A total of 400 actinomycetes were isolated from marine sediments collected from the Madeira Archipelago, six of which belong to the *S. aculeolatus* species, as determined by a phylogenetic analysis based on the sequencing of the 16S rRNA gene [3]. These strains were able to produce secondary metabolites with distinct bioactivities. In this context, we aim at characterizing the proteins involved in the synthesis of the main bioactive compounds generated by 4 of these *S. aculeolatus* strains. To achieve this goal, we have been following a differential proteomic analysis based on two-dimensional electrophoresis (2DE) coupled to mass spectrometry techniques (MS).
Materials and methods: The total and extracellular proteomes of *S. aculeolatus* strains were analysed in the pH range 4–7, in 12.5% acrylamide gels stained with Colloidal Commassie Blue.
Results: Preliminary results showed similar protein profiles for the total proteomes, with most of the spots in the pH range 5–7 and having high molecular weight (above 50 kDa). Regarding the extracellular proteomes, they presented very different profile between strains and also revealed several high molecular weight proteins.
Discussion and conclusions: The significant differences observed in protein expression may be due to a number of factors, such as strains belonging to different phylotypes, unidentified differences at the genome level and biosynthetic efficiency of each strain. The great diversity of protein species found in the exoproteomes provides a good opportunity for investigating novel bioactive metabolites. As future work, we intend to analyse in detail the 2D gels from the *S. aculeolatus* strains and to identify the differentially expressed proteins by mass spectrometry (MS) techniques.

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Toward the understanding of the relationship between amyloid fibril formation and the protein precursor hierarchy level of organization

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Introduction: Amyloid fiber formation has long been associated with several degenerative diseases. Currently there are approximately fifty reported human diseases linked with amyloid, including both localized and systemic amyloidosis as well as transmissible amyloidosis [1]. The general mechanistic view of amyloid formation implies native peptides or proteins to undergo conformational changes that lead to the formation of amyloidogenic, partially structured, β-sheet rich intermediates that upon aggregation lead to nucleus formation and subsequent fibril growth [2]. For these conformational changes to occur an energy barrier defined by the relative conformational stability of both the polypeptide precursor and the amyloidogenic intermediate, need to be overcome [3]. In the present study, we hypothesize that different protein hierarchical organizational levels have different propensities to form amyloid. The hypothesis stands that oligomeric proteins have lower propensity for amyloid formation than monomeric proteins. To assess this hypothesis amyloid formation kinetics of transthyretin (TTR), an oligomeric protein and insulin, a monomeric protein was obtained with thioflavin-t (ThT) fluorescence spectroscopy.
Material and methods: TTR wt was obtained through expression in E. coli BL21, followed by purification through ionic exchange and size exclusion FPLC. Monomeric insulin was acquired from Sigma. Amyloid formation of both proteins was induced through incubation of 80 μM of insulin and TTR in PBS solution (pH 7.0) at 37 °C with constant agitation (1300 rpm) for 48 h. Fibril formation was monitored by the ThT binding assay as previously described [4]. Fluorescence measurements were performed in a Perkin Elmer LS50B spectrofluorometer at 482 nm, with excitation at 450 nm.
Results: Figure 1 shows the ThT fluorescence intensity variation in relation to time during the process of amyloid fibril formation by TTR and insulin. These preliminary results show that ThT fluorescence signal is lower in TTR when compared to insulin. Moreover, this time dependent assay also show that TTR has a longer lag time than insulin.
Discussion and conclusions: The results obtained suggest that the amyloid forming precursor protein hierarchical organizational level might be important for the kinetics and thermodynamics of amyloidogenesis.

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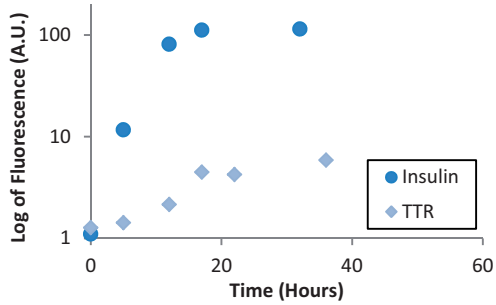

Figure 1. Time dependence of the ThT fluorescence intensity for amyloid fibril formation in insulin and TTR.

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Identification of hippuric acid in urine samples from aircraft cleaning workers exposed to Toluene

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Introduction: Toluene is a very common chemical which has been extensively used as a cleaning agent and as a solvent in many consumer products such as paints and glues. However, toluene produces central nervous system toxicity and fetotoxicity [1]. The urinary metabolite of toluene, hippuric acid (HA) is widely used as a biological marker of toluene exposure. Airborne toluene exposure is regulated in many countries and in 2014, the standard threshold limit value (TLV) of toluene in working environment established by ACGIH was 20 ppm, corresponding to 1.6 g HA/g creatinine for urinary samples [2]. The aim of this work was to detect HA, in urine samples of aircraft cleaning workers to evaluate its occupational exposure.
Materials and methods: Biologic monitoring of toluene exposure by urinary hippuric acid determination was performed with 25 aircraft cleaning workers. Urine samples were collected at the beginning (Monday 8 a.m.) and at the end of the week (Friday 6 p.m., end of shift), through 2 months and were kept at −20 °C until analysis (until now, the total of examined samples was 92). Before analysis of urinary hippuric acid, urine samples were prepared by solid phase extraction, derivatized with *N*-Methyl-*N*-(Trimethylsilyl)-Trifluoroacetamide (MSTFA), to obtain HA-MSTFA, and then analyzed by GC-MS.
Results: GC/MS analysis of urine of workers exposed to toluene indicates the presence of HA. The chromatograms showed a peak at 8.156 min which was identified as HA trough the product-ions mass spectrum from the molecular ion HA-TMS compound. SCAN mode was used to choose the fragmentation ions of HA-MSTFA. Its spectrum was characterized by a [C_6H_5-CO] ion at *m/z* 105, [HA-Si] ion at *m/z* 206 and an M^+-77 [C_6H_5] ion at *m/z* 147. To confirm the results of fragmentation ions an interpretation on mass-spectrum GC-MS was conducted using standard solutions of HA and the database of National Institute Standard and Technology. In the present study, 91% of urinary samples had hippuric acid levels >1.6 g/g creatinine, the ACGIH-recommended biological exposure index for hippuric acid in urine. The result showed that the average concentration of hippuric acid in urine samples of aircraft cleaning workers exposed to toluene was 23.1 (SD = 20.7) ppm, corresponding to the average level of hippuric acid in urine of 11.1 (SD = 9.6) g/g creatinine.
Discussion and conclusions: The detection of HA in urine samples collected from aircraft cleaning workers confirm the possible occupational exposure to toluene. After detection, we intend to measure levels of this biomarker in all urinary samples and also to validate the analytical procedure according to the International Conference on Harmonization (ICH) guidelines [3].

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To the memory of late Professor José Martins dos Santos.

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Co-fermentation of glucose and maltose by the derepressed mutant strain *Saccharomyces cerevisiae* PYCC 5334 – a proteomic insight

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Introduction: *Saccharomyces cerevisiae* (*S. cerevisiae*) are able to ferment industrial substrates like starch hydrolysates, which are composed of the sugars maltose, maltotriose, glucose, sucrose and fructose, together with other nonfermentable carbohydrates However, due to the repressive effect of glucose in the metabolism of alternative carbon sources, all other fermentable sugars remain unused by the yeast until glucose has been depleted [1,2]. In order to improve fermentation performance, we followed a random UV mutagenesis to obtain a mutant, designated PYCC 5334, with the ability to co-ferment glucose and an alternative sugar [3]. Previous characterization of the mutant showed that the derepressed phenotype of this strain resulted from a failure in induction, repression and inactivation pathways whose extension can only be assessed by global screening approaches such as transcriptomics and proteomics. *Saccharomyces cerevisiae* is a model organism and the identification of the molecular determinants. Understanding its phenotype will be extremely useful to illuminate new aspects of the glucose signalling pathways. In this context, we aim to identify proteins differentially expressed between *S. cerevisiae* wild type and mutant strains by proteomic analysis based on two-dimensional electrophoresis (2DE) coupled to mass spectrometry techniques (MS).
Materials and methods: Both strains, the wild type and the derepressed mutants, were grown on glucose to exponential phase. After cells disruption, total protein extracts were analysed by isoelectric focusing in the pH range 4–7, followed by a separation on the second dimension by SDS-PAGE in 12.5% acrylamide gels. Gels were stained with Colloidal Commassie Blue and analysed. Gel image analysis was performed with Melanie 7.0 software.
Results: The 2D gels of the total protein fractions of wild type and diploid mutant strains showed 544 and 618 spots, respectively. In the same conditions, the protein profile of haploid mutant strain showed 670 spots. The wild type and diploid mutant strains presents about 70 spots in common, while the haploid strain has about 400 common spots with the wild type.
Discussion and conclusions: Surprisingly, the proteome of the wild type and mutant displayed rather different profiles with only 10–15% of common spots. Most probably, the mutagenic treatment that was used account for so many changes. Instead, the proteome of the haploid strain, obtained from PYCC 5334 after sporulation in which the glucose repression phenotype is also present, showed stronger similarities. Therefore, the comparative analysis and statistical validation of the protein profiles from the haploid strain and wild type is currently underway. A selection of spots exclusive of one condition or exhibiting different volumes in each profile will be identified by MS.

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Real-time detection of nitrite in the brain using carbon fibre microelectrodes

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Introduction: The inorganic anions nitrate and nitrite are emerging as biological mediators via reduction in blood and tissues to the ubiquitous cell messenger, nitric oxide (NO). This molecule is linked to a number of important biological processes in the brain and is also involved in mechanisms associated with excitotoxicity, neurodegenerative diseases (Parkinson and Alzheimer's) and neuroinflammation. NO may have either beneficial or deleterious effects depending upon its concentration dynamics or its rate of production and inactivation [1,2]. Recently, ascorbate and NO have been shown to be temporarily and spatially related in a quantitative fashion [3]. Given the fact that ascorbate is considered the ultimate reductant, the hypothesis that ascorbate may reduce nitrite to NO in the extracellular space of neurons under activation is a challenging one [4]. Herein, we used carbon microsensors to perform *in vivo* real time measurements of nitrite concentration in the brain's extracellular space. The application of effective microsensors for nitrite, NO and ascorbate would allow testing the hypothesis that nitrite may generate NO upon reduction by ascorbate in the brain's extracellular space.
Materials and methods: Homemade carbon fibre microelectrodes (CFMs) were used to perform fast cycling voltammetry (FSCV) measurements of nitrite at +1.1 V (versus Ag/AgCl) in buffer media and in the cerebral extracellular space of an anesthetized adult male Wistar rat.
Results: CFMs showed good *in vitro* linear response to nitrite (5–500 μM), with sensitivity dropping 8%, 12% and 21% when 100, 200 and 500 μM ascorbate was present, respectively. Transient current signals at +1.1 V (versus Ag/AgCl) were recorded upon pressure injection of 38 and 125 nL of a 5 mM nitrite standard solution in the cerebral extracellular space of an adult male Wistar rat.
Discussion and conclusions: By using FSCV, a technique that allows very fast scan rates and sampling current values at a specific hold potential, reproducible transient current variations were observed upon localized nitrite pressure ejection in the rat's brain. To the best of the authors' knowledge, exogenous nitrite clearance values were recorded *in vivo* for the first time in brain tissue. These preliminary results are very promising and might be a breakthrough in the understanding of *in vivo* (sub)second nitrite changes.

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Adiposity-associated markers in women with multiple sclerosis taking oral contraceptives

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Introduction: Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of unknown etiology. A large body of evidence indicates that sex hormones have a role in the development and clinical course of the disease [1]. Recent data have also suggested that adiposity levels and plasma lipoprotein metabolism could be implicated in the pathogenesis of MS [2]. The goal of the present study was to investigate whether oral contraceptive (OC) use is associated with the age at disease onset (DO) and adiposity, plasma lipid and adipokines levels.
Material and methods: The studied population included a cohort of 59 relapsing-remitting MS patients (age 33 (SD ± 7) years with a mean disease duration 4.6 years (SD ± 4) before the beginning of immunomodulatory therapy. Menarche age, BMI and waist and hip circumferences were obtained and the subjects stratified in OC users at the time of DO (current users) and never OC users or who stopped its intake before DO. Leptin and adiponectin levels were determined by ELISA (Mercodia) and the lipid profile by enzymatic reagent kits. Two-sample t-tests and multiple regression analysis were used.

Results: Younger age at the time of OC use onset and longer duration of OC intake were associated, respectively, with lower ($p = .034$) and higher ($p = .003$) ages at DO. In overweight patients (BMI $\geq 25\,kg\,m^{-2}$), the age at DO was lower in OC current users ($p = .024$). In current users, higher adiponectin levels and ApoB/ApoA-1 ratios were associated with later ($p = .040$) and earlier ($p = .043$) ages at DO, respectively. Younger menarche age was associated with higher BMI ($p = .028$) and leptin levels ($p = .042$) but not with age at DO ($p = .608$).
Discussion and conclusions: These results suggest that OC use affect the age at DO probably in association with alterations in adiposity-associated markers. These new findings support the view that an interplay between sex hormone exposure and adiposity may be implicated in the pathogenesis of MS.

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PHARMACY

Beneficial effects of an aqueous extract from *Cinnamomum burmannii* in STZ-induced type 1 diabetic rats

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Introduction: There is evidence in the literature that cinnamon can reduce blood glucose level in diabetes *mellitus* [1]. This hypoglycemic effect of cinnamon is attributed to its bioactive compounds with antioxidant properties [2]. The aim of this study was to investigate the effects of aqueous cinnamon extract (ACE) on Streptozotocin (STZ)-induced type 1 diabetic rats and to identify its bioactive compound(s).
Materials and methods: The animals were rendered diabetic with a single injection of STZ and subsequently divided into three groups. One group was given saline (untreated group) and two groups were given aqueous cinnamon extract (ACE) orally (75 mg/kg and 150 mg/kg) (treated groups), for 11 weeks. Blood glucose levels (BGL), body weight and food consumption were monitored weekly. After 11 weeks, animals were humanely killed and blood was taken by cardiac puncture and pancreas isolated for analysis. HPLC analyses were performed to identify the compounds of 0.337g cinnamon/25 mL methanol. All animal experiments were performed in accordance with the European Legislation for use and care of laboratory animals, with the agreement of the national authority and Faculdade de Medicina Veterinária ethics committee.
Results: The results showed that ACE decreased BGL on type 1 diabetic rats. Moreover, diabetic rats treated with ACE showed a significant ($p < .05$) increase of body weight and a significantly decrease ($p < .05$) of food intake, compared with untreated diabetic rats. Daily ACE intake resulted in an improvement of the number of insulin containing positive cells in diabetic rats. HPLC analyses revealed the presence of phenolic compounds such as cinnamaldehyde (2.47%), cinnamic acid (0.37%), coumarin (0.16%) and cinnamyl alcohol (0.02%).
Discussion and conclusions: The data revealed that ACE can significantly improve the body weight of diabetic rats compared to untreated rats, and this could be attributed to the insulin-mimetic effect of cinnamon through improving insulin receptor signalling [3] or repairing the pancreatic beta cell [4]. Although that result, food intake decreased with ACE treatment, which may be occur as a result of enhancing insulin-regulated glucose utilization by cellular glucose uptake through GLUT 4 translocation [1]. ACE can also increase the number of positive beta cell in the pancreas of diabetic rats, suggesting that its bioactive compounds could repair the pancreatic beta cell. In conclusion, the current data reveal that aqueous cinnamon extract may exert a protective effect on diabetes *mellitus* by preventing beta cell death.

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Identification of phytochemical compounds with antioxidant activity in *Physalis peruviana* L. extracts

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Introduction: *Physalis peruviana* L. chemical content, rich on carotenoids, flavonoids and other phenolic compounds, has been associated with health beneficial properties such as anti-inflammatory, anticancer and antioxidant activity [1]. Therefore, this study aims to determine the antioxidant activity of different extracts of *Physalis peruviana* L. fruit, grown in Portugal, and to identify some of the compounds responsible for this activity.
Materials and methods: Extractions were performed with hexane, methanol:water:acetone and ethanol to obtain carotenoids, phenols and flavonoids, respectively. Extracts were evaluated for carotenoids, total phenols, total flavonoids and dry weight was determined. After acidic hydrolysis, HPLC-DAD analysis was performed in order to identify bioactive compounds in phenolic extracts by comparison with UV-spectra and retention time of standard compounds. The antioxidant activity was determined through the FRAP reducing power test and inhibition of radicals ABTS, $NO^{\bullet}$ and $O_2^{\bullet -}$.
Results: Phenols, flavonoids and carotenoids concentrations were 2.78 ± 0.06 mg gallic acid/g, 1.61 ± 0.01 mg quercetin/g and 20.01 ± 0.03 mg β-carotene/g of dry fruit weight, respectively. In which concerns the antioxidant activity, reducing power is achieved with 2.83 ± 0.09 μmol of trolox/g of dry weight in phenol extract and 0.39 ± 0.11 μmol of Trolox/g of dry weight in carotenoid extract. Inhibition percentage for free radical ABTS was, respectively, $14.3 \pm 0.3\%$ and $11.9 \pm 0.5\%$ with concentrations of 11.11 mg/L of gallic acid and 42.7 mg/L of β-carotene. For the same extracts, inhibition of $O_2^{\bullet -}$ radical was $22.5 \pm 0.3\%$ and $42.4 \pm 0.4\%$, respectively. For $NO^{\bullet}$ radical, percentages were $12.8 \pm 2.8\%$ for 11.1mg/L phenol extract and $33.2 \pm 0.8\%$ for 14.3 mg/L carotenoid extract. HPLC-DAD identified catechins as the major flavonoid compound with estimated concentration of 1.34 mg/g of dry weight.
Discussion and conclusions: Findings show that *Physalis peruviana* L. is rich in carotenoids and its main flavonol is catechin that exhibits high antioxidant activity through hydrogen bond [2]. The findings also suggest that reducing power is due to the presence of phenols, as already described for this fruit [3]. Phenols are also responsible for the higher inhibition percentages of ABTS and $O_2^{\bullet -}$ radicals, due to their chemical structure that can prevent carcinogenesis through this antioxidant mechanism as shown *in vitro* [4]. Moreover, results show that carotenoids are stronger inhibitors of the $NO^{\bullet}$ free radical than phenols in similar amounts, which suggests that *Physalis peruviana* L. antioxidant activity can be due to a synergy between carotenoids and phenols.

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Preliminary study of *Actinidia arguta* leaves as source of bioactive compounds

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Introduction: *Actinidia arguta*, commonly known as baby kiwi, is an abundant seasonal fruit in Asia, being recently introduced in Europe. The fruit is now available in Portugal, leading to a high consumer attention. During the baby kiwi harvest, a large amount of leaves falls and stay in the soil. To the best of our knowledge, few studies concerning their composition are reported. The aim of this study is to determine and compare the total phenolic and flavonoid contents (TPC and TFC, respectively) as well as the antioxidant activity (through DPPH and FRAP assays) of leaf extracts from different baby kiwi cultivars. The extracts were obtained taking into consideration sustainable conditions (namely solvents and temperatures).
Materials and methods: Samples were harvested in May 2016 in Famalicão (Portugal). Three different extracts were prepared according to Costa et al., namely, an aqueous (100% water), a hydroalcoholic (50%:50%) and an alcoholic (100% ethanol) one [1]. Two different biochemical assays were used to screen the antioxidant properties: scavenging activity on DPPH radicals and ferric reducing antioxidant power (FRAP). TPC and TFC were also evaluated.
Results: The alcoholic extract displayed the highest antioxidant activity in DPPH assay ($IC_{50} =$ 53.7 μg/mL). For the aqueous extract, it was not possible to determine the IC50, as the highest concentration tested (2630 μg/mL) leads to an inhibition of 41.0%. In what concerns FRAP assay, the results agree with the DPPH assay as the alcoholic extract showed the highest value. The TPC ranged between 140.7 and 440.7 mg gallic acid equivalents per gram of dry basis sample (mg GAE/g db) for hydroalcoholic and alcoholic extract, respectively. Regarding TFC, the values obtained were between 137.0 and 318.1 mg of catechin equivalents per gram db sample (mg CEQ/g db) for aqueous and alcoholic extract, respectively.
Discussion and conclusions: This preliminary study demonstrates the richness of baby kiwi leaves in antioxidants and polyphenols. According to the obtained results, the alcoholic extract could be considered the most promising, presenting a good antioxidant activity as well as TFC and TPC. This study showed the high potential of baby kiwi leaves as active ingredients for different products, such as cosmetics or pharmaceuticals. Nevertheless, more detailed studies are needed to identify and quantify the bioactive compounds responsible for this activity.

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Synthesis of new glycoconjugate compounds with potential bioactivity

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Introduction: In order to obtain new carbohydrate based compounds with potential bioactivity [1] a "Click Chemistry" methodology was applied to prepare 1,2,3-triazole linkages, which, by themselves, can show interesting bioactivity to other bioactive moieties or to biomarkers [2]. Coumarins are fluorescent heterocyclic compounds that have been shown to present biological activity against cancer and inflammatory processes among other ailments. Their fluorescent nature also favors their use as potential biological probes [3].

Materials and methods: The new glycoconjugate compounds prepared were constructed from several monosaccharides and coumarins that were connected by triazole rings. The targeted final compounds were characterized by MS, NMR, IR and Optical Rotation. Some of these compounds were tested to determine their *in vitro* antitumoral, antibacterial and antifungal activities.
Results: We have successfully synthesized 11 new compounds in good yields. Some compounds were tested for activity against cancer cell lines such as HeLa, HepG2, NCI-H460 and MCF-7, and against non-tumour cells (PLP2). These compounds were also tested against bacteria and fungi, and one of them was a better antibacterial agent than the standard streptomycin against *Protus mirabilis* and *Pseudomonas aeruginosa*, and presented similar effects in four other bacteria. Another compound showed activity against *Streptococcus salivarius* and *Protus mirabilis*. Also, it was found that some of the synthesised compounds showed similar activity to that of streptomycin to eliminate *Proteus mirabilis*.
In summary, one of the compounds prepared showed considerable activity against human tumour cell lines, namely HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) cell lines, as well as some antibacterial and antifungal activities.
Discussion and conclusions: Liver cancer is the sixth most common tumor worldwide, with approximately 1 million new cases annually, and it is the third most frequent cause of cancer death in the world. These compounds seem to be promising in the fight against this illness, but more tests need to be done, as well as preparing new compounds to add to this library of glycoconjugates.
The approaches to the synthesis of these promising glycoconjugates, as well as their biological test results will be discussed.

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Synthesis of new sugar derivatives with potential biological activity

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Introduction: Carbohydrates are biocompatible and have an important role in energy storage and metabolism, which makes them very interesting for the synthesis of potential drugs. Glucose and mannose are two monosaccharides that have different binding interactions with the organism [1]. The sugar derivatives described here contain specific units such as: PEG, a polymer chain that provides high specificity in interactions with the organism receptors; coumarins, fluorescent compounds that can be exploited as biological probes [2] and lastly, thymidine, a promising compound in the study of stem cell therapies in Alzheimer's disease [3].
Materials and methods: Commercially available compounds such as carbohydrates (glucose and mannose), PEG-2000, coumarins and thymidine were used. The obtained compounds were characterized using NMR and IR spectroscopy techniques.
Results: The different units were connected by employing coupling reactions, mainly "click chemistry", to form triazole rings as binding bridges, between azides and alkyne derivatives, as well as esterification reactions. When necessary, protective groups were used to optimize the selectivity.
Discussion and conclusions: Using this strategy new bioconjugates were obtained and submitted to biological testing. The monosaccharide moiety was always positioned at one end of the final molecule (Figure 1) in order to be recognized and interact with the receptors in the organism to be studied. The thymidine moiety was located at the other polymer terminal. Alternating the position of the remaining units, it was possible to construct a library of compounds that should permit the determination of structure-activity relationships, depending on the biological tests to be performed. If these

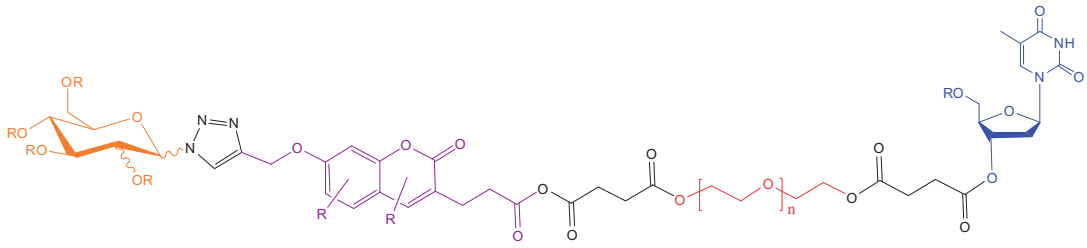

Figure 1. Structure of the generic skeleton of the desired final molecules (orange = carbohydrate; purple = coumarin; red = PEG; blue = thymidine).

tests are positive, then these compounds will be prepared as polymer nanoparticles for further studies related to their biodegradability and their use as fluorescent bioactivity probes.

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Pharmacogenetic: characterization of phase I drug-metabolising enzymes polymorphisms in a HIV-infected Portuguese population

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Introduction: The phase I drug metabolizing enzymes (DMEs), Cytochrome P450 (CYP450) are a superfamily of mono-oxygenases involved in the metabolism of many therapeutic drugs and endogenous substrates [1]. The DMEs are polymorphic and may cause pharmacokinetic variability, namely in antiretroviral (ARVs) therapies, leading to interpatient variability for drug absorption pathways [2]. Knowledge of the frequency distribution of functional polymorphisms in a HIV-infected population can guide to a better selection of therapeutic options. The aim of the present study is the characterization of phase I drug-metabolising enzymes polymorphisms in a HIV-infected Portuguese Population, from Santa Maria Hospital, Lisbon, Portugal, for featuring the interindividuality variability in antiretroviral therapy.
Materials and methods: The study includes 349 Portuguese HIV-infected adult patients receiving ARV therapy at Hospital de Santa Maria, between 2011 and 2014. The informed consents and the study protocol were approved by the ethics committee from Centro Hospitalar Lisboa Norte/Lisbon Medical school. A total of six SNPs from three different genes with pharmacogenetic and clinical relevance were genotyped by Microarray analysis and Taqman methodology. The allelic and genotype frequencies were analysed and calculated by *Hardy–Weinberg R package* based on χ^2-test, with a 95% CI and significative at 5% level. The database *1000 Genomes Project* and *HapMap Project* was used to compare the allelic and genotype frequencies between the study sample and sample among different populations.
Results: Allelic frequencies derived for non-functional allele of cytochrome P450 were (CYP)2A6*9B(16.23%), CYP2A6*14(5.32%), CYP2B6*6(28.4%), CYP2B6*18(1.40%), CYP3A5*3(82.63%), CYP3A5*7(98.04%), respectively. Genotype frequencies for mutant homozygous genotype in CYP2A6*9B, CYP2A6*14, CYP2B6*6, CYP2B6*18, CYP3A5*3 and CYP3A5*7, were 11.88%, 0.29%, 7.28%, 0.28%, 73.16% and 96.69%, respectively.
Discussion and conclusions: Comparison of all frequencies distribution among our sample fitted the patterns commonly found in the European population except for CYP3A5*3 patients which is similar to the African population. In our study we also found that CYP3A5 gene polymorphisms (referring to the presence of *3/*3 and *7/*7 variant genotypes in our

population), was significantly associated with poor metabolizer phenotype. For those patients, it is recommended to initiate therapy with a standard dose, and use therapeutic drug monitoring to guide dose adjustments. Our study indicates individual differences in gene characteristics contributing for personalized medicine treatment optimization in HIV therapeutics.

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Nanotechnology applied in the development of drug target formulation to treat breast cancer

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Introduction: The development of drug target formulations involving nanotechnology is one of the leading research areas in the treatment of cancer. Liposomes are lipidic nanostructures known to accumulate preferentially in tumour areas, leading to a prolonged drug release in the tumour microenvironment and a limited accumulation in healthy tissues. Anthracyclines, such as doxorubicin and idarubicin, are an important class of chemotherapeutic agents. Unfortunately, their efficacy in treating cancer is limited by a cumulative dose-dependent cardiotoxicity, which can cause irreversible heart failure. Nanoparticulated systems have shown to be very promising for the improvement of the therapeutic effect of these drugs, mainly due to the longer retention time in solid tumours, minimizing the systemic exposure, which improves the drug efficacy and reduces the nonspecific toxicity. The objective of this work is to make a preliminary evaluation of the potential of specific nanoparticles and liposomes to encapsulate and release idarubicin.
Materials and methods: An aqueous solution of Idarubicin (1 mM) was mixed with 45mL of poly(dimethylsiloxane)-graft-polyacrylates (0.1% w/v) with pH 8.2 for solubilization. The sample was spray-dried using an atomization membrane with 4 μm of porosity. The particles were collected by an electrostatic chamber. Unilamellar liposomes were prepared by the extrusion method. DMPC and DSPE-2000 (95:5 mol%) were dissolved in chloroform and the film formed was dried overnight in vacuum, to ensure the complete removal of the solvent. Hydration of the film was done with HEPES buffer containing 1mM of Idarubicin at 65 °C. The obtained suspension was subjected to alternate cycles of freezing and thawing and extruded several times at the hydration temperature through poly-carbonate membranes (5 times 600 nm, 10 times 200 nm), under pressurized nitrogen. Both samples were characterized concerning size using a zeta-sizer and SEM/TEM. QCM-D experiments allowed us to investigate the effect of the pH on these structures. Drug release experiments were also carried out.
Results: In order to mimic the environment of the stomach and of the intestine region, the drug release was carried out in 0.1M HCl solution (pH 1.0) and phosphate buffer (pH 8.4), respectively. For the nanoparticles, a release of ≈93% was observed in the first minute in the acidic pH. In the alkaline pH, the study was performed up to 24 h and showed a release of ≈52%. According to zeta-sizer data, the particles have 3582 ± 450 nm. SEM images showed that idarubicin was efficiently encapsulated. Regarding liposomes, their size was 199.5 ± 9.2 nm. QCM-D analysis showed that the change to pH 6.5 (mimicking tumor microenvironment) leads to a loss of mass. Drug-release studies are still on-going.
Discussion and conclusions: The results show that nanoparticles with idarubicin exhibit a rapid release at acidic pH, indicating a possible follow-up of the studies for oral formulations. Regarding liposomes, these were shown to be adequate in size to be absorbed in breast tumours. No data are yet available on their drug release behaviour.

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WDXRF spectrometry for elemental impurities analysis in drug products and dietary supplements

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Introduction: Actual regulatory requirements by the European Medicines Agency (EMA) [1] and the United States Pharmacopeia (USP) [2] for elemental impurities monitoring in drug products highlight the importance of analytical techniques able to determine the concentration of these impurities in the ppm range with a quantitative, fast and accurate analysis. In this study, the feasibility of Wavelength Dispersive X-ray Fluorescence (WDXRF) spectrometry was investigated for the measurement of several elements in drug products and dietary supplements, following the requirements set by international bodies.

Materials and methods: In this study, 27 drug products and 25 dietary supplements for weight loss marketed in Portugal were analysed. Measurements were performed using a 4 kW WDXRF spectrometer and the following elements were analysed: Cu, Cr, Ir, Mo, Mn, Ni, Os, Pb, Pt, Rh and Ru. The validation parameters of the analytical procedure were performed according to the International Conference on Harmonization (ICH) guidelines [3] for this purpose. Risk assessment for non-carcinogenic effects for dietary supplements was calculated using the Hazard Index (HI) following the Environmental Protection Agency (EPA) guidelines [4].

Results: Results showed that the WDXRF method complies with the validation requirements for the elements under study. The analysis of drug products did not show elemental impurities above the limits set by regulatory authorities. However, in some dietary supplements high levels of Cr, Mn and Pb were detected, with high levels of both Mn and Pb being measured in the same product. Unconformities were also detected between the labelled and the determined values. The HI (1.1E-1) obtained for dietary supplements showed no potential risk of non-carcinogenic effects to human health.

Discussion and conclusions: Results underline that WDXRF spectrometry is a very promising technique, and meets the international regulatory requirements. The results obtained for drug products revealed that those products complied with the limits established by the authorities. However, in regards to the dietary supplements, high levels of Cr, Mn and Pb were measured in some products, which emphasizes the need for a more strict regulation for dietary supplements. The simultaneous presence of high levels of Pb and Mn in the same supplement increases the concern about the potential toxic effects on human health, since both metals affect the central nervous system. In spite of the obtained HI, humans are often exposed, by different routes and/or sources, to toxic elements, and supplementary consumption of these products may promote potential toxicological risks that cannot be overlooked.

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Anticoagulants utilization in Portugal: an overview of the past, present and future

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Introduction: Anticoagulation (AC), indicated in different clinical diagnosis, may be assured through heparins, antivitamin K antagonists (AVK) or, more recently through novel anticoagulants (NOACs). The correct use of AC is of utmost importance, both for public and individual health, and also given their impact on costs assigned to the National Health Service (NHS).

Objective: This study analyses the utilization patterns of AC in Portugal, in the outpatient market, considering national, regional and international asymmetries; aside with the evolution of costs (for the NHS and for patients) and costs of treatment per day.

Materials and methods: A descriptive study was used, where outpatient sales data obtained from the drug regulatory agency (INFARMED) was used to characterise AC use between 2006 and 2015. These data were treated annually and disaggregated by the 18 districts of Portugal, using the anatomical therapeutic and chemical (ATC) classification and respective defined daily doses (DDD) [1]. ATC 3 and ATC 5 levels were used according to the purpose of analysis. Several utilization indicators were used, presented as DDD, DID (DDDs per 1000 inhabitants per day, calculated considering the mean annual resident population in mainland Portugal) [2] and the ratio of NOACs on the total market of ACs compared to international data obtained. Cost indicators were also used, expressed as total costs, NHS costs, out-of-pocket costs and costs of treatment per day (CTD).

Results: Data show that Portugal is the seventh country of Europe with the largest % ratio of NOACs, with a 32% mean in 2015, well above the 21.8% UE average [3]. A large disparity of this same ratio was observed comparing regions within Portugal, also visible between districts. The Algarve and Lisbon region had ratios twice as high as the North. The per capita consumption of AC (total market) increased nearly fourfold in 10 years, from 4.28 DID in 2006 to 17.03 in 2015, mostly due to the introduction of NOACs. The costs increased from 6.3 to 74.2 million euros in this same period (reimbursed by the NHS), mostly due to the costs of NOACs, which represented in 2015, 55 M€. The CTD was considerably higher for NOACs.

Discussion and conclusions: Cross-sectional studies indicate that 30–40% of patients with atrial fibrillation are still untreated [4]. The market increase in ACs suggests more naïve patients are being treated [5], although due to the inexistence of a patient registry in Portugal, it is unknown if high-risk patients are reached. Future studies should focus on savings imputed to prevention of stroke, so that the cost-effectiveness of NOACs is confirmed with real world evidence.

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Development of a core set of potentially inappropriate medications associated with major adverse cardio and cerebrovascular events

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Introduction: One of the current priorities of Portuguese health policy is tackling cardio and cerebrovascular diseases given their high morbi-mortality [1]. Portuguese life expectancy is high, as well as unhealthy life years; hence the safety of polypharmacy is of concern. Several screening tools describing Potentially Inappropriate Medications (PIM) have been developed [2,3]. However, no risk on Major Adverse Cardio and Cerebrovascular Events (MACCE; e.g. stroke, cardiovascular death, myocardial infarction) has been presented in these tools. Thus, this study aims to identify a core set of medicines with MACCE risk of occurrence.

Materials and methods: A systematic review was undertaken (PubMed and Google Scholar; 1991–2017) using the PICOS method. Titles and abstracts were reviewed, excluding duplicates, papers describing tools using exclusively implicit criteria or exclusively focusing on drug-drug interactions. A preliminary PIM list was created. The list of outcomes described was reduced to those affecting the cerebrovascular and cardiovascular system. All of these were then checked if available on for the Portuguese market. Criteria were organized in drugs that should be avoided in the elderly and drug-disease interaction. Intra-class correlation (ICC) coefficient was calculated, considering the different tools as raters for this analysis (IBM SPSS v.24.0). Finally, outcomes were classified into MACCE and all others.

Results: The literature search enabled the identification of 1005 papers, from which 26 were retained describing different tools. From those, 92.3% ($n = 24$) were based on explicit criteria and only 7.7% ($n = 2$) on mixed approach (explicit and implicit criteria). A total of 1208 PIM and 309 drug–disease interaction were initially considered. After excluding irrelevant outcomes, 263 PIM and 76 drug–disease interaction were retained, 61 corresponding to PIMs on the market (25.6% from the initial 1208) and 29 (9.4%) drug–disease interaction. Non-steroids anti-inflammatory drugs (NSAIDs) and tricyclic antidepressants were the most frequently described drug classes in different tools associated with cardio and cerebrovascular events. The most frequently reported PIMs were amitriptyline (described in 17 tools), digoxin (13 tools), methyldopa (12 tools) and non-sustained release nifedipine (11 tools). A low relative agreement was obtained for the PIMs considered in all analysed tools (ICC = 0.212; 95%CI: 0.112–0.768). Furthermore, only five PIMs (8.2%) were associated with MACCE risk of occurrence.

Discussion and conclusions: These preliminary results show that the existing lists focus on minor adverse cardio and cerebrovascular events and poorly describe the potential of these PIMs to cause major negative outcomes. Future work will focus on this list's validation, followed by the risk quantification.

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Epidemiology and empirical antibiotic therapy for the treatment of HA-MRSA infections in Alentejo – Portugal

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Introduction: Infections caused by hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are a significant public health problem, especially in Portugal, one of the European countries with the highest rates of resistance [1]. These infections are associated with very high costs to the healthcare system, and increased morbidity and mortality [2]. This study aimed to: determine the incidence of HA-MRSA infections, in ULSNA (Unidade Local de Saúde do Norte Alentejano), evaluate the implemented treatment and de-escalation, and estimate the error rate associated with the empirical therapy for HA-MRSA infections.

Materials and methods: This study was implemented through a retrospective cross-sectional study, which included analysis of clinical, microbiological and therapeutic data of patients diagnosed with HA-MRSA infections who were hospitalized in ULSNA, during 2013. In addition, data from patients who underwent empirical therapy for the treatment of HA-MRSA infections were also studied, to determine the error rate associated with this therapy. The study protocol was approved by ULSNA's ethics committee.
Results: Two samples were considered: one with 150 patients diagnosed with HA-MRSA and another with 105 patients who underwent empirical therapy for the treatment of suspected HA-MRSA infections. In the first sample, HA-MRSA incidence was 1.8% of all hospitalized patients, while the estimated resistance rate of *S. aureus* was 90.4%. Noteworthy, prior antibiotic consumption was 46.7% and focused on quinolones, third-generation cephalosporins and penicillins. The most commonly prescribed empirical treatment was PIP/TAZ (19.6%), followed by levofloxacin (13.8%) and de-escalation occurred in 71.7% of cases. Following microbiological confirmation of HA-MRSA infection, a directed therapy with vancomycin was applied in 89.8% of patients. Polymicrobial infections were detected in 56% of patients, mainly caused by *Klebsiella pneumoniae*, *Enterococcus* sp. and *Acinetobacter baumannii*. Linezolide use was required in 19.2% of cases that had been previously treated with vancomycin, which suggests a clinical treatment failure with the latter. Concerning the second sample, empiric antibiotic therapy was found unnecessary in 27.6% of the cases after confirming the inexistence of HA-MRSA infection in patients treated with vancomycin or linezolide.
Discussion and conclusions: HA-MRSA rates observed in this study are significantly higher than described in the literature, although strict measures of infection control have been implemented in the last few years, with good results. Considering vancomycin use and the suspected treatment failure, an appropriate monitorization of VISA strains is advised. Despite the presence of polymicrobial infections, a possibly unnecessary broad-spectrum therapeutics was maintained and de-escalation was not followed in a wide sample, which stresses the need for a tighter antimicrobial stewardship. Finally, microbiological tests should ideally precede and accompany treatment, as to prevent the emergence of multidrug resistant strains.

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Potentially inappropriate prescribing in domiciliary hospitalization – medication review using GheOP^{3}S tool

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Introduction: The Portuguese Health Care System (PHCS), although considered efficient, can improve in hospital care provision [1]. A new model of hospitalization, shifting care to the community, has emerged, named Domiciliary Hospitalization (DH) [2]. This study aimed to determine the prevalence of Potentially Inappropriate Prescribing (PIP) in elders referred to DH.
Materials and methods: An exposure cohort was created, including patients hospitalized from August to September 2016 ($n = 33$). Clinical and therapeutic information was obtained through the hospital database and information on actual drug use was acquired by overt observation at patient's home. Inclusion criteria were to have been transferred from conventional hospitalization to DH; being $\geq$65 years. Patients without ambulatory medication and those where the pharmaceutical visit occurred during the first day of DH were excluded. The PIP was analyzed using the GheOP^{3}S Screening tool [3]. Spearman's rho was used to test the association between polypharmacy and PIP (SPSS v.24.0).
Results: A sample of 17 patients met the inclusion criteria; with a mean age of 77.1 years {65–94; SD = 8.8}, 76.5% being male. Mean number of hospitalization days was 11.3 ± 7.1, during which patients were prescribed approximately 9.9 ± 3.9 drugs. A total of 167 drugs were analysed, 55 of which considered PIP (32.9%). Among these, 40% were inappropriate independent of the diagnosis (PIP-ID), 23.6% were inappropriate dependent on the diagnosis, 25.5% were drug-drug interactions and 10.9% were potential prescribing omissions. The excessive use of benzodiazepines remains alarming, since among 21 PIP-ID, 47.6% were benzodiazepines, mostly intermediate acting (lorazepam and bromazepam). The use of antidepressants for longer periods than 1 year is also worrisome corresponding to 6 PIP-ID (28.6%). 57% of drug–drug interactions were between antidiabetic or insulin and beta-blockers (35.7% cardio selective). The number of PIP was strongly correlated with the number of prescribed drugs ($r = 0.648$, $p = .005$).

Discussion and conclusions: Medication review enabled the detection of various PIPs in patients discharged from the DH. The inclusion of a pharmacist in this unit was determinant to increase patient safety. The impact of this intervention is substantial on patients' health (reduction of adverse effects and increased adherence to therapy) but also on the economy of the PHCS (reduction in drug costs and hospital readmissions) [4].

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PRACTISE – PhaRmAcist-led CogniTIve Services in Europe: preliminary results

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Introduction: The scope of community pharmacy practice varies widely across different countries and health care systems. Cognitive pharmaceutical services are daily provided to the patients in community pharmacies [1]. According to the PGEU, pharmacy services can be divided into: "Core services" (essential services provided by all pharmacies), "Basic services" (may require separate facilities and pharmacy staff training) and "Advanced services" (require accredited pharmacist) [2]. Published literature focuses on pharmacist-led cognitive services available in Europe, but fails to report the implementation level in detail. The main aims of this project are to develop a map of existing pharmacist-led cognitive services in Europe.
Materials and methods: A cross-sectional study was conducted where data were collected using an online survey, sent to a sample of 49 countries. The survey comprised three questions for each of the 22 services listed: provision; implementation level and remuneration. The survey was sent to three representatives per country (community pharmacist, researcher and policy maker), to ensure data triangulation, which also considered official documents publicly available. Consensus was sought using the Delphi adapted method. Preliminary results presented here focus on the implementation level, where the PGEU classification of services was used. Data were expressed by numbers of countries where the service is available and proportion of pharmacies providing it [2]. Ethics approval was obtained from "Comissão de Ética Egas Moniz" (Proc. 515).
Results: Data were obtained from 75 participants in 35 European countries (response rate = 71%). "Core services" ($n = 9$): 57% of the countries provide at least six of these services, including "medicines dispensing" ($n = 35$; 100%), "provision of information on medicines" ($n = 34$; 97%), "generic substitution" ($n = 29$; 85%), "provision of emergency oral contraception" ($n = 27$; 77%), "home delivery of medicines" ($n = 21$; 60%) and "health screening" ($n = 17$; 49%). "Basic services" ($n = 4$): 66% of the countries provide at least 3 of these services including, "assessment of the inhalation technique" ($n = 28$; 80%), "pharmaceutical care" ($n = 23$; 66%), "adherence support and monitoring" ($n = 22$; 63%). "Advanced services" ($n = 9$): Only 12% of the countries provide at least 6 of these services, including "medication review" ($n = 25$; 71%), "opioid substitution management", "new medicines services" ($n = 18$; 51%), "prescription renewal" ($n = 17$; 37%), "immunization" ($n = 7$; 20%) and "prescribing" ($n = 6$; 17%). The implementation level varied widely for all 3 categories; for example, "health screening" was reported as implemented in 5–100% of pharmacies; "assessment of the inhalation technique" was

reported as implemented in 5–100% of pharmacies and "opioid substitution management" was reported as implemented in 10–100%.
Discussion and conclusions: Preliminary data indicate that "advanced services" are provided in lower proportion than "core and basic services". The variability found in the implementation level supports the idea that this data is essential to describe reality in accurate terms. This is an ongoing project that aims to fill the current gap in the literature.

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MICROBIOLOGY

APOBEC3 are host defenses against HIV-2 infections

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Introduction: Members of human APOBEC3 (A3) protein family are potent cellular restriction factors against HIV-1viral infection. In scientific community it is consensual that A3G is a potent inhibitor of Vif defective HIV-1 as well as A3D, A3F and A3HhapII [1]. There are few works using HIV-2 and the restriction ability of A3 members was not experimentally confirmed. Since A3 has a tight control of HIV-1 it is assumed that the same happens with HIV-2. However, these two viruses present several differences namely in the protein Vif that shares only 30% of identity between HIV-1 and HIV-2. Moreover, they show different replication and infectivity in some cell lines (H9), suggesting either different threshold requirements for the same cellular factor A3 or the involvement of different A3 to compensate for the differences in Vif1 and Vif2 structure [2,3]. In this work, we aim to identify the effect of different A3 in the production and infectivity of HIV-2.
Materials and methods: HIV-2 wild type (wt) and Vif- viruses were produced in HEK293T cells in the absence or presence of different A3 proteins. Production was measured by viral titer quantification (anti-p24Gag ELISA) and infectivity determined by one cycle infectivity assays in TZM-bl cells. The capacity of Vif2 to induce A3 degradation in co-transfected HEK 293T cells with constant amounts of A3 and increased amounts of Vif2 expression vectors was analyzed by SDS-PAGE.
Results: Viral protein Vif2 induces A3B and A3G degradation in co-transfection assays and in virus-producing cells lysates. These two host factors also inhibit HIV-2 production and infectivity (Figure 1). Moreover, A3G inhibition of HIV-2 viral production and infectivity is dose-dependent.
Discussion and conclusions: In this work, we showed that of 6 APOBEC proteins tested, only A3B and A3G are potent host restriction factors against HIV-2 infections. Our results also show that A3B inhibits HIV-2, differently from what was observed with HIV-1.

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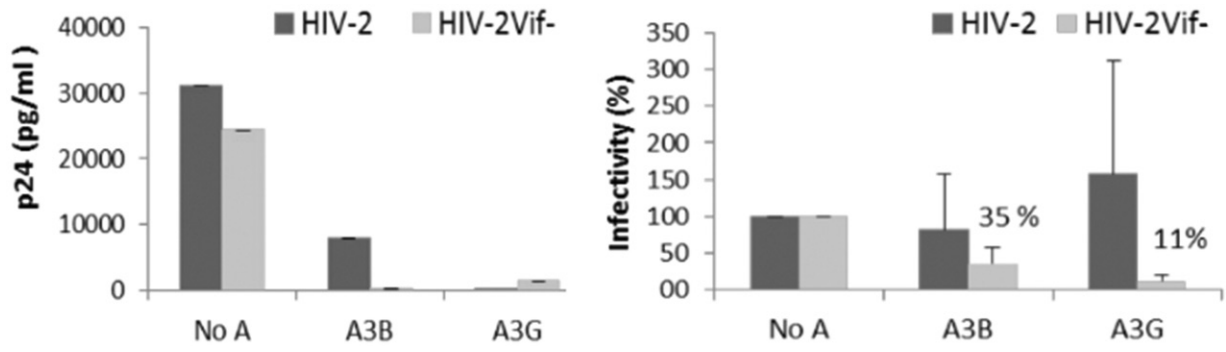

Figure 1.

Frequency of PXR 63396C > T polymorphism in Portuguese HIV-1 infected patients subject to Atazanavir treatment

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Introduction: Atazanavir (ATV) is used as part of a highly active antiretroviral therapy and selectively inhibits virus-specific processing of viral Gag and Gag-Pol precursor polyproteins by binding to the active site of HIV-1 protease enzyme, thus preventing the formation of an infectious HIV-1 viral particle [1]. ATV is metabolized by the cytochrome P450 3A4 whose expression is regulated by the pregnant X receptor (PXR). Homozygosity of PXR 63396C > T has been associated with sub-optimal ATV Ctrough, potentially accounting for interindividual differences in drug metabolism [2]. The present study aims to analyze the frequency of PXR 63396C > T in Portuguese HIV-1 infected patients and compare the results to other ethnic groups.

Materials and methods: 116 adult patients receiving ATV treatment were selected at Hospital de Santa Maria. All participants gave their written informed consent and the study protocol was previously approved by the hospital ethics committee. Genotyping was performed by real-time PCR, following TaqMan™ methodology. PCR conditions are available on request. Results were tested for Hardy-Weinberg equilibrium by goodness off fit χ^2 test with a 95% confidence interval, using The R Project for Statistical Computing. Obtained frequencies were then compared with 2504 unrelated individuals among five ethnic groups gathered from The Ensemble project using a χ^2 test. p Values $<.05$ were considered to be statistically significant.

Results: All frequencies in our study group were in Hardy–Weinberg equilibrium ($p = .863$). The allelic frequencies obtained for PXR 63396C and PXR 63396T were 0.41 and 0.59, respectively, being significantly different from African ($p < .0001$), American ($p = .001$) and European ($p = .025$) ethnic groups. Regarding genotype frequencies, 0.16 for C/C, 0.49 for C/T and 0.35 for T/T were observed in our sample. These frequencies were comparable to the East Asian ($p = .307$), European ($p = .082$) and South Asian ($p = .650$) ethnic groups and discordant with the American ($p = .006$) and African ($p < .0001$) populations.

Discussion and conclusions: The individuals with African ancestry present in our sample amount to 23.2% which may explain the difference observed with the European allelic frequency. Obtained genotype frequencies were similar to the ones observed in European and Asian ethnic groups and statistically different from those of African and American origin, leading to the hypothesis that the PXR 63396T allele may be native to Eurasia and disseminated to the rest of the world via migrations, however further studies are required to validate this theory. Patients with the T/T genotype in our group may be at risk of therapeutic failure, consequently drug monitoring is recommended to guide dose and/or drug adjustment.

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HIV-1/HIV-2 chimeric envelope glycoprotein elicits strong antibody response in mice

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Introduction: The development of immunogens that elicit the production of broadly reactive neutralizing antibodies (Nabs) is the number one priority for an HIV vaccine. A Vaccinia virus-Envelope prime and C2V3C3-boost vaccination strategy elicits bNAbs targeting the V3 envelope region of HIV-2. Given that bNAb epitopes in HIV-1 gp120 lie outside the V3 region, we hypothesized that a chimeric envelope gp120 glycoprotein containing the V3 region of HIV-2 and the remaining parts of HIV-1 would elicit a bNAb response against both HIV-1 and HIV-2.
Materials and methods: We therefore constructed such a chimeric envelope gp120 and expressed it in the context of a full-length envelope complex in a recombinant vaccinia virus. This new chimeric envelope was analyzed by Western-blot (WB) using HIV-1 and HIV-2 positive sera. Recombinant polypeptide comprising HIV-1 gp120 region and C2–C3 polypeptide were produced and reacted with HIV-1 and HIV-2 positive sera in WB analysis. Female BALB/c mice were primed with recombinant vaccinia virus expressing the chimeric env and boosted four times with purified recombinant HIV-2 C2V3C3–polypeptide and/or HIV-1 monomeric gp120. Sera obtained of all mice were tested for antigenicity by binding assays.
Results: The chimeric HIV-1/HIV-2 envelope gp120 was produced to high levels and potently reacted with sera from HIV-1 and HIV-2 infected individuals in ELISA and Western Blot analysis. All mice produced antibodies that bound potently to the autologous and heterologous antigens (C2V3C3 polypeptide and/or monomeric gp120 HIV-1).
Discussion and conclusions: These results show that priming followed by four boosts are strictly necessary to achieve and maintain a sustained binding antibody response against both antigens (gp120 and C2C3 HIV-2 ALI). Neutralization assays are underway to determine if the chimeric HIV-1/HIV-2 envelope glycoproteins as vaccine immunogens can direct the neutralizing antibody response against the most potent bNAb epitopes of both HIV-1 and HIV-2.

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Infection of mice tissues with the Zika virus

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Introduction: Zika virus is a flavivirus discovered in 1947 in Africa [1]. It is pathogenicity is considered low for humans, although it is associated with microcephaly in newborns and the Guilan–Barre syndrome in adults [2]. However an early

histopathological study signaled alterations on other organs and tissues that have not been further explored [3]. A recent study also showed infection of skin immune cells, including dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells with Zika virus [4]. We searched for the virus in Zika inoculated adult and new-borne mice by ultrastructure and immunocytochemistry.
Materials and methods: Newborne mice were inoculated intracerebrally with 0.10 ml of Zika MR766 Vero E6 infected cells supernatant. Control mice were inoculated with 0.10 ml of uninfected Vero E6 cells supernatant. Immunofluorescence antigen detection assay and H + E was accomplished using sections of paraffin embedded histologic samples from brain, liver, lung and kidney. 10 µl of Zika human positive sera were incubated for 30 min at 37 °C, washed twice in PBS, then incubated for 30 min after the addition of fluorescein-conjugated rabbit immunoglobulin to human IgG (Dako). Slides were then washed, dried and covered after mounting with 10% glycerol in PBS. Slides were examined using an Olympus fluorescence microscope BH2 (10 × 40). Electron microscopy was performed with standard embedding and thin section methods described elsewhere [5].
Results: Neurons and glial cells from brain and vascular endothelial cells from all the observed organs, as well as alveolar type II cells from lungs, displayed strong fluorescence staining, not observed in the controls. Zika virus extracellular and intracellular particles were found by TEM in the brain, but not in the other organs displaying fluorescencent cells. Coiled endoplasmic reticulum membranes, but no virus particles were found in the type II alveolar cells from infected mice.
Discussion and conclusions: The pathogenesis of Zika virus infection remains poorly understood. Apart from CNS cells where the virus is known to replicate after experimental infection of mice, little is known about other implicated cell types that occur in infections. Our results show the expression of antibody in endothelial and alveolar type II cells, but failed to demonstrate virus particles in these cells. Modification of target cells by expression of viral antigens may elicit immune responses that can be important for the pathogenesis of Zika infection.

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Magnetically ordered and electrostatic assembly of Pf1 virus and Cytochrome c

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Introduction: The interplay between macromolecular shape and charge in solution is of critical importance to biochemistry or nanotechnology. In this work we describe a new method to create anisotropically aligned proteins samples, which can be used for advanced spectroscopic studies. The new method is based on the electrostatic complex formed (depending on salt concentration) between Pf1 virus and horse heart cytochrome c (cyt c). Pf1 virus has a peculiar rod shape strong anionic character ($-0.475 e/nm^2$), semi rigid nature, molecular mass 37 MDa [1,2]. Its tubular structure has ∼4700 protein capsid subunits [3], and is capable of inducing alignment to other proteins in solution [4].
Materials and methods: NMR spectroscopy was used to monitor the chemical shift of the amino acids of cyt c in the presence or absence of Pf1 virus at different NaCl concentrations. Gran canonical Monte Carlo computer simulations of this system (Pf1, cyt c and NaCl) were performed to mimic the NMR experiments.
Results: NMR salt titration of cyt c with NaCl showed unaffected cyt c resonances. In the presence of Pf1 at high ionic strength the cyt c spectra does not reveal any modification. Upon decrease of salt concentration, the chemical shift of the resonances closer to the heme group of cyt c change, peaks became broader, and the spectra disappears when the concentration of NaCl is lower than 150 mM. Gran canonical Monte Carlo simulations of this system (using the same concentrations of Pf1 cyt c and NaCl) were able to calculate an overall osmotic pressure of the system, replicating the experimental behaviour of association/dissociation of the NMR experiments upon change of salt concentration.
Discussion and conclusions: Monitoring the NMR resonances of cyt c, we observe the process of association Pf1 virus and cytochrome c. The conversion of a system where cyt c is free in solution (observed by liquid state NMR) to a solid association with Pf1 virus (no longer observable by liquid state NMR) was predicted by Monte Carlo simulations. In this experiments Pf1 virus are magnetically aligned due to the NMR magnetic field (9.4 Tesla) and the binding of cyt c to

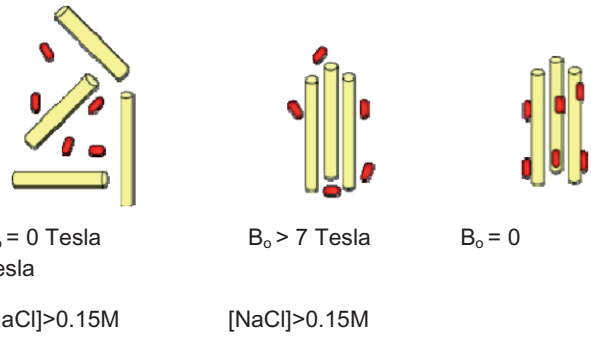

Figure 1.

these alignment matrix should produce solid aligned array of cyt c molecules. This partially aligned sample of cyt c molecules can provide unique and valuable correlation between structural and spectroscopical data.

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Phenotypic identification of multidrug-resistant *Enterobacteriaceae* from hospital SSTI in the Lisbon area

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Introduction: In the past few years, a significant increase of infections caused by multidrug-resistant Gram-negative bacteria has been observed, becoming a serious health problem worldwide. Simple and accessible diagnosis of these bacteria is essential to identify, monitor and treat these infections.
Materials and methods: For the detection of Extended-Spectrum β-lactamases (ESBL) and carbapenemase in *Enterobacteriaceae* (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp.), 100 samples were collected from skin and soft tissue infections (SSTI) in a Lisbon Hospital, between 2011 and 2015. The diagnostic methods used were: the Double Disc Synergy Test (DDST) and a modification of the ESBL Nordmann/Dortet/Poirel (NDP) Test for the detection of ESBL [1]; and the Modified Hodge Test (MHT) and an adaptation of the Carba NP (Nordmann/Poirel) Test for the detection of carbapenemase producing strains [2]. Both adaptations of the tests were especially developed for the study, aiming for simplicity and reduced costs.
Results: Overall, 51.6% of *E. coli* strains ($n = 31$) were ESBL producers, the prevalence almost doubling, from 33% in 2013 to 64% in 2015. 20.8%, of *Klebsiella* spp. strains ($n = 24$) were ESBL positive, while no *Enterobacter* spp. ($n = 16$) and *Proteus* spp. ($n = 29$) tested positive. The number of ESBL positive samples using the two methods was very similar: 20 (20%) and 21 (21%), for the DDST and the modified ESBL NDP Test, respectively. In the detection of carbapenemase strains, both modified Carba NP Test and MHT, identified one positive result (1.4%) – *E. coli* strains, although not coincident. Despite MHT having a described sensitivity >90%, the identified strain can possibly be a false positive, for in the modified Carba NP Test (reported 100% sensitivity and specificity), it tested negative. MHT has the disadvantage of sometimes not being able to differentiate carbapenemases from ESBL and AmpC β-lactamases.

Discussion and conclusions: Portugal is one of the countries with the highest rate of ESBL positive isolates in Europe, contrary to the rate of infections caused by carbapenamase-producing *Enterobacteriaceae* [3]. This pattern can also be seen in the analyzed SSTI bacteria collection obtained in a Lisbon Hospital. Both developed tests demonstrated a very good performance in detecting ESBL and carbapenemase producing strains. Since SSTI can progress to serious systemic infections, it is important to monitor resistance rates of bacteria responsible for these infections, as well as to perform an early diagnosis in order to institute the best adequate antibiotic therapy.

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Healthcare-associated infections versus free-living amoebae – are they related? – A concise review

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Introduction: In this work, it was done a general review about healthcare-associated infections and the knowledge that relates them with amoebas, in order to call attention to the fact that these protozoa may constitute one of the factors that are the basis of a public health problem [1]. The term Healthcare-associated infection (HCAI) refers to the infection acquired by a patient, wherever healthcare services are provided, that was not present or incubating at the time of admission. These infections are highly prevalent worldwide, leading to significant costs to health systems. They can be caused by various microorganisms, such as bacteria, viruses, fungi and parasites. Free-living amoebae (FLA) are protozoa that cause infections that are not prominent in the medical community because they are uncommon, although they may be serious. They are non-parasitic amoebas that do not need a host to survive or complete their life cycle, and can act as reservoir for other pathogenic microorganisms, particularly bacteria that acquire resistances to their destructive mechanisms, surviving in trophozoites or cysts. Thus, since amoebae are organisms that are resistant to several environmental factors, they offer protection to the microorganisms they harbour, which favours their permanence in the environment and increases the probability of causing human infections [2].
Materials and methods: This work was carried out between June and November of 2016, the research for bibliography was made through the platform B-on – Online Knowledge Library and we used scientific articles published between 1996 and 2016.
Results: The existence of amoebae in the environment of health care settings not only facilitates the survival of multiple pathogens, but also contributes to their dissemination and even prepares them to survive within macrophages and to escape the defences of the human organism. Pathogenic bacteria, such as Legionella spp., Mycobacterium spp. and Campylobacter spp., are examples of microorganisms that benefit from the interactions they establish with free-living amoebae [3].
Discussion and conclusions: Free-living amoebae (FLA), being ubiquitous, may be present in places where health care is provided, often where individuals with compromised immune systems are found. Thus, these amoebae have the opportunity to induce infection more easily, when they belong to species infecting humans, and also to increase the likelihood of infections caused by the microorganisms that they transport and shelter. The fact that FLA protects the micro-organisms they contain also poses other problems for health services, including the possibility of contributing to the resistance of bacteria to antibiotics. Another issue is related to disinfection and cleaning procedures, since these are focused on the elimination of pathogenic microorganisms, with disinfectants to which FLA are resistant. Thus, it is important to consider

an approach for elimination of biological agents that contaminate the various materials, instruments and the hospital environment and other health services, also effective for FLA, because only in this way the HCAI risk can be truly reduced.

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PATHOLOGY

Mauriac syndrome: the contribution of electron microscopy

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Introduction: Mauriac's syndrome (MS) is a glycogenic hepatopathy associated with poorly controlled diabetes mellitus type 1 (DM1), hepatomegaly, elevated aminotransferases, hypertriglyceridemia and delayed puberty. In the era of insulin therapy, this entity represents a rare complication, attributed to low therapeutic compliance [1]. Ultrastructural changes in MS were previously reported but the changes found on Transmission Electron Microscopy (TEM) are still a mother of debate. The aim of this study was to contribute to elucidate the usefulness of TEM in the evaluation of MS patients.
Materials and methods: The authors describe a case of a 26-years old female with MS. Liver biopsy material for TEM was obtained and TEM was performed with standard embedding and thin section methods previously described [2].
Results: A 26-years old female, with a history of anorexia nervosa, late menarche and DM1 with 15 years of evolution, complicated by severe retinopathy and neuropathy. The patient maintained poor glycemic control, presenting glycated hemoglobin (HbA1c) persistently higher than 10% and recurrent episodes of hypoglycaemia. In adolescence she developed hepatomegaly, fluctuating elevation of aminotransferases (up to 15 times the upper limit) and triglycerides (511–861 mg/dL), and hepatic biopsy revealed macrovacuolar steatosis and mild fibrosis. At 14 years of evolution of DM1 the patient was referred for gastroenterology clinic due to chronic diarrhea. She presented cushingoid facies, body mass index (BMI) of 17.8 kg/m^2 and exuberant hepatomegaly. Laboratory data showed persistent elevation of aminotransferases and triglycerides (1066 mg/dL). Bilirubin, iron metabolism, thyroid function and coagulation were normal; viral serologies, autoimmune study and anti-transglutaminase antibody were negative. Genetic study for hereditary glycogen storage diseases (GSD) was negative. Upper endoscopy, ileocolonoscopy and capsule enteroscopy had no lesions. Abdominal magnetic resonance imaging (MRI) showed massive hepatomegaly with severe steatosis. Liver biopsy was repeated (10-year interval from the first) showing marked nuclear glycogenization, macrovacuolar steatosis and slight reticulin reinforcement. Transmission electron microscopy (TEM) revealed voluminous deposits of glycogen, pleomorphic mitochondria with an unusually dense matrix and collagen fibers signaling Disse space fibrosis. The diagnosis of MS and diarrhea due to autonomic neuropathy was assumed. Glycemic control was enhanced, with a decrease in the steatosis fraction on MRI.
Discussion and conclusions: The ultrastructural features found in our study included pleomorphic mitochondria with an unusually dense matrix, features that were never reported. Fitzpatrick's review [3] showed that in Mauriac syndrome there was correlation between the severity of liver disease (fibrosis, transaminases levels) and the presence of megamitochondria. The presence of mitochondria disorders in TEM strongly suggests that chronic poor glycaemic control may have significantly affected the hepatocytes at a functional level.

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The impact of electron microscopy in kidney biopsy diagnosis

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Introduction: Electron microscopy (EM), a complementary diagnostic technique in disuse by the advent and progress of Immunohistochemical techniques and molecular biology, has, nowadays, a restricted use (1–3% of the activity of a pathology department) but has considerable maintenance costs. Currently, in the medical field, this technique is primarily used in renal biopsies, mainly for the diagnosis of glomerular pathology. However it is also used in neuropathology, in the study of hereditary metabolic diseases/malformations, in some cases of dermatopathology and in tumoral pathology [1–4]. It is intended with this work to evaluate the value and demonstrate the impact of ultrastructural examination in renal pathology.
Materials and methods: About 201 cases of kidney biopsies "de novo" at our institution were evaluated retrospectively (179 native kidney biopsies and 22 transplant kidney biopsies from years 2014, 2015 and 2016), confronting the ultrastructural diagnosis with the diagnosis of the light microscopy and immunofluorescence study of the same cases. In each case, the ultrastructural assessment was classified as "essential for diagnosis" when the EM confirmed or redirected the diagnosis, "important contribution for diagnosis" when it added some feature useful to the initial diagnosis and "no value for diagnosis" when it did not added any new information to the initial diagnosis.
Results: In 201 biopsies, 40 (19.9%) EM were classified as "essential", 89 (44.3%) as "important contribution" and 72 (35.8%) as "no value" in the diagnosis of kidney biopsy. Adding the "essential" to the "important contribution" cases, the global percentage rose to 64.2%. Additionally, when joining the cases "essential" to the "important contribution" in the native kidney and in the kidney transplanted, the values reached 64.8% and 59.1%, respectively. The cases of EM "no value" confirmed the diagnosis proposed by the study in optical microscopy and Immunofluorescence, excluding unexpected ultrastructural changes that could redirect the initial diagnosis.
Discussion and conclusions: Although there were 35.8% of cases "no value", the present study demonstrates the importance of conducting EM in all renal biopsies of native kidney "de novo" and transplant kidney.

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A case of Ménétrier's disease with ultrastructural follow-up

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Introduction: Ménétrieŕs disease (MD) is a rare form of hypertrophic gastropathy characterized by massive enlargement of gastric folds causing marked protein and mucus exudation [1]. Ultrastructural changes in MD were previously reported but the importance of transmission electron microscopy (TEM) is still a matter of debate. The aim of this study was to contribute to elucidate the usefulness of TEM in the follow up of MD patients.
Materials and methods: The authors describe a 31 years old male with MD. Gastric biopsy material was obtained for TEM during clinical recover of the patient. Electron microscopy was performed with standard embedding and thin section methods previously described [1].
Results: A 31 years old male was admitted for hematemesis, epigastric pain and lower limb edema. Laboratorial data showed haemoglobin 18.4 g/dl, total proteins 2.8 g/dl, albumin 1.6 g/dl and hipogamalobulinemia. Twenty-four hour urinary proteins were normal. Human Immunodeficiency virus (HIV) and cytomegalovirus (CMV) serology were negative. Transthoracic echocardiogram did not reveal signs of heart failure but a mild pericardial effusion was detected. Upper GI endoscopy revealed markedly enlarged gastric body folds covered by abundant exudative fluid, sparing the antrum. Thorax and abdominal CT scan showed ascites, bilateral pleural effusion, marked gastric wall thickening and perigastric adenopathies. Endoscopic ultrasound confirmed gastric wall thickening (6 mm) due to mucosa expansion (3.2 mm) with intact submucosa. In gastric biopsies foveolar hyperplastic and regenerative mucosa was observed being suggestive of Ménétrieŕs disease and this diagnosis was accepted. *Helicobacter pylori* and CMV were not detected. Albumin replacement and diuretics corrected anasarca and long-acting octreotide 20 mg monthly was further instituted. Four months after starting octreotide therapy the patient was asymptomatic and serum proteins were normal (albumin 4.6 g/dl and total proteins 6.5 g/dl). Signs of endoscopic improvement were evident with marked reduction in gastric folds and improving of mucosal inflammation. Gastric specimens were taken and sent for Transmission Electron Microscopy. No ultrastructural changes detected at this time. After one year of follow-up, the patient remains in clinical remission.
Discussion and conclusions: The increase in tight junction width is the most reported ultraestrutural feature associated with MD that seems to justify high mucosal permeability, contributing to protein losing phenomena [2,3]. Absence of ultrastructural changes in our patient could be explained by ongoing octreotide therapy when TEM analysis was requested with already established clinical remission and normal serum proteins at this period. This case report highlights the effectiveness of long-acting octreotide for MD treatment and the potential role of TEM for MD diagnosis and follow-up, and confirms that TEM normalization parallels with clinical improvement.

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Ultrastructural effects of graft pretensioning in anterior cruciate ligament reconstruction

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Introduction: Graft pretensioning (traction load before implantation in the patient) is used in anterior cruciate ligament (ACL) reconstruction to prevent secondary slackening, especially when using quadrupled graft technique. Its effects on collagen fibrillar ultrastructure are still not well known, but considering current data in the literature, in this study we hypothesized that quadrupled semitendinosus (ST) pretensioning at 300N for 2 min, would not create significant ultrastructural changes in scanning electron microscopy (SEM) evaluation.
Materials and methods: Eight constructions using a 4-strand semitendinosus fresh cadaveric graft on 2 adjustable loops (as described by Silva et al. [1]) were used. Samples were harvested in the lab before and after pretensioning on a servo-hidraulic machine for 2 min [2]. The images produced in SEM were analyzed using an original semi-quantitative score

taking into account collagen cohesion, integrity, and parallelism («CIP» score) developed by Guillard et al. [3] (the only existing classification, to our knowledge).
Results: The CIP scores are not concluded in all the samples but presently, we could analyze that although there is a slight decrease in the score, the majority of collagen disorganization occurred in the more superficial fibers, with preservation of cohesion, integrity and parallelism of the deepest ones, when ACL grafts where pretensioned at 300 N for 2 min.
Discussion and conclusion: Ultrastructural changes depend on the load and the amount of time the graft is submitted to that load [4,5]. Considering that we are studying a graft construct containing interfaces that adjust and stabilize after elongation under load, higher loads may be more efficient on achieving this goal. However, we know from the literature that 500 N for 2 and 5 min can lead to significant collagen destruction [5]. The most important finding was that pretensioning ACL grafts at 300 N for 2 min that resulted in small changes of the superficial collagen fibrillar ultrastructure of the graft in SEM. Biomechanical data suggest that they may not influence significantly the tendon mechanical properties.

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Mesenchymal stem cells combined with biomaterial scaffolds and glycosaminoglycan supplementation as an integrated strategy towards articular cartilage repair: biochemical, structural and morphological analysis

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Introduction: The lack of effective and lasting treatments for articular cartilage defects resultant from trauma or highly prevalent degenerative diseases (e.g. osteoarthritis) has increased the interest for innovative tissue engineering strategies to address this unmet clinical need. Tissue engineering strategies following the paradigm of integrating cells, 3D biomaterial scaffolds for structural support and biochemical/physical factors to promote cell growth and differentiation allow for a better mimicking of the native tissue microenvironment and hold the promise of generating fully functional hyaline cartilage with appropriate mechanical properties [1].
Materials and methods: Human bone marrow-derived mesenchymal stem cells (hBMSC) were cultured in poly (ε-caprolactone) (PCL) scaffolds under hypoxia (5% O_2 tension) and using chondrogenic media supplemented with physiological concentrations of glycosaminoglycans (GAG), (chondroitin sulphate (CS) and/or hyaluronic acid (HA)) to develop an integrated strategy for the *in vitro* generation of articular cartilage. Cultures were maintained and monitored throughout 3 weeks for cell proliferation and extracellular matrix production. The final cartilage tissue engineered constructs were evaluated in terms of their biochemistry (gene expression analysis of chondrogenic markers), structure and cell morphology (electronic microscopy, histological and immunohistochemistry analysis) and mechanical properties.
Results: hBMSC proliferation in the PCL scaffolds was observed for all the conditions tested, without any detrimental effects resulting from GAG supplementation. Chondrogenesis was verified for all the conditions, however it was more pronounced in the HA-supplementation group as demonstrated by Alcian Blue staining and increased extracellular matrix production. Gene expression analysis showed the beneficial effect of both HA and CS supplementation by the enhanced

expression of the chondrogenic marker genes (Collagen type II, Sox9 and Aggrecan). However, the enhanced expression verified for collagen type X suggested the presence of hypertrophic tissue characteristic of the deep zone of articular cartilage. The pores of PCL scaffolds were highly populated by cells embedded in a cartilage-like matrix as indicated by SEM and TEM analysis. The final constructs stained positively for GAG deposition and showed the presence of collagen type II and aggrecan, two major components of articular cartilage, as confirmed by histological and immunohistochemistry analysis, respectively.
Discussion and conclusions: In overall, our results suggested the beneficial effect of CS/HA supplementation integrated in a biomimetic tissue engineering strategy for articular cartilage repair. Successful *in vitro* production of native-like articular cartilage will provide platforms for disease modelling and pave the way for novel therapeutic approaches.

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MATERIALS

Production of co-amorphous materials with therapeutic activity

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Introduction: Orally administered drugs must have sufficient water solubility to ensure that they are absorbed in the gut, as required for bioavailability and therapeutic activity. As the number of poorly water-soluble drug molecules entering the clinic rises, the strategies to enhance solubility also become increasingly important. Conversion of a crystalline drug into the amorphous form is one way to improve its apparent solubility and dissolution rate (a measure of bioavailability). Unfortunately, the large internal energy and molecular movement of the molecules in the amorphous state may also cause the material to convert spontaneously back to its stable crystalline form during processing, storage or dissolution [1,2]. Co-amorphous structures containing one, or more, small molecular weight compounds, which are homogenously mixed to form a new entity with a single amorphous phase [2,3], have been shown to stabilize the amorphous form of a drug in the solid state [2]. Finding low molecular weight excipients compatible with the model drug may, however, be challenging [3]. In this respect, amino acids have proved their potential to form co-amorphous systems with some drugs and are the object of the present work [1,2]. Different techniques are available to obtain co-amorphous systems, namely ball milling or quench cooling, since these techniques inflict limited chemical degradation to the drug [1,2].
Materials and methods: Mixtures of olanzapine and L-arginine and L-tryptophan (1:1) and paroxetine or gabapentin and L-arginine, L-tryptophan, L-proline, L-lysine, L-tyrosine and L-phenylalanine (1:1) were processed by ball milling and quench cooling. The new products were characterized by Differential Scanning Calorimetry (DSC) in order to identify the formation of co-amorphous between each drug and the amino acid.
Results: Results have shown that olanzapine could not be converted into the amorphous state in combination with arginine or tryptophan, either by ball milling, or quench cooling. Similarly, gabapentin did not convert into the amorphous state neither by ball milling, nor by quench cooling. Interestingly, olanzapine became amorphous only by quench cooling, whereas paroxetine became amorphous by both ball milling and quench cooling. In turn, paroxetine was converted into the amorphous form when in combination with the amino acid L-proline.
Discussion and conclusions: The study has shown that the conversion of crystalline drugs into the amorphous state is dependent on their chemical structures and interactions with other chemical entities. For the model drugs studied the use of specific amino acids, in combination with the appropriate processing technique, has confirmed the potential to form co-amorphous systems. The use of amino acids thus indeed seem to be a promising approach, provided the right combination (drug:amino acid) and processing technique have been identified.

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Nanomedicine for inhalation therapy

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Introduction: Drug delivery via the lung offers an attractive, non-invasive mean of administration to achieve local therapeutic responses, high systemic bioavailability, avoidance of hepatic first-pass metabolism and a rapid onset of therapeutic action. However, delivery of some drug molecules such as large molecular weight biotherapeutics and small drug molecules with limited solubility through the lung is problematic. Delivery of drugs as nanocrystals may overcome these challenges.
Materials and methods: A prednisolone nanosuspension was prepared through a bottom-up nanoprecipitation technique using microfluidic reactors. Particle size, polydispersity index and zeta-potential of nanosuspensions were measured using a combination of the Nanosight® LM14 microscope and Zetasizer Nano ZS model 3600. Nanocrystals were tested for toxicity and transepithelial transport using a fully validated Calu-3 air-interface culture (AIC) model.
Results: Prednisolone nanocrystals synthesised with SDS, HPMC and PVP (0.0001% to 10% (w/v) for the 3 parameters). Prednisolone nanocrystals synthesised with CTAB, HPMC and PVP (0.01–0.2% (w/v), 0.0001–10% (w/v) and 0.0001–10% (w/v), respectively). The MTS cytotoxic assay showed no cytotoxicity attributed to the prednisolone nanocrystals. Permeability experiments revealed the transport of prednisolone nanocrystals across the cell barrier.
Discussion and conclusions: This study demonstrates that it is possible to successfully formulate nanosized prednisolone particulates through a microfluidic nanoprecipitation technique.

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Hinokinin and Cubebin loaded PLGA and PLGA-co-PEG nanoparticles: synthesis and characterization

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Introduction: Hinokinin (HKN) and Cubebin (CUB) are biologically active lignans extracted from *piper cubeba* that display significant cytotoxic activity against several cancer lines [1,2], but possess low solubility and stability at acid pH. In order to reduce these problems and increase the potency of these compounds, they were encapsulated in polymeric nanoparticles (NPs) of PLGA and PLGA-co-PEG block copolymers. Nanoparticles of PLGA and PLGA-co-PEG are biodegradable and not metabolized by digestive enzymes and this allows the unchanged drug to reach the intestine where it will be absorbed slowly during decomposition of the nanoparticles [3].

Table 1. Characteristics of various NP formulations.

	NP characteristics		
NP formulation	Average size (nm)	PDI	Encapsulation efficiency (%)
PLGA			
Empty PLGA (V1)	242.3	0.087	–
PLGA-HNK (H1)	221.8	0.083	29.3
PLGA-CUB (C1)	218.1	0.077	35.3
PLGA-co PEG			
Empty PLGA-co-PEG (V2)	240.9	0.087	–
PLGA-co-PEG-HNK (H2)	244.9	0.091	52.2
PLGA-co-PEG-CUB (C2)	188	0.057	98.4

Materials and methods: PLGA polymer, lactide:glycolide monomer ratio of 65:35 and PLGA-co-PEG diblock copolymers of poly((d,l-lactide-co-glycolide)-co-PEG) Resomer® RGP d 50105 (10% PEG, 5 kDa)) were used. The seeds of *Piper cubeba*, were imported from Floral Seed Company, Dehradun, India. Lignans of *Piper cubeba* were extracted with a hydroalcoholic solution. Hinokinin and cubebin were incorporated in PLGA and PLGA-co-PEG nanoparticles using a modified emulsification solvent evaporation technique [3]. The incorporation of the lignans in the NPs was confirmed through UV spectroscopy and by comparison between the data obtained for the empty nanoparticles and pure hinokinin and cubebin.
Results: In Table 1 are resumed the results obtained for the particle size and encapsulation efficiency of HNK and CUB loaded nanoparticles with the drug.
Discussion and conclusions: The NPs PLGA and PLGA-co-PEG were successfully prepared. The particle sizes were examined using DLS. Our results show that encapsulation efficiency for cubebin is better than for hinokinin.

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Sizing and morphology analysis of milled olanzapine, paroxetine and gabapentin particles using hot stage microscopy and laser diffractometry

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Introduction: Paroxetine, gabapentine and olanzapine are drugs used in mental disorders (e.g. epilepsy, aggressive behaviour, schizophrenic and bipolar dysfunctions), very often in prolonged, life-long, therapies. However, since these drugs are poorly water soluble and exist in several polymorphic forms, they exhibit remarkably different clinical outcomes [1]. Amorphization is a strategy used over the last decade to enhance the water solubility of drugs [2], in contrast with previous strategies of crystal engineering. Amorphization can be achieved by reducing the particle size to the nanometric scale, using a milling ball technique [3]. The goal of the present work is to obtain amorphous particles of the three drugs, using different milling conditions, and characterize (size and morphology) these particles by hot-stage microscopy and laser diffraction.
Materials and methods: Particles were obtained using a planetary ball mill (PM 100 CM, Retsch) operated in different conditions (time of milling, speed of the mill, size of the spheres used, dry or wet milling, presence or absence of Tween

60, as a surfactant). The size and shape of olanzapine, paroxetine and gabapentin particles were determined by microscopy (Olympus BX51, Japan, fitted with polarized light and a heating stage, Linkam THMS350V, UK) and laser diffractometry (Mastersizer 2000, Malvern, equipped with a Hydro 2000S sample dispersion unit). Particle size and size distribution were determined using the manufacturers' (Olympus and Malvern) software.
Results: The size of particles (laser diffractometry and microscopic analyses) decreased with the milling time for both dry and wet milling, for all drugs. For olanzapine, dry milling seemed to be more effective in size reduction than wet (water) milling for all the rotations tested (100, 250, 400, 650 rpm). Milling (dry and wet) of the other drugs, for 10 min, either at 400 or 650 rpm, resulted in a particle size reduction to almost half. When wet milling was considered the most effective amount of water was 2.5 mL for all speeds (10 min milling). Results have also shown that the most effective diameter of the milling spheres was 3mm diameter, for both dry and wet milling. Microscopy has shown for paroxetine and gabapentin a reduction in the size of particles of 40% and 16%, respectively (dry milling for 30 or 60 min, at 650 rpm).
Discussion and conclusions: The minimum size achieved was on the micrometer scale and the milled particles of all the drugs remained in the crystalline state. In fact, the longest milling time tested (60 min) and/or the highest mill speed considered (650 rpm) in these preliminary studies were not sufficient to attain particles in the nanometer range, nor the amorphous state for the three drugs. In future studies, different processing conditions, such as higher speed and milling time, should thus be considered.

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Diclofenac eluting contact lenses with antibacterial coating

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Introduction: Silicon-based contact lenses established a new vision corrective form. Currently, over 128 million people use these hydrogel devices worldwide. Due to their biocompatibility and prolonged contact with the eye, they can be used as drug carriers for therapeutic purposes. The coatings of the lenses with layer-by-layer (LbL) may be used to create a surface barrier and get control over the drug release. If, additionally, these coatings are able to prevent the adhesion of biological material (e.g. proteins and bacteria) unto the contact lenses surface, it will be an added value. The main objective of this work is to develop LbL coated lenses loaded with an anti-inflammatory drug (diclofenac) whose surface present antifouling properties.
Materials and methods: A silicone-based hydrogel (TRIS/NVP/HEMA, 40:40:20 w/w) was produced, loaded with diclofenac by soaking in the drug solution and coated by a layer-by-layer surface modification process with different polyelectrolytes. Three coatings were done, involving the combination of alginate (ALG), chitosan (CHI), hyaluronate (HA) and poly-L-lysine (PLL), and using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as a cross-linking agent. The coatings consisted in one double layer of ALG/PLL/EDC, HA/EDC/CHI or HA/PLL + EDC + Drug. The drug release kinetics in NaCl solution was investigated in sink conditions. Material properties such as wettability, surface roughness and optical properties (transmittance and refractive index) were studied. Additionally the hydrogel–lachrymal proteins interaction was evaluated by quartz-crystal microbalance with dissipation (QCM-D) as well as the antibacterial activity against *S. aureus* and *P. aeruginosa*.
Results: The three coatings revealed a controlled release and slightly reduce the albumin adsorption, while maintaining the biological and physical properties necessary for contact lenses application. QCM-D data revealed possible enzymatic degradation of the coatings by lysozyme. A new top layer of HA was added to the ALG/PLL(EDC) coating, which offered a protection against lysozyme, maintained the properties and the controlled release of the drug.

Discussion and conclusions: The release of diclofenac presented a favourable kinetics in the coated samples, as a result from the reversible bonding between the carboxyl groups of diclofenac and the amine groups of PLL or CHI through the activating action of EDC to sustain the drug release. The characterization of coated samples showed some changes in the properties of the material, nevertheless the samples maintained adequate characteristics to be used in therapeutic contact lenses, concerning the evaluated properties.

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Chlorhexidine soft contact lenses: towards the development of safe and efficient devices

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Introduction: Contact lenses are considered to be promising platforms for topical corneal drug delivery, capable of increasing bioavailability in at least 50% when compared to eyedrops [1]. However, before use, sterilization is a mandatory step. The impact of sterilization methods on these drug delivery systems, as a whole, remains barely investigated. This study provides insight into this relevant topic, by evaluating the effect of different sterilization methods on a silicone-based hydrogel (SiHy) loaded with chlorhexidine (CHX), a drug generally used in the treatment of Acanthamoeba keratitis [2].
Materials and methods: The SiHy was prepared by thermopolymerization and loaded with chlorohexidine (CHX) by soaking in the drug solution. The CHX-SiHy was sterilized by steam heat, gamma irradiation (10 kGy) and ozone gas. A complete characterization protocol was carried out, before and after each sterilization method, as follows: swelling capacity was determined by water uptake studies, transparency was accessed by UV–Vis spectroscopy, surface morphology by scanning electron microscopy (SEM) and mechanical properties through tensile tests. Sterilization assurance was accessed by performing sterility tests on previously contaminated samples. Drug release assays were carried out in sink conditions in NaCl 0.9% solution and the CHX released was quantified by high performance liquid chromatography (HPLC).
Results: Unsterilized samples became more rigid after drug loading, which indicates specific interactions between the drug and the polymeric matrix. Swelling capacity and surface morphography of the CHX-SiHy after the sterilization processes were not affected. Steam heat did not significantly affect the CHX release profile, however, signs of mild degradation were observed. Gamma irradiation and ozonation led to similar release profiles among them. However, in both cases, a significant decrease in the drug-released amount was observed, relatively to the unsterilized and steam heat sterilized samples, with formation of several degradation products.
Discussion and conclusions: Within the frame of the experimental conditions used, steam heat was the sterilization method with less impact on the properties of the studied drug delivery system. More importantly, the present work shows that the development of efficient and functional drug delivery devices for ophthalmic purposes cannot be done independently of a careful analysis of the influence of the sterilization procedures and methods on the degradation of these polymeric systems as a whole.

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Effect of sterilization on ketorolac loaded intraocular lenses

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Introduction: Control the inflammation process in the eye, after cataract surgery, currently includes the administration of topical or intracamerular anti-inflammatory drugs. An alternative could be the implantation of drug loaded intraocular lenses (IOLs) with the ability to deliver a controlled amount of drug during an adequate period of time. IOL sterilization is mandatory to fulfil the microbiological safety requirements, but it must not compromise the biomaterial properties nor the drug activity. The goal of this study was to investigate the effect of two sterilization methods (steam heat and γ-irradiation) on IOLs loaded with a non-steroid anti-inflammatory, ketorolac.

Materials and methods: The work comprised three stages: evaluation of the sterilization effects on the drug, on the material intrinsic properties and on the drug release profile. A solution of ketorolac (2 mg/mL prepared in NaCl 0.9%), an acrylic IOL material provided by PhysIOL and the drug-loaded IOLs (soaked in ketorolac loading solution, 2 mg/mL) were sterilized by steam heat (60 min, 121 °C, 1 bar) and γ-irradiation (5 kGy). The stability of the drug was studied by high performance liquid chromatography (HPLC) and its pH was measured before and after sterilization. Relevant properties of the loaded and unloaded IOLs, as transparency (UV-Vis spectroscopy) and morphology (SEM), were characterized. The efficiency of the sterilization methods was determined through the membrane filtration method, for the ketorolac solutions and direct inoculation method for the IOLs material. Drug release experiments were carried out at 36 °C in sink conditions (3 mL NaCl solution per lens). The quantification of the released drug was performed by HPLC.

Results: Results of HPLC and pH measurements showed that steam heat sterilization did not induce drug degradation. However, γ-irradiation at 5 kGy led to some changes on drug stability. Concerning the material properties, steam heat and γ-irradiation at 5 kGy did not affect morphology, but γ-irradiation at 5 kGy slightly decreased transmittance of loaded IOLs. Both, drug solutions and lenses were effectively sterilized with both methods. Contrarily to γ-irradiation, steam heat sterilization induced strong changes in the drug release profile, being released a higher amount of drug than from non-sterilized samples and with a more sustained kinetics.

Discussion and conclusions: The sterilization conditions may be critical for drug-loaded IOLs. In this study, steam sterilization revealed to be the best sterilization method, since it did not affect neither the drug stability nor the material properties and significantly improved the drug release profile.

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Effect of loading time and temperature on diclofenac release from intraocular lens

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Introduction: Cataracts are the first leading cause of blindness worldwide, affecting especially the elderly population. The current treatment for cataracts involves surgery (removal of the cataract and implantation of an intraocular lens (IOL).

In the post-surgery period, anti-inflammatories such as diclofenac (DFN) are prescribed in the form of eye drops, during an extended period of time. This drug delivery method is not very effective and requires patient compliance and ability to correctly instill the drops. The use of drug-loaded ophthalmic lenses is an alternative widely investigated to overcome this problem. However, the burst experimented in the beginning of the release and the inadequate kinetics is a problem [1]. Our objective is the optimization of the loading conditions (time and temperature) of IOLs to obtain a controlled and extended delivery of DFN.

Materials and methods: Diclofenac sodium salt (Sigma-Aldrich) and the material CI26Y for IOLs (Contamac UK) were used. CI26Y disks (5 mm diameter, 1 mm thickness) were loaded with DFN by soaking in 3 mL of drug solution at 4 °C, for 4 d, 2 weeks and 1, 2 and 3 months and, at 60 °C, for 4 d and 1 and 2 weeks. Drug release experiments were carried out in 3 mL of PBS, at 36 °C and 180 rpm. The concentration of the drug released was determined by UV–Vis spectroscopy. The samples without and with DFN (loaded for 2 weeks at 4 °C and 60 °C) were analysed with NMR and DSC, as well as Young's modulus measurements. Transmittance of the material loaded using the best conditions was determined with a UV–Vis spectrophotometer.

Results: The release of DFN loaded at 4 °C was affected by the loading time, being the maximum loading achieved only after 2 months. However, at 60 °C, the different loading times tested did not have any effect on the release being the maximum loading achieved after 4 days. To further understand the effect of the temperature, a fixed loading time of 2 weeks was chosen at temperatures of 4 °C and 60 °C. The release was higher and more controlled for the samples loaded at 60 °C. The NMR, DSC and Young's modulus studies demonstrated the existence of reversible interactions between the polymer chains and DFN, more significant at 60 °C. The transmittance was above 90% above 550 nm.

Discussion and conclusions: With a fixed loading time of 2 weeks, the increase of the loading temperature led to an increase in the released amount and release duration of DFN. NMR and DSC analyses confirm the existence of reversible and endothermic reactions between CI26Y and DFN at higher temperatures, which explain the differences in the drug release behaviour. However, the best loading conditions were achieved when the material was loaded at 4 °C for 2 months. These conditions allow achieving a sustained release of DFN for at least 15 days.

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Surface grafting with monomers to control drug release from intraocular lenses

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Introduction: After cataract surgery, topical administration of antibiotic is needed to prevent endophthalmitis. Intraocular lenses (IOLs) may be used as vehicles for drug delivery, with higher efficacy and less side effects than the common eye drops. In this work, we have evaluated the possibility of controlling the release of the antibiotic moxifloxacin (MFX) from a commercial IOL material through surface modification by graft polymerization.

Materials and methods: Surface modification of an acrylic-based IOL material by plasma-assisted grafting involved surface activation through exposure to argon plasma followed by immersion in a 10% (v/v) solution of HEMA (2-hydroxyethyl methacrylate) or AMPS (2-acrylamido-2-methylpropane sulphonic acid) in HBSS (Hank's balanced salt solution) containing MFX at 5 mg/mL (1 h, 60 °C). Antibiotic loading was done by immersion in a MFX solution (5 mg/mL, HBSS) for 4 d at 60 °C. The material was afterwards sterilized by steam vapour (121 °C, 30 min) and stored for 30 d at room temperature in a MFX solution with the same concentration. Characterization of the modified materials was done regarding transmittance, wettability, morphological and topographical properties. Coating thickness was determined through ellipsometry. Drug release studies were performed in a microfluidic cell to approximate the hydrodynamic conditions found in the eye.

Solutions collected from drug release experiments were tested against *S. aureus* and *S. epidermidis* to evaluate their antibacterial activity.
Results: Ellipsometry results confirmed the presence of coating layers in both HEMA (52 ± 5 nm) and AMPS (44 ± 12 nm) grafted samples. In both cases, MFX was released until the 16th day of the *in vitro* tests. Samples collected until the 15th day (last day evaluated) were effective against the tested bacteria, except those from the AMPS-coated material, which was active until the 12th day against *S. epidermidis*.
Discussion and conclusions: Since the recommended duration of antibiotic therapy is about two weeks after surgery and the materials herein tested released effective antibiotic concentration for both bacteria until day 12, they can be an alternative to conventional postoperative topical antibiotic application.

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Determination of median lethal dose of three bulk-fill resin composites

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Introduction: Over the years, amalgam dental fillings have been progressively replaced for aesthetic resin based composite materials. Currently, a new range of resin composites termed bulk-fill has been developed with the aim to facilitate the application of these materials. Bulk-fill materials allow the practitioner to place blocks of composite up to 4 mm thick, instead of the 2 mm commonly used on regular composites. This ultimately results in an easier application method and reduced appointment time [1]. In this study, we aimed to quantify bulk-fill cytotoxicity, determining the median Letal Dose (LD_{50}) using fibroblast cell line NIH/3T3 (ATCC® CRL-1658™). These cells are recommended for cytotoxicity studies, since they present features such as contact inhibition and absence of tumorigenicity, thus making them similar to regular cells [2].
Materials and methods: Three bulk-fill resin composites were utilized: Tetric EvoCeram® Bulk Fill (Ivoclar Vivadent), Filtek™ Bulk Fill (3M ESPE) and Fill-Up!™ (Coltene). These composites were light cured with Elipar™ DeepCure-S LED and then immersed in cell culture medium to prepare extracts. Seven relative concentrations of the extracts (1–75%) were tested through the crystal violet (CV) and the MTT cytotoxicity assays.
Results: Median lethal dose values, LD_{50} (relative concentration of the extracts), determined by two different assays was 59.5 ± 6.6 for Tetric EvoCeram, 62.3 ± 0.4 for Filtek and 26.0 ± 9.4 for Fill Up! (Figure 1). When compared with non-exposed cells, all bulk-fill resins were significantly toxic, mainly for relative concentrations of extracts range between 20% and 75% (*t*-tests, $p < .001$).
Discussion and conclusions: All the bulk-fill resin composites were toxic according to ISO 10993-5 criteria [2] as we have shown previously [3]. In our experimental conditions, the most toxic bulk-fill resin was Fill Up!. Moreover, our preliminary results also suggest that these materials are genotoxic and induce cell death by necrosis. Determination of median lethal dose is fundamental to define experimental conditions to enable cytotoxicity comparisons with others materials.

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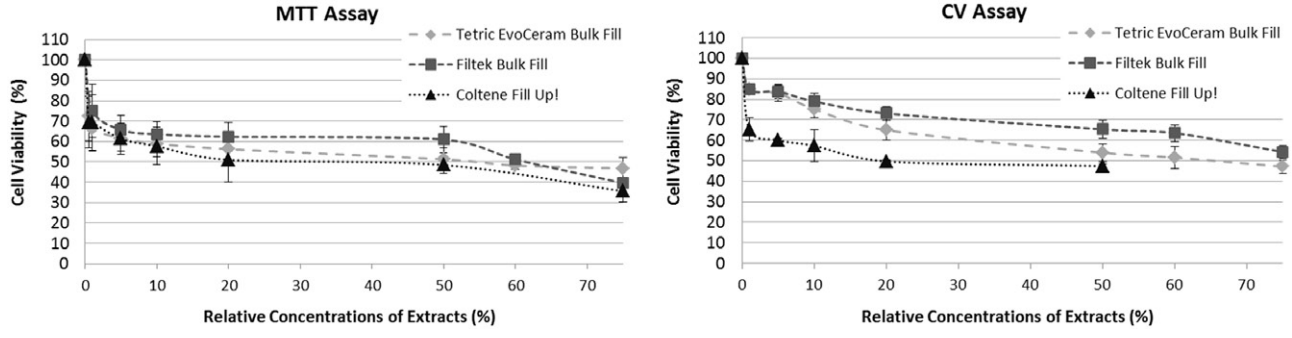

Figure 1. Cytotoxicity of Bulk Fill resins extracts in NIH/3T3 fibroblasts.

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Inhibitors for matrix metalloproteinases, molecular design for dental restoration

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Introduction: Adhesive resins are the most common human-synthetic material interface. Their application enables tooth restoration. Dental caries affects 90% of the world's population. Clinical application of these materials has encountered durability limitations. The adhesive resin is attached to collagen fibers that are exposed on the hydroxyapatite surface. During the following years after restoration, pulp pressure infuses liquid in the dentinal channels defining an intricate frontier of wettability. In the long term, this interface allows activation of matrix metalloproteinases (MMPs) that degrade collagen fibers inducing failure of the restoration. The presence of endogenous MMPs have been identified has a main cause for restoration failure. Furthermore, inhibition of MMP is important for other diseases and cancer.
Materials and methods: The workflow included (1) computational studies molecular docking of inhibitor to the MMP active site to define the most promising candidates for synthesis. (2) Organic chemistry synthesis of new compounds. (3) Biochemical test of enzymatic activity inhibition of the compounds towards different MMPs. (4) Cell toxicity evaluation of the compounds and in silico prediction of their potential passage of the blood brain barrier. (5) Tensile resistance of the hybrid tooth-resin and their fracture analysis by ultramicroscopy.
Results: The initial proposed several hundred compounds had in common the same synthetic strategy of central moiety with two hydroxyl groups that are stepwise substituted with two side groups to yield the final molecule. Compounds were synthesized, purified and characterized. About 60 new molecules [1,2] with vinyl substituents that enable copolymerization with the current dental resins were obtained. Biochemical activity tests revealed the most promising inhibitors. A few selected molecules were included in the mechanical tensile resistance test of the tooth-resin interface, and an increased resistance was found in several samples aged up to 12 months.
Discussion and conclusions: The copolymerization of the inhibitor with the resin limits its potential toxicity since most resin compounds were determined to pass the blood-brain barrier [3,4].

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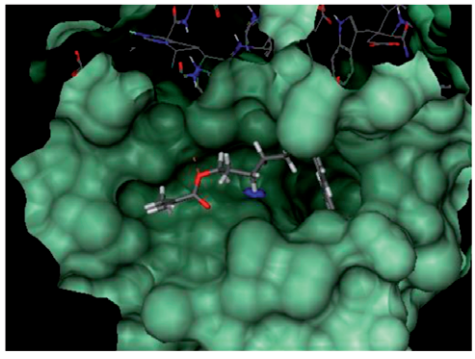

Figure 1. Computationally predicted docking site of synthesized inhibitor in the active site of MMP-8 (LeadIT/FlexX).

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Influence of chlorhexidine as a metalloproteinases inhibitor on the color change of a resin composite

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Introduction: Chlorhexidine (CHX) is a potent inhibitor of matrix metalloproteinases (MMPs) [1]. Numerous *in vitro* studies demonstrated that CHX has beneficial effects on the preservation of resin-dentin bond [1]. Untreated control mineralizes was able to hydrolyze fluoresceinlabeled soluble type I collagen, while CHX inhibited that collageneolytic activity by 99% [2]. The aim of this study was to evaluate, *in vitro*, the effect of CHX, as a metalloproteinases inhibitor, on the color change of a resin composite.
Materials and methods: Twenty freshly third molars were randomly divided into four groups with five teeth. Each group was restored with Filtek Z100 composite resin and previously treated with an adhesive system protocol forming the following groups: OSC (OptiBond FL without CHX), OCC (OptiBond FL with CHX), ASC (All Bond Universal without CHX) and ACC (All Bond Universal with CHX). The teeth were stored in distilled water for 24 h. The color stability was determined by the difference (ΔE) between the L*a*b* coordinates obtained after the CHX use.
Results: The results were analyzed with the *t*-Test and ANOVA test to $p < .05$. After 24 h none of the groups showed a statistically significant change. However, for group ASC, ACC and OSC, $\Delta E > 1$, it was noticed color change visible to the human eye. For group OCC $\Delta E > 3.3$, it was noticed clinically unacceptable color. The *t*-Test and ANOVA between groups ASC and OSC and groups ACC and OCC showed adhesive system had influence in color change.
Discussion and conclusions: CHX didn't change the color of the composite in any of our groups. However, All Bond Universal adhesive system causes more color variation than the OptiBond FL adhesive system.

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Evaluation of microtensile bond strength of an Ormocer® adhesive system – Admira Bond®

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Introduction: Since 1955, when Buonocore introduced adhesion to tooth structure, the use of adhesive properties of dental materials has been growing presenting several advantages such as a minimally invasive approach and prevention of secondary caries [1,2]. Adhesion to tooth structure works as a double bridge between tooth/adhesive and adhesive/restoration. Thus, adhesion, besides providing continuity between tooth and restoration, preventing the infiltration of microorganisms, must support mechanical forces that occur during function and curing process [3]. However, it is known that bond strength durability is the biggest problem associated with adhesion and that adhesive failure is one of the main reasons of restoration failure [4,5]. The aim of this study was to evaluate the microtensile bond strength of an Ormocer® adhesive system.
Materials and methods: Twelve healthy human teeth were divided into four groups according to the adhesive system and aging procedure: G1 – Admira® Bond (Voco) (37 °C for 24 h in an incubator); G2 – Admira® Bond (10,000 cycles of termocycling at 5 °C and 55 °C); G3 – Solobond Plus (Voco) (37 °C for 24 h) and G4 – Solobond Plus (10,000 cycles of termocycling). All specimens were sectioned to expose superficial dentin. Smear layer was simulated with a 600-grit SiC paper. Adhesive systems were applied according to manufacturer's instructions and resin composite build-ups were obtained with Filtek Z250 (3MESPE) (2 mm increments; total height: 6 mm). After aging, samples were sectioned to obtain $1 \pm 0.2\,mm^2$ slabs. The cross-sectional area was measured using a digital caliper. Specimens were fixed to a jig using a cyanoacrylate adhesive (Zapit) and submitted to stress until failure in a universal testing machine at 0.5 mm/min. Failure mode and MPa adhesive forces were recorded. Bond strength data were analysed using Two Way ANOVA, at the 5% significance level.
Results: The values of Solobond Plus were significantly higher than Admira® Bond ($p = .007$), as well as the results at 24 h comparably to those after 10,000 cycles ($p < .001$).
Discussion and conclusions: Long-term success of restoration materials is based on their capacity of resisting oral cavity conditions [6]. Termocycling aging occurs by accelerating hydrolysis and contraction/expansion phenomena leading to the presence of failures. Microtensile bond strength was higher for Solobond Plus. Aging with thermocycling significantly decreased bond strength.

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Resin-enamel microtensile bond strength evaluation of two universal adhesive systems in both etch-&-rinse and self-etch approach

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Introduction: Teeth adhesion had a great developing in the last few years. Hybrid layer is considered as the main factor to achieve a better adhesion [1]. To obtain dental adhesion we have two adhesive systems strategies, etch-&-rinse and self-etch [2], with different ways of application. Recently, universal adhesive systems were introduced in the market that combine both etch-&-rinse and self-etch approach. The purpose of this study was to evaluate microtensile bond strength

(MTBS) to enamel of two universal adhesive systems: Xeno Select (Dentsply) and Futurabond M+(Voco), in both etch-&-rinse and self-etch approach.
Materials and methods: Human third molars were sectioned perpendicularly to teeth long axis in two halves, to separate crown and roots. Furthermore, crowns were longitudinally sectioned in a buccal-lingual direction from their central part, yielding two halves, mesial and distal, both prepared with 600-grit silicon carbide papers in order to simulate smear-layer and obtain a flat surface. Adhesives were applied to enamel according to manufacturer's instructions, both as etch-&-rinse and self-etch. Resin build-ups, 6 mm in height, were obtained with Z250 (3MESPE) light-cured composite resin (2 mm increments). Each layer of composite was separately light-activated for 40 s with an Optilux 501 halogen light-curing unit. Light intensity output was monitored with a Curing Radiometer to be at least 600 mW/cm^2. After storage in distilled water at 37 °C for 24 h, specimens were vertically sectioned into serial slabs, and further into beams with cross-sectional areas of 1 mm^2, with a slow-speed diamond saw Accutom-50, following the method described by Shono et al. [3] (20–24 beams per group). All beams were attached to a customized jig testing apparatus with a cyanoacrylate adhesive (Zapit) and stressed to failure in tension using a universal testing machine at a crosshead speed of 0.5 mm/min. The fractured beams were removed from the apparatus and the cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a digital caliper. The MTBS values were calculated by dividing the load at failure by the cross-sectional bonding area. Fractured specimens were examined with a stereomicroscope at 40× magnification to determine the mode of failure (adhesive, cohesive or mixed). Bond strength data were analysed using Two-Way ANOVA, after validation of model assumptions. All statistical procedures were implemented at the 5% significance level.
Results: No significant differences in MTBS were observed, either between the different adhesives ($p = .375$) or between the different treatments used ($p = .170$).
Discussion and conclusions: Previous work [4] concluded that MTBS to enamel is higher when a universal adhesive is applied in the etch-&-rinse approach compared with self-etch application. These results are not in accordance with what was found in this study, as no significant differences between groups were found. Due to the fact that the adhesives above were new in the market, there are no studies to compare results. We conclude that there are no significant differences in the MTBS to enamel of the different universal adhesives systems studied in both etch-&-rinse or self-etch approach.

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Effect of different gel bleaching agents on microtensile bond strength to enamel

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Introduction: Tooth bleaching is a common procedure in aesthetic dentistry [1]. Following tooth bleaching, many adhesive procedures are also commonly performed in order to improve the overall aesthetics, such as anterior direct or indirect restorations, anatomical recontouring or diastema closure. The bleaching agents are known to induce alterations on the microhardness, chemical composition and surface roughness of the tooth structure [2]. Consequently, this has a detrimental effect on the adhesion of subsequent restorations [3]. Considering this, it is necessary to research their overall effect on the microtensile bond strength to enamel.
Materials and methods: 13 molars extracted for periodontal or orthodontic reasons, were longitudinally sectioned into 25 fragments total. The fragments were randomly distributed into 5 groups ($n = 5$), depending on the application of the bleaching gel: Group 1 – placebo gel (control); Group 2 – 35% hydrogen peroxide gel Perfect Bleach Office + (Voco); Group 3 – 37.5% hydrogen peroxide gel Norblanc Office Automix (Normon); Group 4 – 16% carbamide peroxide gel Norblanc Home (Normon); Group 5 – 16% carbamide peroxide gel Perfect Bleach (Voco). The bleaching gels were applied

three times within a week between each application. After the third application, the fragments were restored with composite resin. After 24 h, the fragments were cut with a diamond saw with slow rotation speed in *X* and *Y* directions in order to obtain sticks with $1 \pm 0.2\,mm^2$ section. The sticks were stressed to failure in tension using a universal testing machine at a crosshead speed of 0.5 mm/min. For the statistical analysis, ANOVA one-way and Tukey's HSD post-hoc tests ($\alpha = 0.05$) (SPSS 20.0) were used.
Results: The microtensile bond strength of the control samples (Group 1) is significantly higher than the samples treated with gels 2 ($p = .006$) and 3 ($p = .047$) and differs significantly from the samples treated with the gels 4 ($p = .087$) and 5 ($p = .385$). The samples treated with gels 2, 3, 4 and 5 do not differ significantly from each other with regard to microtensile bond strength ($p > .05$) in either comparison.
Discussion and conclusions: This *in vitro* study suggests that there is a reduction in the enamel's bond strength after bleaching treatment with hydrogen peroxide and carbamide peroxide gels that can be explained by the presence of residual oxygen in the enamel after tooth bleaching which may inhibit the adhesive and composite resin complete polymerization.

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Surface treatments effect on μTBS of a resin-nano-ceramic

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Introduction: In the adhesive cementation process, it is recommended that the internal surface of the prosthetic restoration be treated in order to optimize the micromechanical bond strengths between the ceramic and the resin-based materials [1–3]. However, it is not clear how the different surface treatments influence the adhesive cementation process. The objective of this work was to evaluate the influence of different surface treatments on the bond strength (μTBS) between a resin-nano-ceramic (RNC) material and a resin-based cement.
Materials and methods: Sixteen blocks of Lava™ Ultimate (3M) were cut into samples of 6 mm and randomly divided into three main groups, according to the cutting method: G1) Digital microtome; G2) Digital microtome + Aluminium oxide sandblasting (50 μm); G3) Milling through the CEREC®system (Sirona). All samples were submitted to a salivary contamination, after which each group was divided into three subgroups ($n = 4$), according to surface treatment: S1) Cleaning with alcohol; S2) Aluminium oxide sandblasting (50μm, 10 s); S3) Conditioning with hydrofluoric acid (9%, 30 s). This procedures were followed by placing the Scotchbond™ Universal adhesive (3M). Thirty-two blocks of Filtek™ Supreme XTE (3M) were done using a silicone mold and cemented with the resin-based cement RelyX™ Ultimate (3M), to the previously RNC samples. The blocks were divided in two groups: the control, stored for 24 h in distilled water in an oven at 37 °C and the thermocycled (10,000 cycles, 30 s, 5–55 °C). The samples were cut in the "*X*" and "*Y*" directions, to obtain beams with a cross-section of $1 \pm 0.2\,mm^2$ and tested in tension (1 mm/min rate) until failure with an universal testing machine. ANOVA one-way and post-hoc tests were used at a 5% significance level.
Results: The highest bond strengths were obtained in both the control (C) and thermocycled (T) groups for G1S3 (C: 91 MPa; T: 88.13 MPa), G2S2 (C: 91.12 MPa; T: 89.74 MPa) and G3S3 (C: 94.12 MPa; T: 94.85 MPa). Significant differences ($p = .001$) were observed between the control and thermocycled groups for G1S1 and G3S1.
Discussion and conclusions: Different surface treatments show different levels of bond strength. The highest bond strength values were obtained in the groups conditioned with hydrofluoric acid.

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Physicochemical and dentin adhesion studies of a new experimental universal dental adhesive without Bis-GMA

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Introduction: Regardless the advantages presented by universal adhesives [1], they still present limitations that lead to the degradation of the interface between the tooth and the restoration [2]. The formation of a secondary caries is the main reason for the replacement of dental restorations and many strategies have been reported in the literature to develop adhesive materials and techniques envisaging the reduction of micro-and nanoleakage phenomena [2]. Low polymerization shrinkage, high double bond conversion, high resin monomers penetration and high hydrophobicity are important characteristics to be considered for the improvement of dental adhesive's bonding efficacy [3]. Due to their polymerization characteristics, hyper-branched macromers are promising candidates to be used in dental materials [4]. In this study, a new Bis-GMA (Bisphenol A-glycidyl methacrylate) free dental adhesive system was prepared, using a synthesized dendritic macromer (G-IEMA – G(2)-isocyanatoethyl methacrylate), and their dentin adhesion efficacy and physicochemical properties were investigated.
Materials and methods: G-IEMA was prepared according to the procedure described by Biao et al. (2014), with minor changes, and was subsequently incorporated into an experimental adhesive (AE2). Their physicochemical properties, such as conversion degree, polymerization shrinkage, and water sorption and solubility were compared with three other adhesives: – AE1(formulated with Bis-GMA), SBU (Scotchbond Universal, 3M ESPE, St. Paul, USA) and FUT (Futurabond M+, VOCO GmbH, Cuxhaven, Germany). Additionally, micro-tensile bond strength (μTBS) tests were used to study dentin adhesion, and values obtained were compared among groups.
Results: Experimental results suggest comparable monomer conversion degree and polymerization shrinkage for AE2 in relation to the other adhesives. Dentin adhesion seems to depend on the adhesive application method; AE2 presented higher values for microtensile bond strength with *etch-and-rinse* protocol, when compared with SBU and FUT groups. However, with self-etch procedures, AE2 presented weaker bond strength compared to AE1.
Discussion and conclusions: Experimental adhesive AE2 showed better adhesive behaviour than the SBU and FUT adhesives when *etch-and-rinse* protocol was applied. According to the results obtained so far, G-IEMA seems to be a good candidate to prepare Bis-GMA free dental adhesives with potential to be used in clinic.

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Adhesion of indirect dental restorations with resin cement and pre-heated resin: effect of film thickness and seating forces

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Introduction: Indirect dental restorations are fabricated outside the mouth. They are indicated when large restorations are needed, and direct restorations tend to fail due to polymerization shrinkage [1]. Originally, luting cements were used to cement these restorations to the teeth. Recently preference is given to pre-heated resins, since better marginal adaptation [2] and esthetics are achieved. To our knowledge, no studies have compared, under the same circumstances, the bond strength of both techniques. Therefore, this study aimed at comparing the bond strength of indirect resin restorations to enamel, using resin cement and pre-heated resin, and to evaluate the effect of seating forces and film thickness on the bond strength.

Materials and methods: Forty healthy bovine incisors were randomly divided into four experimental groups ($n = 10$). Indirect restorations (Filtek™ Supreme XTE, 3M ESPE) were cemented/bonded to the teeth with either resin cement (RC) (Relyx™ Ultimate, 3M ESPE) or pre-heated resin (PHR) at 55 °C (Z100™, 3M ESPE). In two groups (G750-RC and G750-PHR), a 750 g weight was used to standardize the seating force. In the other 2 groups (Gdigital-RC and Gdigital-PHR) finger pressure was used as seating force. The film thickness (FT) was measured (μm) and micro-tensile tests were used to determine the bond strength (BS) in MPa. The failure mode that occurred during the micro-tensile tests was observed with an optical microscope. The dependent variables "BS" and "FT" were statistically analyzed, as well as the failure mode.

Results: A Kruskal–Wallis test showed that there was a statistically significant difference in BS (Figure 1) between the groups. Multiple comparisons indicated that only G750-PHR and Gdigital-PHR did not differ from each other ($p = .066$). There was also a statistically significant difference in FT (Figure 2), being Gdigital-PHR and Gdigital-RC ($p = .455$) and G750-PHR and G750-RC ($p = .089$) not different. Spearman's rank-order correlation showed no association between BS and FT. The Chi-square tests showed a statistically significant association between the failure mode percentages (Figure 3) occurring in each group ($p < .001$).

Discussion and conclusions: The higher values of BS found for the RC groups might be related to a lower number of interfaces involved in this adhesive joint [3]. The seating forces seemed to influence FT, however no effect of FT was seen on BS.

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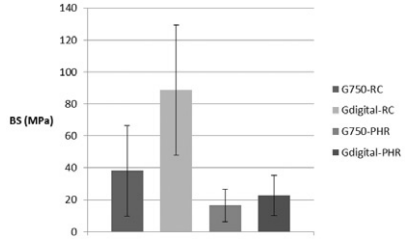

Figure 1.. Average values of BS (±sd).

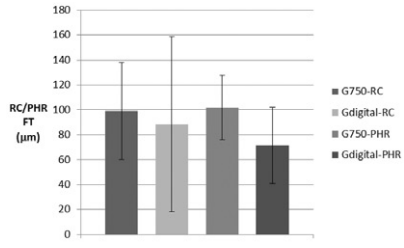

Figure 2.. Average values of FT (±sd).

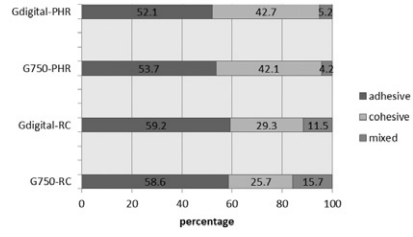

Figure 3. Failure mode percentages.

Influence of atmospheric pressure variation combined with the presence of saltwater in adhesion to dentin of etch-and-rinse and self-etch systems

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Introduction: The aim of this study is to evaluate the influence of atmospheric pressure variations combined with the presence of saltwater in adhesion to dentin of etch-and-rinse and self-etch systems.
Materials and methods: Twenty-four molars extracted were randomized into four groups, according to the adhesive technique: FL-Optibond FL (Kerr Corporation®), SP-Optibond Solo Plus (Kerr Corporation®), XTR-Optibond XTR (Kerr Corporation®); AiO-Optibond All in One (Kerr Corporation®). The restorations were performed with composite resin Filtek Z250 (3M ESPE®, A2 color). Each of these groups was further divided into three subgroups, composed of two teeth each. The first subgroup (FL1, SP1, XTR1, AiO1) corresponds to the control group, the second group (FL2, SP2, XTR2, AiO2) was exposed to thermal fatigue (10,000 cycles in baths of 30 s to 5 to 55 °C), the third (FL3, SP3, XTR3, AiO3) was exposed to the same thermal fatigue together with the pressure display with the addition of saltwater (30 cycles pressure ranging from 0 to 3 atmospheres), to faithfully simulate the activity of a diver. Thereafter, the teeth were stored in an oven for 24 h to proceed after this period to its sectioning in the X and Y directions by means of the diamond blade at low speed to obtain sticks with about $\pm 1 \times 0.2 mm^2$. The samples were individually placed in a stainless-steel jig Geraldeli and subjected to a tensile force until fracture occurs at a speed of 1mm/min. Statistical analysis was applied to using ANOVA with correction Brown-Forsythe test Post-Hoc Tamhane.
Results: Only Optibond Solo Plus adhesive (17.63 MPa) and Optibond All in One (13.18 Mpa) showed a significant decrease in its adhesive strength after the stress procedures.
Discussion and conclusions: When comparing control group (FL1, SP1, XTR1 and AiO1) with those which were exposed only to thermal fatigue (FL2, SP2, XTR2 and AiO2) there were no statistically significant differences. Comparing bond strength among FL3, SP3, XTR3 and AiO3 no statistically significant differences were observed. Although, when comparing each adhesive individually there was a significant decrease of bonding strength between SP1 and SP3 and the same was observed between AiO1 and AiO3, a result which may be attributed to the influence of barometric pressure within saltwater. Adhesives that have demonstrated better resistance after pressure variation on saltwater are Optibond XTR and Optibondl FL (Kerr Corporation®).

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Wear resistance evaluation of prosthetic dental materials

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Introduction: The loss of tooth tissue, besides the aesthetic problems and dental sensitivity, also has consequences in terms of phonetics and food processing. In advanced cases, it can even lead to damage of the temporomandibular joint. Therefore, synthetic materials for dental tissue replacement are commonly used. During chewing, these materials are subjected to forces ranging from 2 to 150 N and temperatures that can vary between 5 °C and 70 °C, as well as to different pH and chemical attack of different agents. Thus, it is essential to ensure a good wear resistance for the prosthetic materials, which also should not cause abnormal wear of antagonists natural teeth. The goal of this work is to perform a comparative evaluation of the wear resistance of two commercial prosthetic materials which are normally used in the manufacture of dental crowns.
Materials and methods: The materials selected for this study were Zirconia (ZrO_2) and the composite commercially known by VITA ENAMIC. Samples of these materials were polished and then characterized in terms of hardness (Vickers hardness), hydrophilicity (sessile drop method) and roughness (AFM). The wear tests were conducted using the chewing simulator at room temperature, in artificial saliva. Cusps of human molars were used as antagonist bodies. 306000 cycles were carried out, corresponding to 2.5 years of mastication. The wear of the cusps and counter materials was determined by volume loss and profilometry analysis, respectively.
Results: Hardness measurements led to 253 ± 29 HV for VITA and 1241 ± 70 for ZrO_2. The water contact angle and the roughness of both materials were similar ($\approx 35^\circ$ and ≈ 20 nm). Concerning the counter materials wear, contrarily to ZrO_2, which did not suffer significant wear, VITA showed high wear rates. However, the wear of the cusps tested against both materials did not differ significantly.

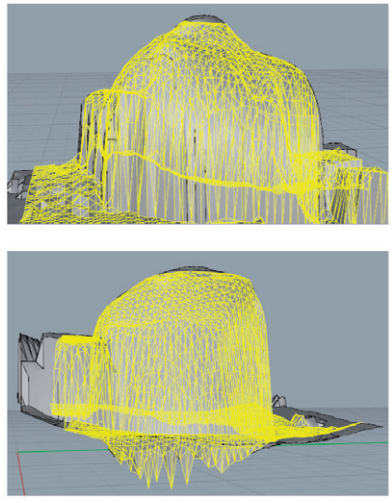

Figure 1. 3D scan of a cusp before (solid gray) and after wear test (yellow mesh) against (a) ZrO2 and (b) VITA.

Discussion and conclusions: The results show that the wear resistance of ZrO_2 is significantly higher than VITA. In fact, VITA is made from composite resin with ceramic filler particles and its hardness is quite lower than that of ZrO_2. More, the fracture toughness of VITA and ZrO_2 are very different, 1.5 MPa $m^{1/2}$ and 8–10 MPa $m^{1/2}$, respectively [1]. This could explain the higher susceptibility of VITA to wear. Although differences are found in the commercial materials wear resistance, no correlation was found between dental wear and the counterface.

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Wear of the implant hexagon with Zirconia and Titanium abutments

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Introduction: In contrast to the high success rates of osseointegration (95–97%), mechanical complications are still common in implant dentistry [1,2]. Mechanical complications such as screw loosening, screw fracture, and framework fracture have been reported to be as high as 44.9% [2,3]. In this study, we will evaluate the wear of the implant-abutments interface after a torsional fatigue test that simulates a loose abutment situation.
Materials and methods: Five external hexagonal implants were embedded with acrylic resin and divided into three groups: Control group: non engaging titanium abutment, Group A: two engaging titanium abutments, Group B: two engaging zirconia abutment. Rotational freedom for each pair was access before and after testing. Each pair was torque to 35 Ncm and placed on an Instron 8874 machine and loaded on a rotational test: +/6.6°, 4Hz, with a compressive force of 100 N for 1 million cycles.
Results: The screws in all groups were loose after testing. In groups A and B, there was an increase in the rotational freedom with destruction of the hexagon but not on the control. Volume loss was higher in group A with the Zirconia abutment in group B suffering limited wear. There was evidence of adhesive wear and abrasion and three body abrasion in both groups.

Discussion and conclusions: The loose screw in an implant prosthesis may lead to the wear of the hexagon on both parts. The wear is sufficient to compromise the implant-abutment connection beyond repair. Studies that evaluate the rotational freedom of implants or abutments show different results due to differences in machining tolerances of the different implant systems [4]. The literature is sparse on torsional tests of implants and abutments [5,6]. The rounding of the implant hexagon is consistent with the findings of Yao and Dhingra [6]. When a patient presents a loose screw restoration, it should be removed and inspected.

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ORAL MEDICINE AND DENTISTRY

Association between overweight/obesity and chronic periodontitis in a Portuguese population

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Introduction: Obesity is a chronic metabolic condition occurring due to an energy imbalance (intake > consumption) [1]. It is an extremely prevalent disorder worldwide, especially in developed countries [2]. Some studies suggest that obesity could be a novel risk factor for periodontitis [3–5]. The aim of this study was to evaluate the possible association between overweight/obesity and periodontal status in an adult Portuguese population.
Materials and methods: A random sample of 380 people, aged between 20 and 90 years, was selected from patients who attended the Egas Moniz Dental Clinic (Monte de Caparica, Portugal), from September 2015 to June 2016. The American Academy of Periodontology (AAP) classification system was used to assess periodontal condition [6]. Body mass index (BMI) was calculated and categorized using the WHO criteria [2]. Data were subjected to statistical analysis by using descriptive and inferential methods. A significance level of 5% was considered in the later.
Results: In this group, 41.8% ($n = 159$), 39.2% ($n = 149$) and 18.9% ($n = 72$) were normal weight, overweight and obese, respectively (Table 1). No association was found between BMI categories and periodontal condition ($p = .198$).
Discussion and conclusions: The results show no association between body mass index and chronic periodontitis extension and severity.

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Table 1. Association between overweight/obseity and chronic periodontitis.

IMC (kg/m^2)	Mild localized (%)	Moderate localized (%)	Severe localized (%)	Mild generalized (%)	Moderate generalized (%)	Severe generalized (%)	Chronic periodontitis (total) (%)
18–24.9	17 (10.7)	8 (5.0)	13 (8.2)	31 (19.5)	24 (15.1)	66 (41.5)	159 (41.8)
25–29.9	6 (4.0)	3 (2.0)	12 (8.1)	31 (20.8)	28 (18.8)	69 (46.3)	149 (39.2)
>30	8 (11.1)	2 (2.8)	11 (15.3)	10 (13.9)	9 (12.5)	32 (44.4)	72 (18.9)

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Association between age and chronic periodontitis in a Portuguese population

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Introduction: Age is considered a non-modifiable risk factor for chronic diseases [1]. The assessment and diagnosis of periodontitis in younger adults may not be fully applicable in older individuals [2]. Recent literature suggest that gingival lesions in the elderly develop differently from gingival lesions in younger adults [2]. The aim of this study was to evaluate the possible association between age and periodontal status in an adult Portuguese population.
Materials and methods: A random sample of 380 people, aged between 20 and 90 years, was selected from patients who attended the Egas Moniz Dental Clinic (Monte de Caparica, Portugal), from September 2015 to June 2016. The American Academy of Periodontology (AAP) classification system was used to assess periodontal conditions [3]. Participants were stratified by age: younger (20–44 years old), middle-aged (45–64 years old) and older (65–90 years old) adults. Data were subjected to statistical analysis by using descriptive and inferential methods. A significance level of 5% was considered in the later.
Results: In this sample, for 20–44, 45–64 and over 65 we had 20.3% ($n = 77$), 56.1% ($n = 213$) and 23.7% ($n = 90$) of chronic periodontitis, respectively (Table 1). Periodontal disease type distribution was found to be associated with age ($p = .043$).
Discussion and conclusions: The results show an association between age and chronic periodontitis extension and severity. The group 20–44 has a slight superior prevalence of mild forms of the disease whereas 45–64 and 65–90 has an increase in the prevalence of more serious types. This could be due to an age cumulative effect rather than age by itself. However, we point out the worrying high values of severe generalized chronic periodontitis among 20–44 year old patients.

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Table 1. Association between age and chronic periodontitis.

Age (interval)	Mild localized (%)	Moderate localized (%)	Severe localized (%)	Mild generalized (%)	Moderate generalized (%)	Severe generalized (%)	Chronic periodontitis (total) (%)
20–44	11 (14.3)	7 (9.1)	8 (10.4)	14 (18.2)	27 (13.0)	27 (35.1)	77 (20.3)
45–64	12 (5.6)	3 (1.4)	21 (9.9)	43 (20.2)	38 (17.8)	98 (45.1)	213 (56.1)
65–90	8 (8.9)	3 (3.3)	7 (7.8)	15 (16.7)	13 (14.4)	44 (48.9)	90 (23.7)

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Association between gender and chronic periodontitis in a Portuguese population

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Introduction: Periodontitis is a multifactorial disease with several known risk factors, such as: gender, smoking, alcohol, metabolic syndromes, diabetes, osteoporosis, stress and genetic factors [1]. According to the literature, male gender is a risk indicator for periodontal disease severity [2]. Furthermore, being male greatly increases the risk for periodontal disease [1]. The goal of this study was to evaluate the association between gender and periodontal disease prevalence in an adult Portuguese population.

Materials and methods: A random sample of 380 participants, aged between 20 and 90 years, was selected from patients who attended the Egas Moniz Dental Clinic (Monte de Caparica, Portugal), from September 2015 to June 2016. The American Academy of Periodontology (AAP) classification system was used to assess periodontal conditions [3]. Data were subjected to statistical analysis by using descriptive and inferential methods. A significance level of 5% was considered.

Results: The studied sample was constituted by 56.1% ($n = 213$) females and 43.9% ($n = 167$) males patients. Severe generalized chronic periodontitis prevalence is higher in men (53.9%, $n = 90$) than in women (36.2%, $n = 77$). In all other forms of disease, higher values of prevalence were found in women (Table 1). Periodontal disease type distribution was found to be associated with gender ($p = .008$). Although there are some controversial literature, in this population gender was a significant factor.

Discussion and conclusions: The results show a significant difference in periodontal disease type distribution by gender, with severe generalized chronic periodontitis condition being more prevalent in men.

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Table 1. Association between gender and chronic periodontitis.

Gender	Mild localized (%)	Moderate localized (%)	Severe localized (%)	Mild generalized (%)	Moderate generalized (%)	Severe generalized (%)	Chronic periodontitis (total) (%)
Female	24 (11.3)	8 (3.8)	22 (10.3)	47 (22.1)	35 (16.4)	77 (36.2)	213 (56.1)
Male	7 (4.2)	5 (3.0)	14 (8.4)	25 (15.0)	26 (15.6)	90 (53.9)	167 (43.9)

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Coadjuvant application of hyaluronic acid in non-surgical treatment of periodontitis – a split mouth study

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Introduction: The application of coadjuvants in the non-surgical treatment of periodontitis has been used to overcome the limitations of mechanical treatment [1]. The application of hyaluronic acid (HA) has been suggested as a potential local chemotherapeutic agent in the treatment of periodontitis due to its chemical properties: hygroscopic and viscoelastic; and its biological properties: bacteriostatic, biocompatibility, anti-edematous, antioxidant, osteoconductive, healing and regenerative capacity [2].
Materials and methods: This split mouth study included 17 subjects with chronic periodontitis. Plaque index (PI), gingival index (GI), bleeding on probing (BoP), probing depht (PD) and clinical attachment level (CAL) were recorded at baseline and 6 and 12 weeks after treatment conclusion. Both groups received scaling and root planning (SRP) in two random quadrants. In the test group an HA gel (Gengigel®, Ricerfarma, Italy) was applied immediately after SRP. Data were subjected to statistical analysis using descriptive and inferential methods with a significance level (α) ≤ 0.05
Results: At 12 weeks, a significant improvement in all clinical parameters was observed ($p < .05$) in both groups. In the test group, bleeding on probing was significantly reduced ($p < .05$) when compared with the control sites (9.16 ± 4.05 versus 14.61 ± 8.69). Despite the improvement in PD and CAL the difference was not statistically significant ($p > .05$).
Discussion and conclusions: The additional improvement in bleeding on probing was probably due to the anti-inflammatory and bacteriostatic properties of HA. Absence of significant differences in PD can be attributed to plaque control fluctuations over time or to the fact that only a single application was made [3,4]. Improvements in CAL may also be due to a reduction in the inflammation of gingival tissues [5]. The local application of 0.8% hyaluronan gel as an adjunct to SRP may have a beneficial effect in patients with chronic periodontitis.

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Prevalence of periodontal disease in a Portuguese population

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Introduction: Periodontitis is a chronic inflammatory disease caused by bacterial pathogens and modified by other factors, with 10% to 15% of adults having advanced forms of disease with significant attachment loss [1,2]. In Portugal, according to the latest Oral National Health Survey, 10.8% of adults and 15.3% of the elderly had periodontitis [3]. The goal of this study was to conduct a retrospective study in order to assess the periodontal condition of an adult Portuguese population.
Materials and methods: A random sample of 3458 individuals, aged between 18 and 90 years, was selected from patients who attended the Egas Moniz Dental Clinic (Monte de Caparica, Portugal), from September 2015 to June 2016. The diagnosis of periodontitis was made through a full mouth periodontal examination, including probing depth, recession and attachment loss evaluation. Periodontitis was classified according to criteria defined by Armitage (1999) [4]. Data were subjected to statistical analysis using descriptive and inferential methods. A significance level of 5% was considered.
Results: The prevalence of periodontitis was found to be 11.7% (CI 95% 10.6–12.8%). Chronic periodontitis (99.7%) was by far the most prevailing type, in contrast with aggressive periodontitis (0.3%). Among the group exhibiting chronic periodontitis, severe generalized was the most prevalent type (43.4%), followed by moderate generalized (19.1%), severe localized (15.9%), moderate localized (9.9%) and mild localized (7.9%). Mild generalized chronic periodontitis was the less prevalent type (3.5%).
Discussion and conclusions: In the studied population, the generalized (severe and moderate) forms of periodontitis were the most prevalent types. Interestingly, mild forms of the disease were less prevalent. Aggressive periodontitis cases where residual. The reported results are in agreement with the Portuguese Oral National Health Survey of 2015.

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Antibiotic regimens for medication-related osteonecrosis of the jaws: a retrospective study

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Introduction: Medication-related osteonecrosis of the jaws (MRONJ) is a rare but serious complication of antiresorptive and antiangiogenic treatment. Conservative management with antibiotics and analgesia is the recommended approach [1], but length of antibiotic treatment or drug associations are not set and no studies address this matter.
Objectives: (a) Describe the antibiotic regimens used and its length, (b) identify factors that may influence antibiotics prescription and (c) search for differences in treatment outcome according to the antibiotic regimen chosen.
Materials and methods: A retrospective study was made including all patients with MRONJ treated with antibiotics in an Oral Surgery Clinic between 2004 and 2016. Exclusion criteria was follow-up inferior to 3 months. Treatment outcome was defined as Healed/Improved versus. Stable/Worse. Chi-square, Student *t* test, Mann–Whithney *U* test and Kaplan–Meier analysis were used as appropriate. All analyses were performed using IBM SPSS for windows version 23.
Results: Sixty-two patients were included in the sample, 37 female and 25 male, with a mean age at diagnosis of 69.9 ± 9.93 years. Median time of administration was 271.5 days (range, 15–2081). The preferred first antibiotic regimen

was amoxicillin with clavulanic acid 875/125 mg in association with metronidazole 500mg in 37% of patients, amoxicillin with clavulanic acid 875/125 mg in 27.4% and amoxicillin 1000mg in 24.2%. 29% of patients were treated with only one type of antibiotic, 33.9% with 2 and 33.9% with 3 types of antibiotics. No differences in first antibiotic were found concerning base pathology, the presence of diabetes, number of comorbidities, presenting symptom or MRONJ staging at presentation. Median healing time seems to be longer for those who started treatment with amoxicillin 1000 mg (13 ± 7.96 months) than for those who started with the other antibiotics (7 ± 1.88 months) ($p = .210$). We found an association between starting treatment with amoxicillin and clavulanic acid associated with metronidazole and worse outcomes ($p = .04$). No differences in outcome concerning number of antibiotics were found.
Discussion and conclusions: As expected concerning the oral microbial flora and its resistance profile, the main antibiotics used for MRONJ are amoxicillin, amoxicillin and clavulanic acid and metronidazole, either alone or in association with each other. Clindamycin seems to be a second choice, probably due to its frequency of administration. More than 70% of patients were treated with more than one antibiotic and the median time of administration was 271.5 d, reflecting the severity of MRONJ and carrying out high costs and a negative impact on quality of life. Although AAOMS guidelines [1] suggest that the penicillin group of antibiotics is the mainstay for MRONJ treatment, isolated amoxicillin may be a bad choice for first antibiotic as it seems to be associated with a longer healing time. This may be due to resistance profiles of oral microbiota. Amoxicillin and clavulanic acid associated with metronidazole seems to be related with worse outcomes in MRONJ, that may reflect diverse degrees of severity at presentation. Antibiotic regimen guidelines should be defined in order to reduce treatment length, minimize antibiotic resistance and improve treatment outcomes.

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Actinomyces in medication-related osteonecrosis of the jaws: a retrospective study

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Introduction: Medication-related osteonecrosis of the jaws (MRONJ) is a multifactorial disease whose pathophysiology is not completely understood. *Actinomyces spp.* (AS) have regularly been found in MRONJ patients suggesting a role in pathogenesis and prognosis [1].
Objectives: (a) Describe the prevalence of AS in MRONJ patients sample; (b) identify differences in antibiotherapy length, healing time and treatment outcome according to presence of AS; (c) describe differences in AS positivity according to time of collection of sample.
Materials and methods: A retrospective study was conducted including all patients with MRONJ submitted to sequestrectomy or marginal resection during treatment in an Oral Surgery Clinic between 2004 and 2016. Exclusion criteria was follow-up inferior to 3 months. All bone samples were evaluated for the presence of AS by histopathological analysis. Treatment outcome was defined as healed/improved versus. Stable/Worse. Chi-square, Student *t*-test, Mann–Whithney *U* test and Kaplan–Meier analysis were used as appropriate. All analyses were performed using IBM SPSS version 23.
Results: Fifty patients were included in the sample, 34 female and 16 male, with a mean age at diagnosis of 68.08 ± 11.38 years. The MRONJ lesion was located in the mandible in 32 patients and in the maxilla in 18 patients. AS was identified in 35 patients (70%), 15 in maxilla and 20 in mandible. Median healing time was longer in patients with AS (360 ± 89.87 days) than their counterparts (150 ± 36.66 days) ($p < .029$). Mean time of antibiotic administration was 493 ± 486.25 days in patients with evidence of AS and 352.30 ± 343.71 days in those without it, although no statistically significant differences were found ($p < .41$). Healed/Improved was achieved in 68.6% of patients with evidence of AS and in 73.3% of their counterparts with no statistically significant differences. Mean time of sample collection since diagnosis of MRONJ was 340.34 ± 466.54 days for those AS positive and 166.33 ± 204.60 days for those AS negative ($p < .179$). Regarding time of collection sample from MRONJ diagnosis, AS was found in 50% (4) of those submitted to surgery within the first month, 72.7% (16) between 1 and 6 months and 75% (15) over 6 months.
Discussion and conclusions: AS was present in most patients as expected from previous studies. Patients with MRONJ lesions located in the maxilla showed a higher prevalence of AS, which may reflect the involvement of anatomical factors. Patients with AS had a longer healing time although there were no statistically significant differences in treatment outcomes and antibiotherapy length. Moreover, AS was present in half of patients that were submitted to surgery within the first month and showed a trend to increase in patients that were operated later. Therefore, the presence of AS in MRONJ lesions does not have a significant impact on treatment outcome and colonization of necrotic bone appears to occur in

an early phase, increasing over time. As an opportunistic agent, AS might not have a significant role in MRONJ pathogenesis and rather be a consequence of prolonged bone exposure.

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Use of transillumination as a diagnostic aid in oral health surveys

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Introdution: The diagnosis of caries is a complex process that involves the interpretation of a set of clinical data and complementary medical exams. Fiber-optic transillumination is increasingly considered as an additional support in epidemiological surveys, where the radiological examination is not available [1,2]. Our objective was to evaluate the performance of visual methods and fiber-optic transillumination (FOTI) in screening of tooth decay in epidemiological studies.
Materials and methods: The study included 68 children (boys and girls with mixed dentition) with ages between 7 and 10 years old, from the elementary school of Valado dos Frades. The observer has been subjected to a prior calibration process. In this process there was uniformity regarding the type of ambient lightning, the examineŕs position and the child examined. The oral cavity observation of each child was made by using a disposable basic observation kit (exploratory probe, mirror and tweezers) and a light device, FOTI (Proface WH®). The first observation was carried out according to the visual inspection, and the second using the transillumination device. A document containing an international dental file chart was filled, after each one of the observations, in order to compare the results.
Results: Six hundred and four deciduous teeth and 983 definitive teeth were observed in this study. The visual method detected 129 dental lesions in deciduous teeth and 36 lesions in the definitive dentition. FOTI detected 173 in the first ones and 61 in the second ones.
Discussion and conclusions: As in other studies, FOTI revealed to be more sensitive than the visual method, specially detecting proximal carie lesions. FOTI could be also considered as a good alternative to X-ray since there is no radiation involved. Additionally, FOTI present several advantages as: is easy to use, can be used repeatedly without risk to the patient and is useful in epidemiological surveys, when there are no other auxiliary diagnostic methods available.

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Imaging assessment of the effect of decompression of odontogenic cysts – preliminary results

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Table. 3D and 2D measurements.

	3D volumetric reconstruction			2D axis reductions (%)			
Patient	Initial V (cc)	Final V (cc)	↓V (%)	↓CC (%)	↓AP (%)	↓T (%)	Months
1	3.4	2	41	9.6	14	20	8.2
2	10.1	3	70	11.2	50.2	24.5	14.9
3	8.7	3.8	56	3.9	19.9	33.8	9.4
4	1.9	0.5	74	43.3	34.8	40.2	8.6
5	5.7	1.3	77	39	19.2	29.9	7
6	24.6	11.9	52	4.3	21.2	11.9	7.2

Introduction: Odontogenic cysts are one of the most frequent maxillary lesions [1]. Large lesions, proximity to vital structures or risk of bone plate perforation strongly recommend decompression followed by cyst enucleation. The decrease of intracystic pressure allows reduction of lesion volume and favors bone growth [1,2]. As cysts are irregular three-dimensional lesions, the aim of this study is to compare 3D and 2D methods in assessing the effect of decompression of odontogenic cysts.
Materials and methods: This ongoing study includes patients with maxillary odontogenic cysts who underwent decompression followed by enucleation. Helical computed tomography was performed at diagnosis and at the end of decompression stage. The widest axis of the lesions were measured in the three planes (craniocaudal (CC) in coronal plane, anteroposterior (AP) in sagittal plane and transversal (T) in axial plane), using Sectra IDS7® workstation measuring tool. Volume measurement was performed using Medtronic StealthStation Surgical Navigation System® software, designed to build three-dimensional models of lesions.
Results: Until now, this study comprises 6 patients (4 male and 2 female), 3 inflammatory cysts and 3 keratocysts. The mean volume (V) of lesions was 9.1 ± 7.5cc at initial diagnosis and 3.75 ± 3.8cc at the end of decompression. The mean percentage reduction of volume was 62 ± 13%. The mean percentage reduction of CC, AP and T axis were 18.55%, 26.55% and 27%, respectively.
Discussion and conclusions: The cyst reduction accomplished with decompression shall not be neglected. The mean percentage of reduction of any of the axis is utterly inferior comparing to the mean percentage of reduction of the volume, thus wrongly discrediting the former to assess decompression effect. We believe this misevaluation of the real effect of decompression may compromise the choice for this type of treatment.

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Inflammatory radicular cyst: clinical case

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Introduction: Radicular inflammatory cyst associated to primary teeth is an injury resulting from pulpal necrosis or pulpectomy treatment. Commonly, the cyst involves the apex of the affected tooth and represents a prevalence of 0.5–3.3% [1,2]. The majority of patients are affected in first decade and in the early second decade of life, the girls have higher incidence than boys, as well as the mandible when compare to the maxilla [1,3,4]. Is usually an asymptomatic lesion detected by routine radiography [2]. The aim of this study is to present a clinical case of a young patient, who was diagnosed, by radiography a radiolucent image associated to a primary tooth with pulpectomy treatment, with a follow-up treatment of six months.
Materials and methods: A 7-year-old girl was referred to Paediatric Dentistry by National Health System to treat a carious lesion on the 52, primary incisor tooth. Intraoral examination revealed buccal cortical plate associated a first right lower primary molar – 84. The tooth had been restored with intermediate restorative material, and had no mobility and no pain. Panoramic and periapical radiographs revealed pulp therapy, well-defined unilocular radiolucency involving the interradicular area and a delay in the development of the successor tooth – 44. After obtained informed consent, the cyst was enucleated along with the involved primary tooth and was sent for histopathologic examination. Surgical exploration confirmed the non-association of the cyst to the successive permanent tooth.

Figure 1. Postreatment diagnosis exams.

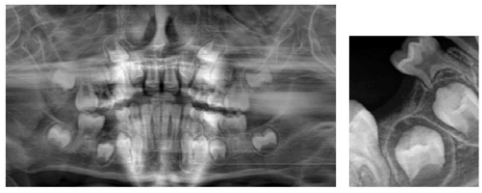

Figure 2. Palatine exposure by a surgical closed technique, traction using a cantilever and fixed appliance.

Results: The histopathological result confirmed the diagnosis of inflammatory radicular cyst. Six months after the treatment, a new radiograph evidenced the disappearance of the lesion, bone formation and a spontaneous development of the definitive successor tooth. A good soft tissue healing was observed in the clinical examination.
Discussion and conclusions: A relationship between intracanal medicaments used for pulp therapy and intraepithelial inclusions in the cystic walls, which might provide a site for continuing antigenic stimulation had been proposed [3]. In the majority of cases the clinical findings are expansion of buccal cortex of the affected tooth, delayed permanent tooth development, radiolucent unilocular lesion with smooth and well-defined borders and extending in the periapical area of primary tooth [1] which match with the clinical findings on the presented case. We conclude that in these cases, pulp therapy did not a good prognosis in agreement with the published literature, demonstrating the importance of periodic radiographic control [3,5].

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Radiopaque mandibular lesions observed in the diagnosis consultation of Egas Moniz University Clinic

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Introduction: The heterogeneity of radiological lesions in the mandible is a frequent reality in the daily clinical practice. These lesions can be considered complex in terms of etiology, clinical behavior, aggressiveness and recurrence rates and

may represent variable malignant potential. Awareness of the demographic distribution of these lesions and their associated clinical features, as well as the radiologic approach, is important to explore radiopaque jaw lesions [1]. The objective of this study was to statistically analyze mandibular radiopaque lesions observed in orthopantomography during the diagnosis consultation at Egas Moniz university clinic.
Materials and methods: 1460 random ortopantomographies of patients who attended the diagnosis consultation at Egas Moniz university clinic were observed. Frequency tables were organized according to the diagnosis and clinical/radiological aspects of each case. The results were gathered in classes, according to radiological diagnosis: (1) remaining intrabony root fragments, (2) focal sclerosing osteomyelitis, (3) impacted supernumerary teeth, (4) static osseous cysts, (5) cementoblastoma/hipercementosis and (6) remaining dental amalgam fragments.
Results: About 112 patients (7.67%) presented radiologically radiopaque mandible lesions observable in orthopantomography. Radiopaque remaining intrabony root fragments were the most frequently found (46 cases or 41.08%.) Focal Sclerosing Osteomyelitis/Focal Petrosis (41 cases or 36.60%) was most frequently observed in the right side of the mandible (23 cases versus 18 on the left side). Impacted Supernumerary Teeth (8 cases or 7.14%) were most frequently observed on the left side of the mandible (6 cases versus 2 cases). Cementoblastoma/Hipercementosis lesions (9 cases or 8.04%) were most frequently observed associated with tooth #45. Remaining amalgam fragments (8 cases or 7.14%) were most frequently observed on the #36 and #46 teeth (3 cases each).
Discussion and conclusions: Radiopaque findings in the mandible are of the upmost importance. Therapeutics for mandible radiopaque lesions may vary according to the diagnosis. Early diagnosis is clinically fundamental. Decision making for the clinical management of these lesions depends on several factors such as radiological diagnosis, growth rate, size and anatomical location.

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Tongue carcinoma: the role of the dentist in early diagnosis

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Introduction: Tongue carcinoma is the most common malignancy of the oral cavity and its incidence has increased. This study aims to characterize patients diagnosed with oral tongue carcinoma at a tertiary referral centre, through a descriptive study and ratio of bio-psycho-social variables such as age, gender, TNM staging, grade of cellular differentiation, type of surgery, adjuvant treatment and survival rate. It also aims to emphasize the importance of the dentist in early diagnosis and subsequent prognosis of such tumours.
Materials and methods: This is a retrospective and comparative study of two periods, carried out at the tertiary referral centre. The statistical analysis of 313 patients diagnosed with oral tongue carcinoma in the years of 2004/2005 and 2014/2015 were performed. For this, we used the Software Package for Social Sciences Software to characterize the sample, correlate variables and make survival curves.
Results: A higher incidence of cases was found in males aged $\geq$65 years, for both periods. The most common histological type was squamous cell carcinoma and, at the time of diagnosis, most of the tumours were at stage IV. The number of early stages (I and II) had increase and the number of advanced stages (III and IV) decreased when compared 2004/2005 with 2014/2015.
Discussion and conclusions: Tongue cancer remains the most common cancer of the oral cavity. Frequency did not decrease when comparing the last decade. The stage they are diagnosed is still the highest (IV), referring to the importance of early diagnosis of oral cancer.

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Contact endoscopy – an adjuvant tool for oral cancer early diagnosis

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Introduction: Potentially malignant oral lesions have a high risk of carcinogenesis. Morbidity after treatment and cancer mortality increases substantially in advanced stage tumors (T3, T4), making early diagnosis highly beneficial. The authors studied the role of contact endoscopy in the diagnosis of oral mucosa malignancy and compare it to conventional biopsy histopathology.
Materials and methods: A prospective study of 148 consecutive patients with oral mucosal lesions was carried out. Patients were assessed with contact endoscopy in outpatient environment during routine procedures. Results were compared to histopathology biopsy. The informed consent of the subjects and acceptance of the study protocol by a local ethics committee has been obtained.
Results: Contact endoscopy revealed a sensitivity of 0.961 (CI 95% −0.811 to –0.993); specificity 0.972 (CI 95% −0.922 to −0.990); predictive positive value 0.892 (CI 95% −0.728 to −0.962); negative predictive value 0.990 (CI 95% −0.949 to −0.998); positive likelihood ratio 38.02; negative likelihood ratio 0.03; accuracy of 0.970 (CI 95%: 0.926 to 0.988); kappa index 0.908 ($p < .001$).
Discussion and conclusions: There were three false negative results in our study. Two of them proved to be, in fact, malignancies after total surgical excision. In these cases, contact endoscopy proved to be superior to conventional biopsy in diagnosing oral cancer. The third case was a non-epithelial submucosal cancer which could not be identified by a surface technique as contact endoscopy. Although some false positive results may occur, combining clinical assessment and contact endoscopy observation can reduce undiagnosed early stage tumors. This study proves that contact endoscopy is a useful diagnostic tool to identify early oral mucosal malignancy.

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Implications of Parkinson disease in oral health

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Introduction: Parkinson's disease (PD), described for the first time 200 years ago, is the second most prevalent neurodegenerative disorder [1] and is increasing in developed and developing countries due to the increase of life expectancy.

This disease is characterized essentially by motor function disorders, such as resting tremor, muscular rigidity, bradykinesia and postural instability and has implications for performing daily life activities, namely, in oral hygiene [2]. Parkinson patients have dysphagia, sialorrhea, xerostomia, burning mouth syndrome, olfactory dysfunction and difficulty in using and adapting to dental prosthesis worsen by cognitive impairment and dementia. In order to treat them properly, the dentist should have the knowledge and competence to identify all signs and symptoms that these patients can develop in their oral cavity and be aware of the adverse effects of the medicines used for the treatment of the disease. The main objective of this study was to integrate and summarize the implications of Parkinson disease in oral health.

Material and methods: Concerning the collection of data for this review, the PubMed database of the U.S. National Library of Medicine was used as the main electronic database. A systematic search of articles published up until May 2016 was performed for the subjects "Parkinsońs Disease" AND "Oral health". Publications written in Portuguese, English or Spanish were included and Mendeley software was used for electronic title management. Seventy-three documents were carefully evaluated and the most important findings related to the oral health alterations in PD are summarized below.

Results: About 9–77% of PD patients have dysphagia [2] which can result in 32–74% of PD patients [3] having sialorrhea due to an incapacity to maintain a closed mouth because of muscle hypotonia and oesophageal reflux enhancing dental erosion and angular cheilitis [4]. However xerostomia (dry mouth) is also frequent in PD patients, and facilitate dental caries, periodontal disease and oral discomfort [4]. The burning sensation is five times more prevalent in PD patients and is related to pharmacological treatment (levodopa). Others factors for bad oral hygiene is a loss of manual dexterity and cognitive dysfunction.

Conclusion: The collaboration between the dentist and the caregiver (formal and/or non-formal) and his experience and skills is essential to obtain a cared supervision in oral and personal hygiene during the course of the disease and improve the patient's quality of life. It would be interesting to evaluate the effect of an early accompaniment and its consequences on the oral complications of these patients but for this, health professionals must be informed about the multiple alterations in oral health revised in this study.

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Impacted maxillary canine – clinical case

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Introduction: Apart from third molars, upper canines are the most frequent impacted teeth [1–3]. The aetiological factors are not clearly defined, but it is believed that there is a polygenic and multifactorial cause. They have a key role in dental and facial aesthetic and in a stable and functional occlusion [4]. Given its importance, it emerged over time the need to develop therapeutic techniques to solve this dental inclusion adopting a conservative attitude towards the positioning of the tooth in the arch. Early diagnosis is essential because the younger the patient the higher the success rate [3]. The purpose of this work is to present a clinical case of impacted maxillary canines, whose clinical approach consisted of orthodontic-surgical traction, with a nine months follow-up.

Materials and methods: A 13-years-old female patient came to Egas Moniz orthodontic clinic, with the main complaint of having primary teeth that have not fallen yet. After clinical examination, it was observed the presence of primary maxillary canines with no mobility and no exposure of definitive maxillary canines. The radiographyc analysis showed palatally impacted canines. After obtained informed consent, the treatment consisted of palatine exposure of canines by a surgical closed technique and its traction using a cantilever, followed by fixed appliance.

Results: After performing a surgical closed technique for the impacted teeth exposure, followed by traction with a cantilever and fixed appliance, it was possible to place both canines in the arch.

Discussion and conclusions: According to several authors, palatally impactation of upper canines is reported as the most frequent, raging among 83% and 85% [3,4], with a rotation between 60° and 90° of their long axis and with a mesial orientation [5]. The surgical exposure of these cases has been one of the treatments of choice [1] when patient's age does not allow a preventive intervention. It can be performed by a closed or open approach. The surgical close

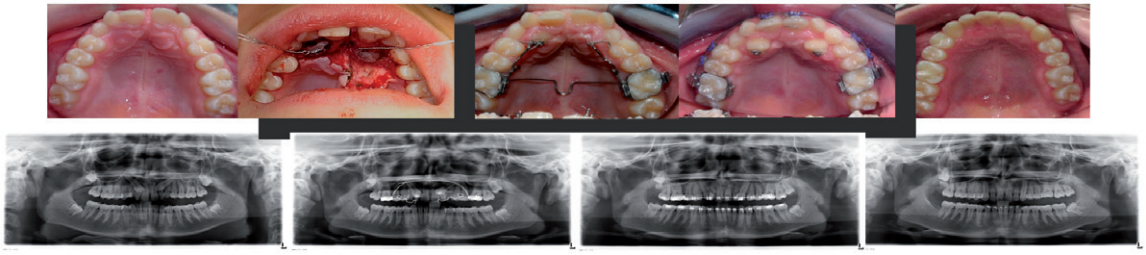

Figure 1. Palatine exposure by a surgical closed technique, traction using a cantilever and fixed appliance.

technique, reported by most authors being associated with an orthodontic traction system, namely cantilevers (which cause initial extrusion forces and crown verticalization), allow a correct alignment, without traumatizing the periodontium and the adjacent teeth [3,5]. In this case, using the technique described above, it was possible to correctly position the canines in the arch without damaging any hard or soft tissue, obtaining a good stability during the nine months follow up.

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Salivary flow rates in young adults in relation to tobacco and oral contraceptive

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Introduction: Saliva plays a vital role in maintaining oral homeostasis. Low salivary flow, or hyposalivation, leads to drying of the oral mucosa, demineralization of dentition, mucosal ulceration, altered oral flora, dysphonia, impaired taste and smell while eating, and discomfort [1]. It is well known that salivary flow rate and saliva's composition may be influenced by exercise, caused by rapid breathing and sweat-induced dehydration [2]. However, the effects of tobacco consumption on some salivary parameters are controversial, some studies reported a decrease in flow rate and pH in exclusive cigarette smokers (ECS) when compared with non-smokers, another study showed similar results between the two groups. Tobacco smoking increases the occurrence of dental caries. One study showed a salivary buffer effect higher, in oral contraceptive users than that seen in non-users, who resembled male controls [3]. The aim of the study was to measure salivary flow rates in young adults; correlate salivary flow rates with tobacco and oral contraceptive; correlate salivary flow rate with xerostomia.
Methods: Several students from ISCSEM, during 2015–2017 were invited to participate voluntarily in a saliva project. Informed consent was signed according to the declaration of Helsinki. Oral complains of xerostomia were asked as also habits of smoking and medical history. Saliva was collected during classes. Saliva volumes were estimated by weighing to the nearest ml. Flow rate was calculated as the volume of saliva collected divided by the collection time. 146 students satisfied the following inclusion criteria: (a) sentistry students, (b) age $\geq$20, (c) criteria for hyposalivation were non-stimulated salivary flow (NSSF) $\leq$ 0.2ml/min and stimulated salivary flow (SSF) $\leq$ 0.5ml/min. A pre-established plan recurring to a statistical package (version 22.0; SPSS Inc., Chicago, IL) was employed to analyze all data.
Results: About 66.7% were women, 27.2% were smokers, and the mean age was 22.6; the prevalence of hyposalivation was 5.5% ($n = 8$), non-reported xerostomia; from the group with hyposalivation five students were smokers, 2 took at

least one xerostomic medication, 4 took oral birth control pill. Mean salivary flow rates were NSSF = 0.38 [0.0–1.60] ml/min and SSF = 1.76 [0.25–5.50] ml/min, no significant differences were observed between genders or ages. A weak negative correlation was found between NSSF and oral birth control pill ($r = -0.057$) and SSF and oral birth control pill ($r = -0.094$).
Discussion and conclusions: Factors like hormone alterations and medications could lead to alterations on salivary flow rates, although in this study no strong correlations were found between medications and salivary flow rate. This could be explained because the students in this study were generally healthy. No statistical differences were observed in salivary flow rates between genders or ages, this is according with several studies. Salivary flow rates are within normal values for this population. The prevalence of hyposalivation was very low, non-reported xerostomia. Birth control pill and tobacco does not impact significantly at salivary flow rates in this age group.

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The efficacy of the *Cinnamomum burmannii* aqueous extract in the reduction of halitosis of intra oral cause, in young adults: a pilot clinical study

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Introduction: Halitosis is still poorly studied in adolescents and young adults albeit being considered as an important oral health issue for these groups [1]. The research on halitosis of intra oral cause has been directed to the plaque that is fixed on the tongue and for the existence of food remains, which are retained in the interproximal spaces, being generally caused by anaerobic oral micro-organisms [2]. The aim of this study was to explore the effect of *C. burmannii* aqueous extract in terms of production of Volatile Sulphur Compounds (VSC) compared to Chlorhexidine 0.06%, over 7-d rinsing period.
Materials and methods: The study was approved by the Ethics Committee for Health, of Egas Moniz. 30 individuals, exhibiting halitosis, were randomly selected, from the 5th year of the Dentistry Master degree, at ISCSEM. All subjects presented halitosis values $\geq$75 ppb (Halimeter®) and $\geq$2 (organoleptic tests) and were divided into three groups. Each group ($n = 10$) performed the mouthwash (15 ml, 2 times a day, for 1 min) during 7 d: Group A – *C. burmannii* aqueous extract (3 g/mL); Group B – *C. burmannii* aqueous extract (6 g/mL) and Group C – Chlorhexidine-based antiplaque agent 0.06% (G.U.M. Sunstar Paroex®), acting as negative control. All individuals were instructed to perform an oral hygiene protocol and on how to apply the respective rinse. The values of Halimeter® levels (ppb), were taken before the first rinse ($t = 0$ min), 15 min after the first rinse (mask effect) and after 7 d (therapeutic effect). Data were analyzed by using descriptive and inferential statistics methods. A significance level of 5% was considered for the later.
Results: At $t = 0$ min, the mean levels of halitosis, in Groups A, B and C, were, respectively: 100.0 ± 95.6; 127.5 ± 119.8 and 153.0 ± 90.6. At $t = 15$ min: 68.0 ± 70.6; 82.1 ± 72.9 and 67.5 ± 49.0. At $t = 7$ d: 61.11 ± 1.84; 79.40 ± 38.35 and 47.7 ± 42.9. Halitosis levels reduction with time was statistically significant for all Groups (A, $p = .008$) (B, $p = .045$) and (C, $p = .001$).
Discussion and conclusions: The mouthwash with chlorhexidine, considered the gold standard, showed better results in reducing halitosis levels; however it is associated with some reversible side effects. The mouthwashes containing *C. burmannii*, showed that their time-effectiveness was not dependent on concentration, however only *C. burmannii* 3 g/mL aqueous extract rinse was effective in reducing halitosis below the level of 75 ppb, which for this type of aqueous extract constitutes a new finding.

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Direct accuracy measurement through repeatability of an intraoral scanner: a clinical study

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Introduction: Digital impressions are becoming more and more popular over the years much due to a clear evolution of the digital Dentistry market [1]. The use of intraoral scanners came to simplify the whole process of impression taking, bypassing and eliminating the need of pouring casts and preventing errors resulting from the impression material's properties, its distortion or even the operator's technique [2]. This improves workflow, patient satisfaction and reduces the total appointment time leading to a more time-efficient technique [3]. In order to fabricate restorations with a precise fit, a high level of accuracy of the impression is needed [4]. The main goal of this study was to clinically assess the accuracy and repeatability of the intraoral scanner 3M^{TM} True Definition Scanner (3M TDS).

Materials and methods: A sample of nine patients received a weekly intraoral scan with the 3M TDS for 12 weeks. The virtual models obtained were imported into a 3D analyzer software Geomagic Control 2014. Corresponding models from each patient were aligned in order to assess the accuracy and repeatability of the scanner. In the statistical analysis, one-way ANOVA, two-way ANOVA, Mann–Whitney test and a regression analysis with $p \leq .05$ (Minitab V17) were used.

Results: The 3M TDS showed high repeatability over time (94% of dots are repeated in a three-dimensional comparison with a deviation of 30 μm). Regarding the dental arch factor, the upper arch showed a better accuracy when compared to the lower. The patient is one of the factors responsible for the variability; however, the maximum interincisal distance does not influence the reproducibility. Over 77% of the observed variability is explained by the combination of the quadrant and the patient's individual factors (ex. tooth individual morphology).

Discussion and Conclusions: The acquisition of a reliable and accurate impression is a crucial step for the manufacturing of precise dental restorations. Regarding trueness and precision, digital impressions have been compared to conventional ones recently [5]. The main aim of this study was to clinically assess the precision through repeatability of the intraoral scanner. The 3M TDS showed high accuracy and repeatability, proving it appropriate to be used for clinical application.

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Subjective interpretation of articulating paper markings cannot accurately determine occlusal force

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Introduction: Subjective Interpretation of articulating paper markings is the most commonly employed method to select tooth contacts for occlusal adjustment even though, no study data has been published that illustrates its accuracy [1,2]. Articulating papers sensitivity and reliability depends on the thickness, strength, ink substrate, plastic deformation and oral environment thus, related to a high prevalence of false positives and negatives. Additionally, its clinical interpretation is based on a long-standing concept that mark size is representative of the load contained within the mark, even though no scientific evidence supports this [3]. Maness et al. [4] first reported (in 1987) the development of the prototype of a new computerized occlusal analysis device (T-Scan® Tekscan Incorporation, Boston, MA). To date, the manufacturer states having improved the system's accuracy, sensitivity and reproducibility [5,6]. The aim of this study was to determine whether Subjective Interpretation of articulating paper markings is a reliable method for clinicians to employ when determining occlusal contact relative force content and timing.
Materials and methods: One hundred and seventy-one Dental Professionals and 5th grade students of the Integrated Master in Dentistry were asked to participate anonymously in an online "picture observation" test. Figures showing various occlusal compositions were presented (natural, acrylic and ceramic teeth) with articulating paper marks and the subjects were instructed to select the corresponding contacts that they thought reflected the "most forceful", "least forceful" and "the first" contact through the employment of Subjective Interpretation. The relative occlusal force and timing was determined by performing digital occlusal analysis with T-Scan®III HD. Four qualifying questions characterized the clinicians in relation to their clinical practice, continuing education courses and predominance of clinical practice in Oral Rehabilitation. The diagnostic validity of articulating paper to assess determination of force was measured performing accuracy tests (sensibility and specificity scores).
Results: The overall score of correct answers was 24.6%; there wasn't found statistical differences between years of experience, number of courses taken nor predominance of clinical practice in Oral Rehabilitation and it was determined a sensibility of 34% for the method of visual determination of force.
Discussion and conclusions: Our results suggest that the use of articulating paper doesn't improve with clinical experience and its subjective interpretation proved to be an unreliable method to guide tooth selection prior to occlusal adjustments.

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T-Scan III® HD sensors' accuracy influenced by *in vitro* occlusal simulation of anterior and posterior teeth

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Introduction: In 1987, the World Health Organization (WHO) emphasized the importance of reliability in clinical measurements regarding oral health care [1]. With this in mind, many investigators have been researching the performance of registration materials and methods in an effort to thoroughly understand the patient's occlusion [1–3]. Modern technology has conquered an important position in daily clinical practice; however, reliable studies on these devices' performance are not always present. The present study aims to test the sensitivity and accuracy of a computerized occlusal analysis system, under different *in vitro* simulated anatomic circumstances.
Materials and methods: Four different occlusal situations were reproduced: a 180 degree table (plane surface) for static and variable sensor placement; a 100 degree table (simulation of the occlusion in anterior teeth) and another of 120 degrees represented by an artificial inferior first molar. Three levels of force (10N, 50N and 150N) were applied, 40 times each, by a universal testing machine (Autograph, AG-I; Shimadzu Co.) with a spherical bur (Ø = 2.2mm) on the sensor film. One-way ANOVAs with Bonferroni corrections for post-hoc tests were used for multiple comparisons ($\alpha = 0.05$) of the acquired RAW-sum values.
Results and discussion: Graphically and statistically sustained differences ($p < .05$; $F = 57.129$) in the recorded data were found, only when the sensor's position was switched between closures. The anatomic morphology did not influence the sensor's sensitivity. However, its accuracy proved to be dependent on the anatomic circumstances and the produced contact area.
Conclusions: The anatomic circumstances did not influence the sensor's sensitivity, but they did influence its accuracy. Further studies on the varying sensitivity throughout its sensing surface are required. While recording, the sensor must be well secured in place in order to obtain useful data. The clinician must be aware that very low forces might not be accurately depicted.

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Internal sinus lifts in the implantology consultation at Egas Moniz University Clinic

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Introduction: After tooth extraction, the bony width loss is due to the resorption of the buccal plate. As the atrophy progresses, a loss in bone density and height are observable, as well as antral pneumatization [1,2]. For these reasons, the proximity between sinus floor and alveolar crest is frequently short [3]. Summers introduced a surgical technique using osteotomes that is indicated when the subsinus residual bone height is between 5 and 10 mm [4]. After progressive preparation of the bone, elevation of the floor of the sinus by several millimeters in height is obtained. The objective of this study is to quantify the internal sinus lifts performed at the implantology consultation Egas Moniz University Clinic, between June 2012 and July 2016.

Materials and methods: Data collection from the surgical records between June 2012 and July 2016. The data were grouped according to sex (male or female), the side of the sinus lift (left, right or bilateral) and by type of rehabilitation intended (partial or total) into frequency tables. Approximations were made to the hundreds.
Results: Sixty-one internal sinus lifts were performed, from which 40 (65.57%) were performed in female patients and 21 (34.43%) were performed in male patients. Two bilateral internal sinus lifts an 51 unilateral sinus lifts were performed. In 78.69% (47), these procedures were performed for partial rehabilitations whereas the remaining 21.31% (14 cases) were performed for full upper arch rehabilitation. No implant placed in these cases was lost (0%).
Discussion and conclusions: Sinus pneumatization as a consequence of edentulism, as well as bone height loss, are clinically challenging to the implant based rehabilitation of this region. The correct use of this technique was successful in both partial and full arch rehabilitations. Internal sinus lifts allow predictable implant based rehabilitations of the posterior maxila. Low bone height due to sinus pneumatization is not a contraindication for implant-based rehabilitations in the posterior maxilla. Clinically, this technique allowed for patients to obtain fixed or hybrid restorations in an anatomical area where, otherwise, only removable prosthesis would be possible.

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Partial dental users at the Egas Moniz University Clinic

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Introduction: Several prevalence studies indicate that the type of tooth loss is associated with multiple factors, like age, gender and sociocultural factors [1–3]. Despite improved healthcare, 37% of the Portuguese population have more than 6 teeth missing [4]. With the exception of the study by Carneiro (2013) carried out in Oporto, there is a lack of Portuguese studies that address this issue. Our goal is to characterize the partial edentulism and the removable partial dentures (RPD) in a sample of patients treated at Egas Moniz University Clinic in Almada.
Materials and methods: All clinical charts (240) of the prosthodontic clinic between September 2014 and June 2015 were evaluated and characterized by: age, sex, Kennedy classification and type of rehabilitation made. A descriptive statistical analysis with crosstabs procedures was applied to check frequencies using the SPSS Statistics 20.0 software
Results: Seventy-three records with fabricated RPD were selected. A total of 95 RPD were planned and delivered. Average age was 59 ± 30 ith the women being younger and the largest gender 68.4%. Kennedy class III was the most common (43%) while class IV the most uncomon (3.2%). Most patients had mandibular gaps (52.6%). Cobalt chrome alloy (CCA) was the most prevalent type of infrastructure (55.8%) and most of them were made on Kennedy class III. Number of teeth replaced by acrylics (7) was higher than Cr-Co (5) regardless of gender.
Discussion/conclusion: Patients who sought oral rehabilitation treatment had an average age of 59 years old. Of these 68% were women. Similar findings were found by Carneiro in Portugal [1], Souza in Brasil [3] and Pun in Greece [8]. Average number of teeth replaced by acrylics was higher (2 more) regardless of gender. The prevalence of edentulous class was Kennedýs class III (43%) and the lowest class IV (3%), similar to the findings of other authors [2,5]. Most of edentulous arches are in the mandible (53%) like several authors [2,3,5,6]. Of the 95 prostheses fabricated, 49 were Cr-Co (56%) and 46 Acrylic (44%) similar to the results of Pun and Polychronakis [7,8].

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Black Merino Sheep: suitable animal model for temporomandibular joint interposal material research

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Introduction: Appropriate animal models are essential to progress in temporomandibular joint (TMJ) interposal materials research [1]. Failures of previous investigations regarding TMJ interposal materials can be related to the use of non-appropriate animal models. Although no animal model can fully replicate human conditions, Black Merino sheep have revealed anatomical, histological and biomechanical resemblance to human TMJ [2]. The main goal of this investigation was to test the Black Merino sheep feasibility to be used in a rigorous preclinical randomized controlled trial respecting ARRIVE guidelines [3].
Materials and methods: A total of 18 adults, female *Black Merino* sheep in good health condition were included in Temporomandibular Joint Interposal Material Study (TEMPOJIMS) [4]. In this randomized, blinded, preclinical trial the authors tested the effect of: bilateral discectomy, bilateral discopexy, three different interposal biomaterials compared in sham surgery control group. The outcomes evaluated were (1) histologic changes; (2) imaging changes; (3) sheep body weight and (4) kinematics jaw movement.
Results: The surgical access and anatomy of TMJ was very similar to human with a preauricular approach. The pioneer masticatory kinematics analysis was evaluated. All the animals survived to study and no adverse events were reported. All the proposed outcomes were possible to measure in *Black Merino* sheep.
Discussion and conclusions: Previous preclinical studies in TMJ domain used contralateral TMJ as control in heterogeneous animal models with no rigorous study design. The use of bilateral intervention, with a rigorous preclinical trial in Black Merino Sheep was an effort to reduce bias on this study. The kinematics evaluation was a challenge. The animal was able to stand peaceful in front of the video cameras for the kinematics evaluation. All the animals survived bilateral interventions and no adverse events were noticed. All the outcomes proposed were feasible in Black Merino sheep animal model introducing a new challenge in TMJ preclinical trials.

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Figure 1. TEMPOJIMS kinematics evaluation.

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TMJ distraction device for patients with arthrogenous TMD – the project

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Introduction: Temporomandibular Joint (TMJ) disk displacements can be effectively reduced through condylar distraction movement [1,2]. Currently there is no device capable of joint distraction, so in cases of disk displacement, with the presence of retrodiscal pain, patients often need a large number of physical therapy sessions to increase mobilization of the joint [3].
Materials and methods: The authors plan to perform 4 major tasks. First, the study of existing devices on the market for mandibular therapy. Second, the conception, design and manufacture of a functional prototype device. This task includes a first step with 3D geometry obtained from image segmentation technique and reverse engineering, followed by the development and optimization of a conceptual 3D geometry for the distraction mechanical device, based on numerical simulation of the mechanical behavior of the device. Finally, the manufacture of the functional prototype. Third, the legal process with the Infarmed for classification and use of this medical device for clinical research. Fourth, the clinical test in patients with temporomandibular disorders (subgroup of the condyle-disc complex dysfunctions) divided in two groups: the conventional therapy group (manual distraction performed by the physiotherapist in office) and the test group with manual distraction performed by the physiotherapist in office and condylar distraction made by the patient with the condylar distraction device in an ambulatory regimen.
Results: The preliminary results led to an ultralight device with a mechanical behavior suitable for the desired objectives. The finite element model, based on linear static analysis, with tetrahedral solid elements (10 nodes and three degrees of freedom at each node), allowed to evaluate comparatively the behavior of the device for several types of materials, showing an adequate strength and stiffness.
Discussion and conclusions: Numerical simulation and initial tests of the functional prototype device are promising. The development of an apparatus capable of assisting patients in condylar distraction maneuver could be a useful adjunct in treating these diseases by increasing patient compliance and reducing costs associated with currently longer treatment times.

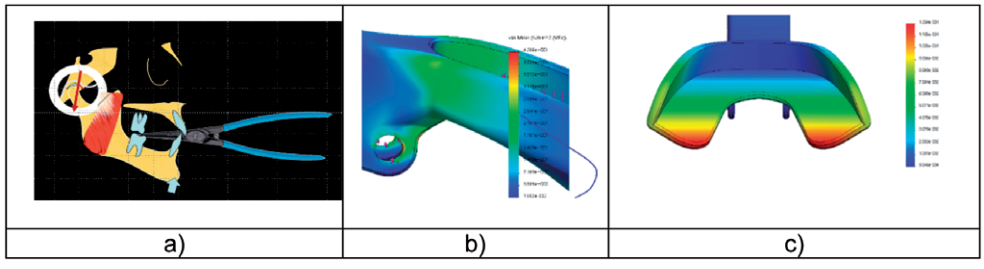

Figure 1. (a) Preliminary conception of the device. (b) Detail of von Mises stress distribution for limit conditions; (c) Distribution of resultant displacements in the upper part of the device.

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The influence of intraoral devices in salivary cortisol levels of golf athletes – a pilot study

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Introduction: A removable intraoral device is often manufactured in hard acrylic that is placed over the incisal and occlusal surfaces of the teeth [1]. The correlation between the intraoral devices and the concentration of cortisol has been demonstrated by some researchers, and suggested to reduce cortisol levels due to a hormonal cascade reaction [2,3]. This study aimed to analyze the changes in salivary cortisol levels and the state of anxiety with the use of intraoral devices in Golf athletes.
Materials and methods: After study approval by the Ethic Commission of the Cooperativa de Ensino Superior Egas Moniz, athletes from the Centro Nacional de Formação de Golfe do Jamor were invited to participate in this study. Following the informed consent a Diagnostic Criteria for Temporomandibular Disorder was applied. Inclusion criteria: Athletes of both genders who have signed informed consent with handicap <20 or professional. Exclusion criteria: Athletes who were under pharmacological treatment with glucocorticoids or anti-inflammatories; altered function of the adrenal glands; Kennedy-Applegate Class I or II. In order to ensure anonymization all data were coded. Individualized intraoral devices (IOD) were developed for each athlete. Athletes held a 9-hole tournament with two phases: one phase without and another with the use of the IOD. To analyze the stress levels, salivary cortisol levels were accessed through a chemiluminescent enzyme immunoassay (Immulite 1000), and requested the completion of the Competitive State Anxiety Inventory-II. Linear mixed effect models were used to evaluate the effect of the use of the intraoral device, evaluation moment and interaction between them and the logarithm of salivary cortisol and anxiety levels. Significance of these effects was assessed through the analysis of variance of type III with Kenward–Roger approximation for degrees of freedom.
Results: The sample was composed of 8 athletes, with an average age of 27.3 (±7.3) years. The developed model to evaluate the effect of the use of the IOD, evaluation moment and interaction between them with the logarithm of the salivary cortisol concentration shown to be significantly different than the null model (χ^2 (3) = 14.752, $p < .002$), moreover, only the effect of the use of the IOD was shown to influence the model significantly (F(1,21) = 15.661, $p < .0007$. The model developed to evaluate the effect of the IOD, evaluation season and their interaction on the anxiety state showed not to be significantly different from the null model (χ^2 (3) = 7.5918, $p < .055$).
Discussion and conclusion: The use of IOD decreased salivary cortisol levels, but didn't change in subjective anxiety levels. These effects over the level of cortisol may result from the athletes closing their teeth with IOD due to a reduced response to stress [3]. Most significant larger samples are required to confirm these results.

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The influence of intraoral devices on dynamic performance of golf athletes – a pilot study

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Introduction: It is widely accepted that the putt in golf depends primarily on the expertise, concentration, and postural control of the performer, in which, stabilization of the mandibular position is of great importance. Previous studies have shown that this stabilization can be optimized with the use of removable intraoral devices that allow precise contact of the teeth from the two arches [1]. Herein we report influence of the use of intraoral devices (IOD) in the putt performance of golf athletes.
Materials and methods: After study approval by the Ethic Commission of the Cooperativa de Ensino Superior Egas Moniz, athletes from the Centro Nacional de Formação de Golfe do Jamor (CNFGJ) were invited to participate in this study. Following the informed consent a Diagnostic Criteria for Temporomandibular Disorder (DC/TMD) was applied. Individualized IOD were developed for each athlete. Athletes were evaluated at two different stages: (a) initial phase (IP) – on the first day of application of the IOD; and (b) adaptation phase (AP) – after 1 week of frequent use of the IOD. In both cases, 20 putts were analyzed with and without IOD. Sam PuttLab (Science & Motion GmbH) was used for task analysis and performance recording. Linear mixed effects models were used to analyze the effects of the utilization of the IOD and adaptation stage in the balls' distance to the hole (BDH), in the percentage of putt success (PPS), and several measures of technique (club angle on impact, local of impact and club trajectory). The significance of these effects was assessed through the analysis of variance of type III with Kenward-Roger approximation for degrees of freedom.
Results: In this study, 17 volunteer golf athletes were included, 88% (15) were male and the average age was 26.2 (±6.74) years. The developed linear mixed effects models for BDH and PPS shown to be significantly different from the null model (χ^2 (3) = 9.6314, $p \leq .022$ and χ^2 (3) = 8.194, $p \leq .042$, respectively). Analysis of variance shown the interaction between the use of the IOD and the adaptation stage to affect significantly the BDH (F (1,34) = 2.128, $p \leq .034$), reducing in average 6.78 cm the BDH from the IP without IOD to the AP with IOD. Moreover, it also shown that the use of IOD affects significantly the PPS (F (1,34) = 7.8140, $p \leq .008454$), increasing in average 1.6% the PPS.
Discussion and conclusions: Our results show that after reaching a balance period of use with an IOD with the purpose of increasing the stabilization of the mandibular position, there was a significant improvement in accuracy, although there have not been changes in technique of the putt, leading to 1.6% increase in putt accuracy and a decrease of 6.78 cm in the distance of the balls close to the hole. Our results confirm Pae and coworkers' findings describing that the use of IOD promotes an improvement in both isokinetic muscular strength and mental capacity for concentration at the time of impact of the golf club with the ball, which leads to greater precision of movement [2].

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The influence of intraoral devices on static performance in golf athletes – pilot study

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Introduction: The use of intraoral removable devices can lead to postural corrections through stomatognatic system information sent to the central nervous system, which interprets and sends to the muscle groups a neuromuscular response [1]. This can bring benefits for golf athletes, because this sport requires a high postural control and its income is dependent directly from the correct alignment of body segments and their dynamic relationship. The aim of this work was to analyse whether the use of an intraoral device, totally adapted, in centric relation, causes changes in static posture.
Materials and methods: After study approval by the Ethic Commission of the Cooperativa de Ensino Superior Egas Moniz, athletes from the Centro Nacional de Formação de Golfe do Jamor (CNFGJ) were invited to participate in this study. After obtaining consent, an oral clinical observation was performed with the application of the Diagnostic Criteria for Temporomandibular Disorder (DC/TMD). Inclusion criteria were athletes of both genders who have signed informed consent with handicap <20 or professional. Exclusion criteria were athletes with Kennedy–Applegate Class I or II edentulous athletes. Individualized and equilibrated intraoral removable devices were developed for each athlete. The postural parameters of each athlete were collected using a pressure platform (RsScan) in four conditions: eyes open and eyes closed, with and without intraoral device. The athletes performed randomly 3 repetitions of each condition for 66 s each, and the centre of pressure (CP) sway velocity was measured. Linear mixed models were used to analyse the effects of the utilization of the intraoral device and presence of visual information on the CP velocity. Significance of these effects was evaluated through a type III analysis of variance with Kenward–Roger approximation for the degrees of freedom.
Results: There were included in this study 17 Golf athletes, 15 men and 2 women with a mean age of 26.2 (± 6.74) years. The developed model showed to be significantly different from the null model (χ^2 (3) = 32.344, $p \leq 4.428e-07$). Analysis of variance shows that both the effect of intraoral device use and the effect of the presence of visual information influences the model significantly ($F(1,45) = 4.235$, $p \leq .045$ and $F(1,45) = 38.895$, $p \leq 1.39e-07$, respectively).
Discussion and conclusion: The use of intraoral devices appears to positively influence the posture, proving the interconnection of the stomatognathic system with the muscular system in balance control, through the neuromuscular responses sent by the central nervous system. In our sample the use of intraoral devices seems to positively influence the posture, meeting the interconnection of the stomatognathic system with the balance control system.

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Class III malocclusion patients: an interceptive treatment

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Introduction: The treatment of skeletal class III malocclusions has been considered a challenge, due to patients' unpredictable and potentially unfavourable growing pattern [1]. Usually, skeletal class III patients present a maxillary hypoplasia or retrusion, with a normal or minimally prognathic mandible [2]. Many orthodontic and orthopaedic treatment approaches regarding skeletal class III malocclusion can be found in the literature, including intraoral and extraoral appliances [3,4]. The objective of this paper is to examine whether maxillary protraction leads to skeletal changes by comparing treatment and control groups during and after facemask therapy.

Materials and methods: Forty-one patients (23 female and 18 male subjects) were divided into two groups. The treatment group ($n = 23$; 13 female and 10 male subjects, mean age 6.2 years) used a facemask (model Delaire, type of anchorage fronto-mentum and force origin on canines) and modified quad-helix during 12 months. The control group ($n = 18$; 10 female and 8 male subjects, mean 8.6 years) underwent no treatment. The results were analysed using a statistical platform (SPSS v.20, SPSS Inc., Chicago, IL). The mean treatment and control data were compared with Student's t-test to determine statistically significant differences. All analyses were performed at the 0.05 level of statistical significance. Males and females were combined either in treatment and control groups because statistical significance has not been shown between them.
Results: Based on the cephalometric alterations observed after 12 months of maxillary expansion and protraction during early mixed dentition, dentoskeletal changes and improvements in dentofacial complex were recorded, mainly by a combination of forward displacement and rotation of the maxilla. These modifications demonstrate that this treatment in an early age is clinically recommended.
Discussion and conclusions: Significant orthopaedic changes can occur with maxillary expansion and facemask therapy. Early correction can improve facial aesthetics and psychosocial development of a child. These results support the importance of early treatment in skeletal class III malocclusions.

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Early treatment of a class III malocclusion with the myobrace system: clinical case

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Introduction: The preventive or interceptive orthodontic treatment in early age patients who present breathing, chewing, swallowing, speaking or face morphology is of extreme importance. Minimizing occlusion problems, improving facial aesthetics and muscle activities, all of which tend to aggravate during growth, is considered essential [1–4]. The use of myofunctional appliances, such as myobrace, allows the practitioner to alter the functional stimulus, altering the shape and bone structures of the jaws [3,5]. This clinical case shows the effects of a myofunctional appliance on a young patient diagnosed with anterior crossbite with a 9-month follow up.
Materials and methods: A 6-year-old girl, was referred to Paediatric Dentistry Department of ISCSEM, the parents main complaint is an anterior crossbite. Clinical and radiologic assessment confirmed a dental anterior crossbite and a class III malocclusion. After obtained informed consent, the treatment consisted in the use of a myofunctional appliance (myobrace system) used for 12 months, in order to correct the patient occlusion.
Discussion and Conclusions: As a myofunctional appliance, myobrace has two main effects, correcting the orientation of the grow of arches and guide the perioral soft tissues. Its main propose is to eliminate the factors related to malocclusion, promoting the stability of both hard and soft tissues and preventing the need of tooth extraction in an older stage of the patient's life [1,5]. In this clinical case, we were able to show the effectiveness of this myofunctional appliance. It was possible to correct the anterior crossbite and to improve the patient's function and aesthetics.

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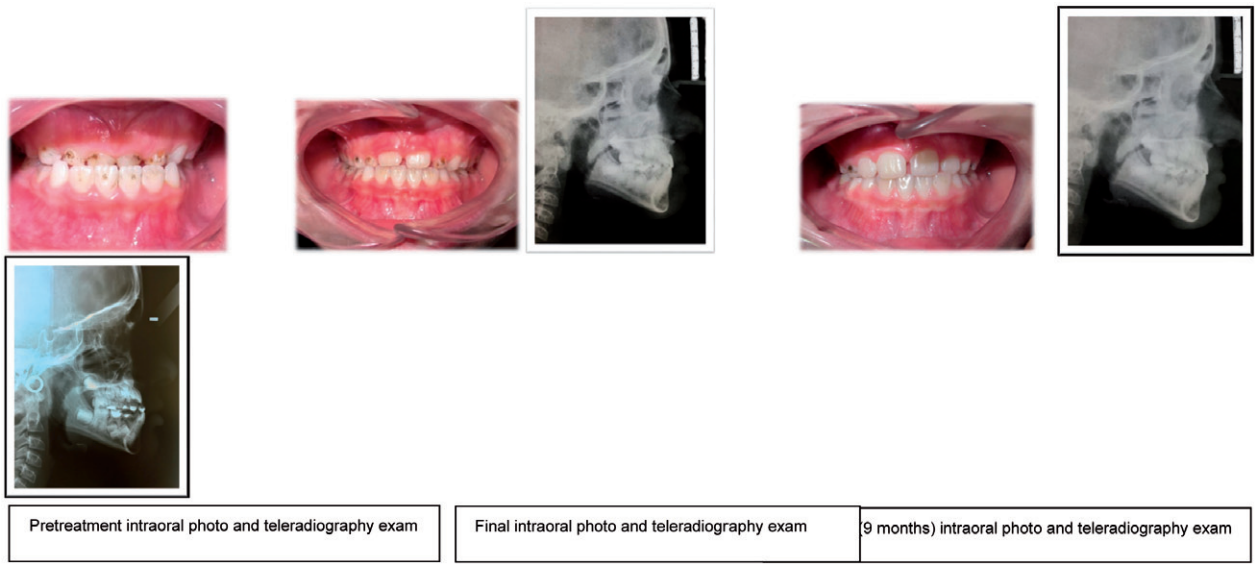

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Sagittal condylar inclination and Anglés sagittal malocclusion classification

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Introduction: Sagittal condylar inclination (SCI) is the angle formed by protrusive condylar path and usually the Frankfort horizontal (or any other horizontal) reference plane [1]. It is an important parameter during oral rehabilitation [2]. For the competent and reproducible investigation of the mandibular movements, computerized axiography (CA) was confirmed as a valuable complementary examination for programming articulators based on the provided values [3]. There is scarcity of data concerning SCI correlated with Anglés sagittal malocclusion classification (ASMC). The purpose of this study was to assess the association of SCI and patient's malocclusion classification.
Materials and methods: About 203 adult participants, 134 females and 69 males with an average age 43.69 were included in this study, approved by the Ethics Committee. Patients with systemic disease, temporomandibular joint (TMJ) disorders, previous orthodontic treatment, fully edentulous and posterior partially edentulous were excluded. For each patient, we determined SCI for right (rTMJ) and left TMJ (lTMJ) with computerized condylography (Cadiax Diagnostic®) and ASMC for both sides obtained through intraoral photography and mounted cast models. Statistical analysis was made using SPSS version 22 (SPSS Inc®) and $p < .05$ was considered significant.
Results: See Table 1.
Discussion and conclusions: Our results are different from those from Santos (2013) [4]. In our study, Class II group presented statistically significant higher SCI mean values than all other groups in both sides. We can conclude that the variability of SCI contraindicates simplified mounting technique for the articulator using average condylar guidance settings.

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Table 1. Dental Class and Class 2 subclasses mean values ± standard deviation.

ASMC	I	II Div 1	II Div 2	III	Asymetrical	*p*
rTMJ (45.4 ± 8.4)	43.9 ± 7.6$^{(b)}$	48.4 ± 8.4$^{(b)}$	48.9 ± 8.9$^{(b)}$	39.6 ± 9.0$^{(a)}$	43.8 ± 7.5$^{(b)}$	<.001*$^{(z)}$
lTMJ (44.9 ± 7.7)	44.2 ± 8.1$^{(a,b)}$	47.1 ± 4.8$^{(b)}$	47.2 ± 7.4$^{(b)}$	40.4 ± 5.1$^{(a)}$	43.4 ± 6.8$^{(a,b)}$	.007*$^{(z)}$

*Statistically significant differences between the mean values, for a level of significance of 5%.
(z)ANOVA one-way/Scheffé *post-hoc* test (a,b homogeneous subgroups of mean values).

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Relation between cranial skeletal pattern and sagittal condyle inclination

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Introduction: Sagittal condylar inclination is an important parameter during oral rehabilitation [1] regarding dental articulator programming. There is lack of data concerning the effect of the skeletal pattern in sagittal condylar inclination (SCI). The purpose of this study was to investigate the variability of SCI, depending on the skeletal pattern.
Materials and methods: About 203 adult participants, 69 male and134 female, average age 43.69, were included in this study, approved by the Ethics Committee. Patients with systemic disease, temporomandibular joint (TMJ) disorders, previous orthodontic treatment, fully edentulous and posterior partially edentulous without presence of at least one molar bilaterally in occlusion with the antagonist were excluded. For each patient, we determined the SCI with computerized condylography (Cadiax Diagnostic®) and the skeletal pattern with cephalometric analysis through teleradiography (GDSW® and Cadias®). Condylar inclination values were obtained by the average of three protrusive jaw movements for right (rTMJ) and left TMJ (lTMJ). Statistical analysis was made using SPSS version 22 (SPSS Inc®) and $p < .05$ was considered significant.
Results: See Table 1.
Discussion and conclusions: Our results are in accordance with Canning et al. [2] and similar to Signh et al. [3]. Class II group presented statistically significant higher SCI mean values than Class I ($p = .00$) and Class III ($p = .012$) groups in both sides, meaning that during extensive prosthodontic treatment especially with noticeable skeletal discrepancy, appropriate considerations should be given to customizing SCI values, because a simplified mounting technique adopting an average condylar guidance setting may lead to error in reproducing mandibular movements accurately and therefore occlusion may not be the more correct one.

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Table 1. Skeletal Class mean values ± standard deviation.

Skeletal pattern	I	II	III	*p*
rTMJ (45.4 ± 8.4)	$43.3 \pm 8.3^{(a)}$	$48.3 \pm 8.6^{(b)}$	$41.2 \pm 3.9^{(a)}$	$<.001^{*(II)}$
lTMJ (44.9 ± 7.7)	$43.7 \pm 7.5^{(a,b)}$	$46.9 \pm 7.8^{(b)}$	$41.1 \pm 6.9^{(a)}$	$.003^{*(I)}$

*Statistically significant differences between the mean values, for a level of significance of 5%.
(I)ANOVA one-way/Scheffé *post-hoc* test (*a*,*b* indicate homogeneous subgroups with mean values).
(II)ANOVA one-way with Brown-Forsythe correction/Test *post-hoc* Dunnett's T3 (*a*,*b* indicate homogeneous subgroups with mean values).

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Bilateral mandibular distraction osteogenesis using a tooth-born device: an experimental study

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Introduction: The distraction osteogenesis (DO) is a surgical orthopedic technic used to lengthen bones. It is the regeneration of new bone between two vascularized bone surfaces that are gradually separated by a mechanical device [1]. DO was first described by the Italian Alessandro Codivilla [2]. The osteogenic distraction is an alternative to mandibular bilateral sagittal split osteotomy and should be considered as a treatment option for mandibular retrognathism. This study aims to assess *in vivo* the biomechanical effects of a new tooth-born distractor, as well as the biological performance and regenerative potential of two different rates of distraction in the formation of new bone.
Materials and methods: Ten beagle dogs, weighing between 15 and 18 kg, were used. Three remained as the control group and seven underwent a mandibular distraction protocol. Both hemi-mandibles were used for experimental purposes. For this study and to evaluate the treatments outcome, radiographic and cephalometric analyses, absorptiometry, histology and histomorphometry techniques were used.
Results: The distractors anchored to the teeth and parallel to the occlusal plane allowed the significant increase of the anterior bone segment, without any side effects at the gonial angle level or alteration of the transverse angulation of the bone segments. Therefore, the tooth-borne distractor produced a correct lengthening direction, which facilitated the creation of the desirable stress force necessary for the osteogenesis process, instead of an undesirable compression force. The frequency of the distraction influenced the coefficient of variation in the groups submitted to osteogenesis distraction protocol, indicating that there was a direct relationship between the increase in the frequency and the acceleration of bone regeneration.
Discussion and conclusions: The distractors anchored on the teeth and parallel to the occlusal plane significantly increased the anterior bone segment without producing lateral or vertical statistically significant changes. The elongation of 1 mm/d is the rhythm of distraction that produces better results in the process of distraction osteogenesis [3]. Subsequent studies [4] demonstrated that a rhythm below 1 mm/d led to premature bone union, and over 1mm/d was detrimental to the healing mechanism, favouring the invasion of fibrous tissue in the zone of distraction. An increase of the rhythm from one to two daily activations changed the quality of the new bone present in the area created by the distraction.

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Assessment of malocclusion and oral health-related quality of life in Portuguese children using the Child Perceptions Questionnaire (CPQ 11–14)

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Introduction: The prevalence of malocclusion has been reported between 43% and 78% in schoolchildren and it has been long recognized that malocclusions are associated with impaired oral health or function [1,2]. The study objective was to assess the impact of malocclusion using the CPQ11–14 in a schoolchildren population at Torres Vedras.
Materials and methods: A sample of 112 school children of 12–14 years old, with a consent form previously signed by their respective guardians fully completed the CPQ11–14 and were examined for malocclusion using Dental Aesthetic Index (DAI). A descriptive analysis was performed using IBM SPSS© statistics 20.0 software.
Results: Only 7.1% of the sample had no need of treatment, while remaining 92.9% needed orthodontic treatment according the severity level of malocclusion. The Dental Aesthetic Index scores ranged from 21 to 72, with mean scores was 39.67 (±11.89). Overall CPQ11–14 scores ranged from 0 to 72. No statistically significant associations were found between the clinical and overall CPQ scores.
Discussion and conclusions: The results indicated that malocclusion did not have a significant impact on quality of life on Portuguese children using CPQ11–14. The relationship of reported quality of life and malocclusion is probably related to others factors.

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Table 1. Absolute and relative frequencies of DAI.

DAI	N	%
Minor/None	8	7.1
Definitive	16	14.3
Severe	25	22.3
Handicapping	63	56.3
Total	112	100.0

Table 2. Descriptive statistics of DAI.

DAI	
Mean	39.67
Median	37.50
Variance	141.37
Standard-deviation	11.89
Minimum	21
Maximum	72

Table 3. Descriptive statistics of CPQ11-14.

CPQ11–14	Min.	Max.	Mean	SD
General Oral health and well-being	0	6	2.27	1.375
Oral Symptoms	0	14	4.32	2.357
Functional Limitations	0	15	2.76	3.433
Emotional well-being	0	23	3.04	4.471
Social well-being	0	14	2.0	3.079
Total	0	72	14.39	14.71

Assessment of dental caries and oral health-related quality of life in Portuguese children using the Child Perceptions Questionnaire (CPQ11–14)

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Introduction: Dental caries is the most prevalent oral disease in children and can have a negative impact on the life of a child [1,2]. The aim of this work was to assess the impact of dental caries using the CPQ11–14 in a schoolchildren population at Torres Vedras.
Materials and methods: A sample of 112 school children of 12 to 14 years old, with a consent form previously signed by their guardians duly completed the CPQ11–14 and were examined for dental caries using DMFT according WHO criteria. A descriptive and inferential analysis was performed using IBM SPSS© statistics 20.0 software.
Results: The prevalence of caries in the permanent dentition was 79.5% and a mean of the DMFT 2.16 (± 1.71). Overall CPQ11–14 scores ranged from 0 to 72 with a mean of 14.39 (± 14.71). The results demonstrate a statistically significant and correlation between the DMFT and functional limitations (,373, $p < 0, 05$) of CPQ 11–14.
Discussion and conclusions: The prevalence and severity of caries may be considered moderate, the children with more extensive dental caries rated their oral health less favorable in oral function.

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Table 1. Characteristics of various NP formulations.

DMFT	N	%	% accumulated
DMFT =0	23	20.5	20.5
DMFT ≥0	89	79.5	79.5
Total	112		100.0
DMFT Mean = 2.16			
DMFT Median = 2.00			
DMFT Standard-deviation = 1.71			

Table 2. Descriptive statistics of CPQ11-14.

CPQ11–14	Min.	Max.	Mean	SD
General Oral health and well-being	0	6	2.27	1.375
Oral Symptoms	0	14	4.32	2.357
Functional Limitations	0	15	2.76	3.433
Emotional well-being	0	23	3.04	4.471
Social well-being	0	14	2.0	3.079
Total	0	72	14.39	14.71

Table 3. Discriminant validity of CPQ11-14/DMFT.

		DMFT
General Oral health and well-being	R de Pearson	,141
	P	,330
	N	50
Oral Symptoms	R de Pearson	,167
	P	,247
	N	50
Functional Limitations	R de Pearson	,373
	P	,008
	N	50
Emotional well-being	R de Pearson	.160
	P	,266
	N	50
Social well-being	R de Pearson	,93
	P	,521
	N	50

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Prevalence and severity of dental caries in a 10–15 years old schoolchildren population, in a Portuguese rural population

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Introduction: Tooth decay is the most prevalent disease in children (and not only) worldwide [1,2]. Although its etiology is well known, there is still no full control of the disease. There are several factors identified as being associated with the disease, as described in recent epidemiological studies [3–5]. The aim of this study was to evaluate the prevalence and severity of dental caries, in a schoolchildren population (10–15 years old), at a public school, in the parish of Freiria (Torres Vedras).
Materials and methods: The study was approved by the Ethics Committee for Health, of Egas Moniz. A sample of 104 individuals (17% of total) was observed in the school of Freiria and data were recorded for DMFT index and International Caries Detection and Assessment System (ICDAS II) calculation. Additionally, a questionnaire about access to oral health care, oral hygiene and food frequency was handed over. Data were subject to statistical analysis by using descriptive and inferential methods. A significance level of 5% was considered in the later.
Results: The prevalence of dental caries was 66.3% (CI 95% 57.3–75.4%), according to DMFT index and 62.5% (CI 95% 53.2–71.8%), according to ICDAS II criteria. DMFT distribution was DMFT =0 (33.7%, $n = 35$), DMFT =1–2 (28.8%, $n = 30$) and DMFT >= 3 (37.5%, $n = 39$). In the surveyed group, the average DMFT index was found to be 2.36 (± 2.81), yielding an average of decayed permanent teeth of 1.38 (± 2.54), lost permanent teeth due to decay of 0.01 (± 0.10) and filled permanent teeth of 0.96 (± 1.46). The severity of dental caries was assessed by ICDAS II scores, with ICDAS II maximum codes ranging from 01 to 86, as follows: ICDAS II =01, 02 (26.0%, $n = 27$), ICDAS II =05, 15, 25, 80–85 (15.4%, $n = 16$), ICDAS II =06, 16, 26, 86 (21.1%, $n = 22$).
Discussion and conclusions: We found a high prevalence of dental caries, with cavity lesions of enamel and dentin. However, no statistically significant correlations were found between the prevalence and severity of dental caries and sociodemographic variables or food frequency intake. A plausible reason for this finding may be due to a confounding factor such the lack of assessment of oral hygiene practices. Nevertheless, caries lesions were underestimating because no radiographs were taken.

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Evaluation of the erosive potential of three national drinks, in human enamel surface – *in vitro* study

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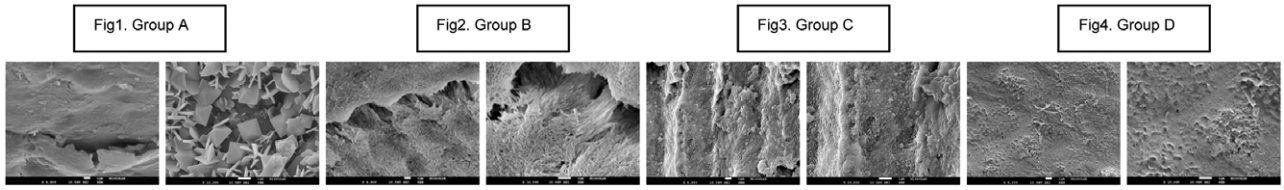
Introduction: Dental Erosion is a progressive and irreversible loss of dental hard tissue, by a chemical process, which does not involve any bacterial activity [1], and in recent years an increasing in acidic drinks has been associated with this

Table 1. Different group pH's with respective temperatures.

	Group A		Group B		Group C		Group D	
Temperature	4 °C	37 °C	4°	37 °C	4 °C	37 °C	Room temp.	37 °C
pH	3.85	3.69	3.24	2.95	4.16	3.95	7	7



event [2]. The purpose of this study was to (a) characterize the pH of three national consumption drinks (Green Juices So Natural®, Somersby® and Kefir So Natural®); (b) compare its pH at different temperatures and its erosive potential, on human enamel surface; (c) compare the enamel topography from a qualitative point of view.
Materials and methods: The study was approved by the Ethics Committee for Health, of Egas Moniz. Eight human enamel specimens were randomly divided in four groups: Group A ($n = 2$) exposed to Green Juices So Natural®; Group B ($n = 2$) exposed to Somersby®; Group C ($n = 2$) exposed to Kefir So Natural®; Group D ($n = 2$) positive control, exposed to deionized water. All groups were subjected to a 25 h exposition, with 5 h demineralization cycles, at 37 °C. The drinks' pH was also measured, at different temperatures. All specimens were visualized with a scanning electron microscope (SEM), JEOL JSM-700001F, at 5000× and 10,000× magnifications.
Results: The results show (a) pH depends directly on the temperature, and in this study, is always below 4.5, in all groups (Table 1); (b) Groups A and B present a greater enamel disorganization, when compared to Group C, with SEM analysis.
Discussion and conclusions: All study groups have demonstrated a high erosive potential (Figures 1–3), comparing with control Group. When the temperature increases there is a pH decreasing. The consumption of acidic products is related to the presence and severity of erosive lesions, with some differences at their microscopic evaluation. Milk-based products have a less erosive potential, in group C were observed collinear spheres of organic material along the crystal (Figure 3).

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Pulp revascularization/revitalization after trauma of permanent tooth: a case report

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Introduction: Pulp revascularization/revitalization is a promising alternative therapy for the treatment of necrotic immature permanent teeth. There are several difficulties in this procedure, namely very thin dentin walls, risk of over-obturation, and canalar irrigation because of the risk of extravasation due to immature apex [1,2]. Other obstacles are found, such as the application of Ca(OH)2 which causes unpredictability on the apical barrier formation and leads to numerous consults, the apexification procedure with MTA, also results in thinning of dentinal walls [2–4].
Material and methods: A 13-year-old boy attended on urgent consultation due to a fall. After clinical and rediologic observation, subluxation was diagnosed to the 21 tooth. Follow up was performed at 1, 3 and 6 months, however after 11 months, the patient appeared in the consult, with grayish coloration on the tooth [5]. After clinical examination, radiological and negative sensitivity tests, it was diagnosed pulp necrosis, as well immature root. The proposed treatment plan was revascularization/revitalization therapy in two sessions. In the first session, the protocol was primary canal

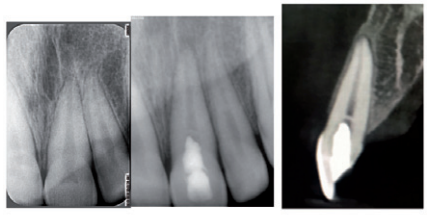

Figure 1. X-Ray of tooth 21 before revascularization/revitalizacion.
Figure 2. X-Ray of tooth 21 after revascularizacion/revitalization (follow up 9 months).
Figure 3. Cone Beam-Computed Tomography of tooth 21 after ravascularization/Revitalization (Follow up 12 months).

disinfection, through irrigation (NaOCL 5%) and later through intracanal medication with a tri-antibiotic paste of Ciprofloxacin, Metronidazole and Minocycline [6]. After 2 weeks, second session occurred where hemorrhage was caused and MTA apllied above the blood cloth [1,2,7]. Informed consent was signed.
Results: After 12 months, by means of complementary diagnostic examination, it was possible to verify dentin bridge formation, root development with consequent apical closure.
Discussion and conclusions: There are a variety of protocols for revascularization/revitalization, and all are valid, although there is still a long path to go on this field.

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Bayesian model averaging in seeking for the best polishing-resin combination and its impact on teeth surface roughness and gloss

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Introduction: An important feature in dentistry is teeth brightness. During the intervention the doctor must apply a resin and then a polishing to achieve the final result, i.e. the highest gloss (brightness) and the lowest roughness possible. The aim of this study was to evaluate the effect of four polishing protocols, p, in teeth surface Roughness and Gloss when considering two different resins, r. Bayesian model averaging [1] was used to improve the posterior parameter estimates.
Materials and methods: 20 discs of a nanofilled resin (1: Filtek Supreme XT®) and 20 of a nanohybrid resin (2: Brilliant Everglow®) were prepared. Sets of 5 discs were randomly assigned to each polishing protocol (1: sof-lex discs/enhance/spiral/diamond paste; 2: Sof-Lex discs/spirals; 3: sof-lex spirals; 4: swiss-flex discs/Diatech Shapeguard®), resulting in 8 groups polishing*resin, $g_1, \ldots, g_8$, with 5 discs each. Analysis of surface roughness was performed using an Atomic Force Microscope (AFM). A 4x4 regular grid per disc was considered. Roughness measurements were obtained for each of the 16 squares of dimension $10\,\mu m \times 10\,\mu m$. A unique gloss measure per disc was considered using a suitable meter with incidence at 60°.
We assumed that for each disc, d_i, $i = 1, \ldots, 40$, given the categorical covariates, p_i and r_i, the two outcomes, roughness, R_i, and gloss, G_i, are conditionally independent. Instead, we might have considered the direct impact of the roughness on the gloss. Although that would preclude us to test the main effect of the polishing*resin interaction on the gloss. Accordingly, we build separate Gaussian hierarchical models for each continuous outcome.

Table 1. Posterior mean and the respective Credibility Interval (CI). The values represent the difference of each group from g_1 (reference group). For g_1 the values represent the estimated posterior mean value.

	r (Resin)			
	1		*2*	
p (Polishing)	*log(roughness)*	*gloss*	*log(roughness)*	*gloss*
1	g_1: 3.08; (2.80, 3.37)	g_1: 36.49; (32.45, 40.32)	g_5: 1.52; (0.89, 2.15)	g_5: 5.55; *(0.08, 11.15)*
2	g_2: 0.94; (0.53, 1.33)	g_2: −11.38; *(−16.73, −5.96)*	g_6: 2.66; *(2.16, 3.16)*	g_6: −21.81; (−27.21, −16.22)
3	g_3: 1.01; (0.67, 1.34)	g_3: −19.47; (−24.65, −13.80)	g_7: 0.97; *(0.41, 1.51)*	g_7: −21.02; (−26.20, −15.56)
4	g_4: 1.36; (0.77, 1.99)	g_4: −22.97; (−28.53, −17.58)	g_8: 1.30; (0.53, 2.12)	g_8: −18.39; (−23.73, −12.85)

As we have 16 roughness measurements per disc, $R_i = \{R_{i1}, \ldots, R_{i16}\}$, we decided to perform sixteen Gaussian hierarchical models [1] and averaging the posterior parameter estimates, considering equals weights for the different models. A similar Gaussian hierarchical model was considered for the only gloss outcome, G_i, in (equation 2).
Interaction between *p* and *r* was modeled considering the new categorical variable, *g*, whose eight categories consist of all possible combinations of the values of *p* and *r*. The reference category, g_1, when defining the 7 dummy variables, $w_1, \ldots, w_7$, was considered the combination *(p,r) = (1,1)*:

$$\log(R_{ij}) \sim N(\beta_1 + \beta_2 w_{ij1} + \beta_3 w_{ij2} + \beta_4 w_{ij3} + \beta_5 w_{ij4} + \beta_6 w_{ij5} + \beta_7 w_{ij6} + \beta_8 w_{ij7},\ \sigma_R^2), i = 1, \ldots, 40; j = 1, \ldots, 16 \quad (1)$$

$$G_i \sim N(\gamma_1 + \gamma_2 w_{i1} + \gamma_3 w_{i2} + \gamma_4 w_{i3} + \gamma_5 w_{i4} + \gamma_6 w_{i5} + \gamma_7 w_{i6} + \gamma_8 w_{i7},\ \sigma_G^2), i = 1, \ldots, 40 \quad (2)$$

Results: See Table 1.
Discussion and conclusions: The best combination to achieve the lowest roughness is *(p,r) = (1,1)*. All the other combinations result in rougher surfaces, because the CI's (Table 1) are strictly positive. The best combination to achieve the highest gloss is *(p,r) = (1,2)*, because its CI (0.08,11.15) is strictly positive, meaning that it is shinier than the reference group, g_1.

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Efficacy evaluation of carbamide and hydrogen peroxide as internal bleaching agents

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Introduction: Root canal therapy often leads to tooth discoloration [1]. The most conservative, minimal invasive and cost effective treatment for those cases is internal bleaching [2,3]. Accordingly the Council of European Dentists tooth whitening products containing more than 6% hydrogen peroxide (present or released) were not considered safe for use by the consumer [4]. The aim of this study was to evaluate and compare, *in vitro*, three bleaching agents, hydrogen peroxide (6%), carbamide peroxide (10% and 16%) as internal bleaching agents.
Materials and methods: Forty-eight anterior teeth were randomly divided into four groups: Group A – 6% hydrogen peroxide *Poladay* (SDI); Group B – 10% carbamide peroxide *Opalescence*® 10% (Ultradent); Group C – 16% carbamide peroxide *Polanight* (SDI) and Group D – control (with no bleaching agent). The bleaching agent was applied every seven days until the colour stopped changing, which was after five times. The colour was measured, with the CIE L*a*b* system, using a spectrophotometer, and Vita® scale, at three different moments: Initially, immediately after bleaching and after three months in water storage. Concerning the CIE L*a*b* each letter represents a different parameter: L* refers to lightness while a* indicates the position of the colour between red and green and b* indicates the position between yellow and blue. The L* parameter was the most relevant to this study [5]. Data were subjected to statistical analysis by using descriptive and inferential methods. A significance level of 5% was considered in the later.
Results: Immediately after bleaching all groups showed a statistically significant change in the Vita® scale. All of them brightened, with Group B achieving the more noticeable change. Regarding the CIE L*a*b*, all groups showed an

increase in the L* parameter, with Group A exhibiting the highest difference. For a* and b* parameters, no statistically significant differences were found between initial and final average values. For ΔE the best results were achieved in Group A, followed by Groups C and B.
Discussion and conclusions: The three bleaching agents were effective immediately after the bleaching process ended and were still effective after three months. Time and time-concentration interaction effects were found to be significant regarding the efficacy of the internal bleaching agents.

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Enamel bleaching with H_2O_2 solutions: effect of pH

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Introduction: Tooth whitening treatments are based on the application of bleaching agents in the teeth surface. Although tooth whitening treatments are efficient, they may impair teeth health, due to its effect on enamel properties. The teeth may become weaker, with lower wear resistance and microhardness.
The purpose of this work is the study of the effect of pH on enamel bleaching and wear. Three pH values (2, 4 and 6) of H_2O_2 solution were tested.
Materials and Methods: Five human molar teeth were disinfected and washed with a toothpaste without any bleaching agent in its composition and stored in distilled and deionized water at 4 °C. Teeth surface was polished with silicon carbide sandpapers. After that, the teeth were cut in four equal pieces and darkened in a black tea solution for 48 h. The colour of each part was determined using a spectrophotometer. Teeth samples were bleached in 30% H_2O_2 solution with the different pH values (10 min of immersion in the solution with exposure to blue light each 2 min, for 30 s) for several bleaching sessions until the same degree of whitening was reached in each one of them.
Enamel microhardness was measured before and after bleaching. The microhardness experiments were done in a microdurometer with an applied load of 1.96N during 15 s. Wear resistance was also tested before and after the bleaching in the different conditions in a nanotribometer with reciprocal linear movement, using an applied load of 25nN and a sliding distance of 1 mm. Tests were carried out at room temperature for 20 min. As counter body, zirconia beads of 3 mm diameter were used. The topography was observed by scanning electron microscopy.
Results: It was found that to achieve the same whitening degree, the same number of sessions was required independently of the pH. A statistically significant decrease in the enamel microhardness values was observed after the several bleaching sessions with H_2O_2 solution at pH =2. The H_2O_2 solution at pH =4 also led to a decrease in the microhardness mean value, but lower than the observed for pH =2. The H_2O_2 solution at pH =6 led to minor changes. SEM images revealed that the demineralization increases as the solution pH decreases. Also the analysis of the wear tracks show that a lower pH leads to higher wear.
Discussion and conclusions: The bleaching efficiency does not depend on the solution pH but the enamel microhardness and wear resistance do. The lower the pH values, the greater the changes in the enamel microhardness after treatment. When bleaching at pH =6, there were also microhardness variations after treatment. Although hydroxyapatite is stable at that pH (stable above 5.4), it was observed variations, which may suggest that there was degradation of the tooth organic matrix. The results point out that there might be an attack of the organic matrix.

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Raising awareness for toothbrush disinfection among future dental professionals

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Introduction: Currently there is a vast disposal of information regarding oral heath hygiene. The toothbrush continues to be the most widely recommended tool for this purpose [1]. However, the important need for basic care, such as toothbrush storage, exchange and disinfection is left aside, these are also important aspects for the conservation of a heathy mouth [1,2]. Microorganisms can be retained in the toothbrushes bristles and remain viable for a day to a week after brushing, allowing disease, infection and transmission [2,3]. Therefore, its elimination through disinfection methods becomes crucial in order to reduce microbial contamination and promote oral health.
The aim of this study was to evaluate the awareness about toothbrush disinfection methods among the students of Dentistry.
Materials and methods: The subjects were composed by students ($n = 60$) attending the 5th year of dentistry in Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM), Portugal. All information was gathered through an anonymous questionnaire, composed of eleven closed and semi-open questions regarding their oral hygiene habits, care and maintenance of toothbrushes and their knowledge about disinfection methods. All subjects signed an informed consent and the research was approved by the Ethics Committee of the ISCSEM.
Results: Sixty students (73% female), ages between 22 and 40 (mean age 24), answered our questionnaire. It was found that the majority, 57%, brush their teeth twice a day and 95% use the Modified Bass brushing technique. Regarding their toothbrush care, 64% exchange their toothbrush every 2–3 months, 23% every 6 months, 10% under a month and 3% yearly. Also, 58% of the students reported to rinse their toothbrush after every use with tap water but don't remove the excess of it. While 25% remove this excess by tapping on the sink and 17% use a hand towel. It was also verified that 53% store their toothbrush without a cap in a cup on top of the sink, while 27% do the same but with a cap, 8% put their toothbrush inside a cabinet and 5% lay them on the sink. Only 15% reported knowing any kind of toothbrush disinfection methods, describing chemical methods only.
Discussion and conclusions: The subjects being future dentists have access to oral and general health information. Regarding their health care most follow the Portuguese (DGS) Directorate-General for Health and the American Dental Association recommendations. But according to their toothbrush storage, only some follow ADA guidelines. Only a few admitted to have knowledge about disinfection methods, being the chemical method the only one mentioned, but don't apply them in their daily oral hygiene routine. These results confirm the knowledge gap on this subject, and the need to reinforce awareness among the students, in order to inform and motivate their role as health educators and promoters in society.

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Prevalence of molar and canine class in children with ages between 6 and 15 years in the paediatric dentistry clinic of ISCSEM

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Introduction: Malocclusion is classified as an irregular development of the dental and/or arches that are established in the deciduous and permanent dentition [1,2]. It may affect a person's appearance, speech, and/or ability to eat [3–5]. Therefore, the aim of this study is to evaluate the prevalence of each molar and canine class in children with ages between 6 and 15 years, present at the pediatric dentistry clinic of Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM).

Materials and methods: The sample consisted of 191 children with a mean age of 11 years, ranging from a minimum of 6 years to a maximum of 15 years. Exclusion criteria: children with orthodontic treatment, children who present agenesis of the first molars and definitive canines, or who have facial asymmetry, craniofacial anomalies or syndromes. Inclusion criteria: Children of both sexes aged 6–15 years that were pacient at the Egas Moniz University Clinic and that the parents have signed an informed consent. This study was approved by ethics committee of ISCSEM. Statistical analysis involved measures of descriptive and inferential statistics. The level of significance to reject the null hypothesis was set at $(\alpha) \leq 0.05$. Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences) version 22.0 for Windows.

Results: The mean age was 11 years old. 74 females and 117 males. The majority had class I (62.4%), followed by class II (27.5%) and finally class III (10.1%), with no statistical differences by age or gender ($p = .984$, $p = .753$).

Discussion and conclusions: The results show that within the malocclusions, class I is the most prevalent. The gender and age did not appear to be a determining factor in the malocclusion of the majority of the children that compose this sample.

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Anterior open bite in a 3-year-old child – clinical case

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Introduction: The anterior open bite is considered a vertical anomaly that is located from canine to canine, which can be asymmetrical and depends on the position of the parafunctional oral habit [1,4]. The main cause of open bite is prolonged digital suction and inhibition of the sucking habit is the most important step in obtaining open bite correction [2,3]. The aim of this clinical case is to present a patient with an anterior open bite being diagnosed at an early age whose treatment consisted in eliminating the parafunctional habit.

Materials and methods: A 3-year-old male was referred to pediatric dentistry clinic, the parents' main complaint being the anterior open bite and a change in the position of the teeth. In the clinical analysis, the anterior open bite was confirmed to be 2 mm, a posterior crossbite of the 6.5 tooth and a small fracture of the tooth 5.1 were diagnosed, the child was treated after the parents have signed an informed consent.

Results: With the elimination of the pacifier habit within a period of 10 months it was possible to observe a closure of

the anterior open bite. The posterior crossbite of the 6.5 tooth has been maintained and can be correct later.
Discussion and conclusions: Prolonged use of the pacifier may lead to an anterior open bite, in other words, a reduced overbite, which may produce a posterior crossbite due to the absence of mastication and consequent extrusion of the molars. Mothers use the pacifier with the purpose of reassuring the baby and, up to the age of three, is considered a normal sucking habit [2,5]. When it remains, it is important and reasonable to consider the cost-effectiveness of its suspension for a number of changes in the dental arches that may arise [3], specifically an anterior open bite and posterior crossbite [5]. In this clinical case, it was verified that after removal of the pacifier the anterior open bite ended spontaneously, and later the crossbite will still have to be corrected.

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The child's self-perception about the loss of deciduous dentition in pediatric dentistry

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Introduction: The loss of deciduous teeth can result in a post-traumatic phenomenon with regard to the identity and creation of the self-image of the child's face [1–3]. This event is experienced transversely in childhood and usually occurs in the age group between 5 and 12 years [4]. The present study aims to understand how the child experiences the loss process and its implications on the self-perception inherent to the change of dentition.
Materials and methods: In this exploratory study, the sample consists of 100 children of both genders, aged between 5 and 12 years old. An instrument for the collection of information (protocol) was originally conceived, which is divided into two distinct sections: (i) section A, in which the child is asked to draw his self-portrait twice – before and after the loss of deciduous teeth; and (ii) section B, a set of open-ended questions. The interpretation of the drawings was carried out through a grid of content analysis, also designed for this purpose.
Results: The results suggest the existence of differences between the self-perception of the face before and after the loss of deciduous teeth. The content analysis also reveals some notable differences in the pictorial investment between the two drawings elaborated by the child.
Discussion and conclusions: The results of this study allow us to verify differences in the self-perception of the face image before and after the loss of deciduous teeth, which lead us to pinpoint possible clues that incite us to understand how the loss is felt and overcome by the child, with the support of the Dentist, through his role as Health Promoter and Educator.

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The language of silence in the therapeutic setting of dental medicine

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Introduction: Treatment adherence strategies, therapeutic outcome and patient satisfaction are intrinsically dependent of the complex relationship established by the dyad Dentist – Patient [1,2]. The aim of the present study is to explore the perceptions associated with the non-verbal communication that is installed in the therapeutic *setting* [3,4], more precisely in relational contexts of the practice of Dental Medicine. The study consists of evaluating the non-verbal language component, in the context of the Dentist–Patient relationship, as an integral part of the entire Dental Medicine consultation *setting*. Thus, we intend to (a) determine the extent to which non-verbal signals are apprehended both by Dentists and Patients; (b) evaluate the responses/perceptions of Dentists and Patients when exposed to the same non-verbal stimuli/signals; (c) to verify the existence of significant differences between Dentists and Patients in the capacity of apprehension of the signs.
Materials and methods: The study is of exploratory nature, has a transversal character and is of the quantitative type. The convenience sample consisted of 150 Dentists and 184 Patients. A measurement instrument was originally developed consisting of (I) two versions of the *Likert* scale to evaluate the perception of non-verbal behavior; one for Dentists and another for Patients, and (II) a Sociodemographic Questionnaire integrating psychosocial data of the participants. The following categories were evaluated: *Personal Space, Look and Facial Expression, Gestures and Postures.*
Results: The results suggest that patients do not seem to be aware of how they communicate their discomfort through nonverbal language. On the other hand, the patient's body language is perceived by the Dentist as a way to demonstrate discomfort and insecurity. Because of the physical proximity between the two dyad actors, the Patient tends not to look directly into the face of the Dentist, so he may not be aware of his facial expressions. However, the results regarding the Dentists show that they are aware that their adopting of a discomforting facial expression – as frowning may represent doubt, fear or surprise – can condition the patient's emotional state.
Discussion and conclusions: Deepening the knowledge over non-verbal communication (NVC) can redeem the ability of Dentists to better understand patients' internal experiences. Learning to read verbal and non-verbal language, recognizing emotional behaviours, doubts and difficulties of expression from the Patients allows the Dentists to develop their communication skills and thus to increase the choice of the most appropriate intervention strategies regarding Dental Medicine health care. It should be noted that, according to the preliminary results obtained in the present investigation, the Dentist stands as a reader of the patient's facial expressions at the level of the paralanguage.

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Evaluation of the training needs in medical emergency among Dentistry final year students, from Portugal and Spain

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Introduction: Several authors state that most dentists will, at some point in their career, be faced with an in-office emergency. Hence, they must have a basic knowledge of emergency medical care [1–4] and be prepared to prevent and manage episodes related to general health.
Material and methods: An online questionnaire, composed of 19 questions of closed answer, was applied to a total of 91 senior students (53 from ISCSEM and 38 from the UB) using the Survey Monkey platform.
Results: Results shown in Tables 1 and 2 indicate that students may have insufficient theoretical background resulting in greater insecurity when performing some medical emergency techniques such as venepuncture, preparation of IV medication, EKG analysis, AED and ALS.
Likewise, there is a suite of medical conditions that the pupils do not feel comfortable acting upon like: asthma crisis, stroke, hypertensive crisis, cardio-respiratory arrest and airway obstruction.
Discussion and conclusions: Overall, senior students state that they have a greater confidence when performing simpler medical emergency techniques (such as BP measurement, RBS test and oxygen administration), while they feel less prepared to execute more complex and specialized ones (such as EKG analysis, preparation of IV drugs, venepuncture, AED and ALS). As evidenced by this study the current workload dedicated to this subject during pre-graduate training is insufficient and there is a lack of knowledge in the medical emergency field. Therefore, the higher education institutions that deliver Master Degrees in Dental Medicine must find the appropriate educational formats to deliver this knowledge and build up self-confidence amongst the senior Dentistry scholars and future dentists, so that they can be prepared to act competently in real life-threatening situations, potentially saving lives in the process.

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Table 1. Summary table with overall result to the question: Do you feel capable of performing the below techniques?

	Answers for the both ISC SEM and UB (%)		
Do you feel capable of performing the below techniques	*Yes*	+/−	*No*
Measurement the Blood Pressure (BP)	92.3	7.7	0
Oxygen Administration	46.2	31.9	22
Non-Invasive Ventilation	38.5	27.5	34.1
Venipuncture	6.6	15.4	78
Preparation of IV Medication	8.8	25.3	65.9
Randam Blood Suger (RBS) Test	54.9	9.9	35.2
Electrocardiogram (EKG) Analysis	8.8	16.5	74.7
Automated External Defibrillation (AED)	22	17.6	60.4
Basic Life Support (BLS)	56	39.6	4.4
Advanced Life Support (ALS)	4.4	12.1	83.5

Table 2. Summary table with the overall result to the question: Do you feel competent to act in the following emergencies?

	Answers from both ISC SEM and UB (%)		
Competent to ACT:	*Yes*	+/−	*No*
Syncope	42.9	28.56	28.6
Seizure	27.5	47.3	25.3
Hypoglycemia	68.1	24.2	7.7
Asthma Crisis	17.6	34.1	48.4
Cardiopulmonary Arrest	17.6	50.5	31.9
Storke	5.5	19.8	74.7
Hypertensive Crisis	11	47.3	41.8
Airway Obstruction	15.4	51.6	33

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The creation of pedagogical instruments in the consultation setting (simulacrums) in the pre-graduate training in dental medicine

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Introduction: The purpose of this work is to manifest the relevance of the Health Education Instruments developed in the Theoretical-Practical component of the Communication in Health curricular unit (3°A MIMD). The objectives of the Curricular Unit and the competences to be developed are based on teaching the pragmatics of relational and pedagogical communication when applied to the communicational competencies of Dentists. Thereby, we consider relevant the acknowledgement of the specific training needs of Behavioral Sciences, when applied to Medical Sciences.
Materials and methods: The applied teaching methodologies consist in the creation of the simulation of "typical situations" in the clinic practice, held in a virtual doctor's office, to enable the student to face up to pedagogical barriers that take place when educating the patient [1] (pedagogical simulacrum). The dual empowerment methodology allows simultaneously to promote the learning and development of the *Other* and of *Self* (peer educators) through the conception and development of pedagogical actions (all participants in the simulacrum are the students themselves). We present as a paradigmatic example of a pedagogical simulacrum a case study, suitable to the target population selected – adolescents –, for which a health education instrument was proposed, using pictograms originally conceived as comics. The teaching act conducted through the proposed pedagogical instrument was limited to the use of a removable orthodontic appliance, exemplifying its hygiene and that of the oral cavity, as well as the prescription of drugs to be adopted in case of pain, in the context of the Orthodontic Care consultation of the Egas Moniz's Dental Clinic.
Results: Based on empirical experience, faculty members use active/participative pedagogical methodologies in the training of future professionals, promoting and validating their creative abilities in the construction of their own pedagogical instruments for the replacement of existing expositive approaches and therefore enabling of the teaching-learning process.
Discussion and conclusions: Training programs should include presentation and "ice breaker" exercises, brainstorming, group discussion, role playing, interactive games [2] and question-and-answer sessions based on the participants' perceptions and analytical thinking. In fact, in the daily life of a clinical practice built around a Patient-centered perspective, the Dentist assumes the role not only of Health Care Provider, but also of the Health Educator.

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BIOINFORMATICS

3D virtual planning and cad/cam technology applied to orthognathic surgery

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Introduction: In this clinical case, the feasibility and precision of tridimensional (3D) virtual planning is tested in one patient with craniofacial microsomia using Nemoceph 3D-OS software (Software Nemotec SL, Madrid, Spain) to predict postoperative outcomes on hard tissue and produce CAD/CAM (Computer Aided Design/Computer Aided Manufacturing) surgical splints.
Materials and methods: The clinical protocol consisted of 3D data acquisition of the craniofacial complex by cone-beam computed tomography (CBCT) and surface scanning of the plaster dental casts. The "virtual patient" created underwent virtual surgery and a simulation of postoperative results on hard tissues. Surgical splints were manufactured using CAD/CAM technology in order to transfer the virtual surgical plan to the operating room. Intraoperatively, both CAD/CAM and conventional surgical splints were compared. A second set of 3D images was obtained after surgery to acquire linear measurements and compare them with measurements obtained when predicting postoperative results virtually.
Results: It was found a high similarity between both types of surgical splints with equal fitting on the dental arches. The linear measurements presented some discrepancies between the actual surgical outcomes and the predicted results from the 3D virtual simulation, but caution must be taken in the analysis of these results due to several variables.
Discussion and conclusions: The reported case confirms the clinical feasibility of the described computer-assisted orthognathic surgical protocol. Further progress in the development of technologies for 3D image acquisition and improvements on software programs to simulate postoperative changes on soft tissue are required.

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Combining 2D and 3D image data for preoperative planning of corrective osteotomies in cubitus varus and cubitus valgus

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Introduction: The continuous progression of cubitus varus and valgus can lead to more severe problems, therefore requiring an efficient corrective method [1]. The most used technique is closing wedge osteotomy at the distal portion of the humerus [1,2]. However, its preoperative planning based on two 2D radiographs is not well suited to deal with a three dimensional bone malformation. This work presents a software interface that relies on the combination of the usual radiographs with a 3D model of the distal portion of the humerus, enabling the simulation of the surgical approach and the 3D representation of the postoperative humerus.
Materials and methods: The application is formed by a 2D panel (Figure 1(a)), for the radiograph-based planning, and a 3D panel, where the model of the bone is presented and the osteotomy simulation is performed (Figure 1(b)). 3D models were generated from the respective CT datasets. A total of three orthopaedists tested the application using a single case study.
Results: All participants found the application useful, easy to use and more precise than conventional methods. Although all methods were considered useful, two were not found to be the most adequate by some users. However, the surgical simulation was uniformly appreciated, as well as the visualization of the postoperative appearance of the humerus.
Discussion and conclusions: This work suggests this application is a viable solution for the planning of corrective osteotomies in cases of cubitus varus and cubitus valgus deformations. Also, results indicate its potential to be a more accurate, easier and faster option when compared to conventional methods, especially for less experienced medical professionals.

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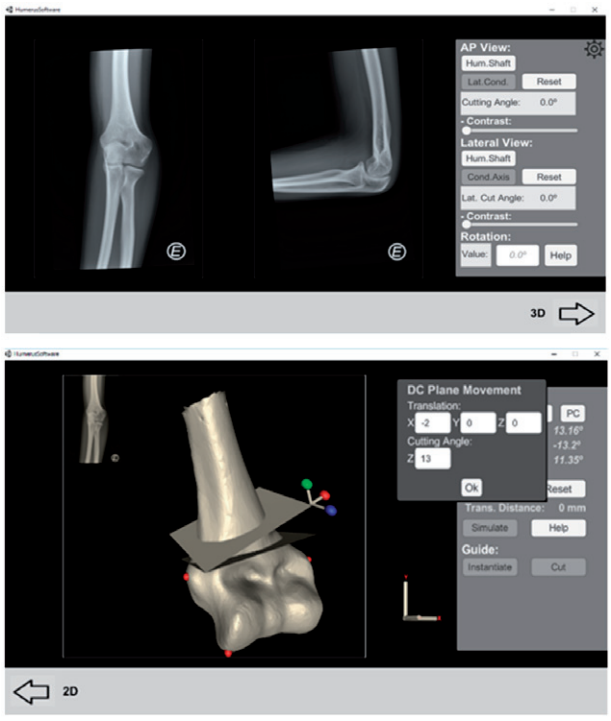

Figure 1. (a) 2D Panel – organized in three sections: the AP view, the lateral view and the rotation commands; (b) 3D Panel – instantiating and positioning the proximal cutting plane.

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Kinect-based biofeedback interfaces to improve upper limb rehabilitation

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Introduction: A sedentary lifestyle and bad eating habits are leading causes of cerebral vascular accidents or stroke [1]. Physical rehabilitation is often required to recover from upper extremity complications that commonly follow stroke. While computer-based interactive methods have been proposed to help defray person-hour costs, most require expensive dedicated hardware, lack good interfaces featuring user-centered biofeedback to better detect compensatory movements or assist the collaboration between patients and physiotherapists [2]. To approach these problems, we propose an inexpensive solution built with commercial off-the-shelf hardware to promote upper limb rehabilitation and collaboration between stroke patients and physiotherapists.

Materials and methods: The proposed system relies on a Kinect v2 to track the patient's body movement, a PC running Windows 10 and a pair of displays. Two distinct but related graphical user interfaces were developed: (i) a physiotherapist interface that contains a rich set of biofeedback portraying important patient kinematic data (e.g. shoulder flexion-extension and abduction-adduction angles) and compensatory movements (e.g. shoulder unevenness) in real-time; and (ii) a patient interface which displays a series of targets to be sequentially reached through arm elevation. Informal evaluations were performed by two physiotherapists and three physical medicine and rehabilitation physicians to validate both interfaces. Each participant was asked to verify if the proposed interfaces could help analyse or aid the treatment of acute stroke patients with physical limitations of the upper limbs.

Results: In the conducted interviews, all the interviewed participants indicated that distinct interfaces for the physiotherapist and patient is a meaningful feature, as the patient interface must be very minimalistic to avoid unnecessary distractions, whereas the physiotherapist must contain the full range of kinematic data. For the patient interface, it is important to track the completed repetitions, the session time of the proposed task and to display a few targets to be reached. As for the physiotherapist interface, it is important to visualize the vertical (sagittal plane) and horizontal (transverse plane) angles of the arm movement and, most noticeably, in which angle ranges the compensatory movements occur.

Discussion and conclusions: The proposed interfaces have received positive response from several rehabilitation

professionals. Although still in a pilot phase, the minimalistic design of the patient interface is devoid of graphical distractions, only delivering the necessary biofeedback for tracking shoulder movements by following predefined targets. On the other hand, the physiotherapist interface presents the necessary kinematic data and activity metrics to assist the evaluation and treatment of acute stroke patients.

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Estimating respiratory frequency by filtering Kinect v2 skeletal data

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Introduction: Monitoring a patient's breathing cycle is very important to identify early signs of serious complications manifested by decreased oxygen saturation, respiratory acidosis, and cardiac disturbances (e.g. cardiac arrest). This work presents a signal processing technique to measure respiratory frequency by relying only the skeletal tracking data of an inexpensive depth camera. Our approach aims to be useful for reading the breath frequency automatically in a non-invasive and markerless manner. This study applies a pass-band filter that magnifies the micro-movements [1] of skeleton's joints to compute the respiratory frequency.
Materials and methods: The Microsoft Kinect v2 was used as a depth camera which computes a skeletal representation of the human body, which consists of 25 notable points (joints and body extremities). Here, we only consider the 5 torso points to analyze the breathing movement.
To evaluate the system, we collected data from 6 subjects along four acquisitions of during 60 s each: seated with a normal breath; standing with a normal breath; seated with a heavy breath; and standing with a heavy breath. To assess our approach, we considered as ground truth data acquired from the BioSignalsPlux Researcher device. Informed consent was received from 6 volunteers (2 female, 4 male) with no history of lung disease or pain.
Results: The results show that it is possible to extract reliable kinematic data from skeletal data to calculate the respiratory rate. Figure 1 shows the differences between the estimated frequency and the data acquired from the BioSignalsPlux device.

Subject	Sitting normal breath	Standing normal breath	Sitting heavy breath	Standing heavy breath
1	0.181 bpm	3.199* bpm	0.050* bpm	0.222 bpm
2	0.037 bpm	0.092 bpm	0.019 bpm	8.661* bpm
3	0.633 bpm	3.618 bpm	1.104 bpm	3.872 bpm
4	0.008 bpm	0.160 bpm	0.543 bpm	5.881 bpm
5	0.133 bpm	7.174* bpm	1.547 bpm	0.069 bpm
6	0.766* bpm	0.036 bpm	0.137 bpm	0.333 bpm

Discussion and conclusions: The preliminary results are very promising, although relatively high discrepancies still persist and should not be ignored. Further acquisitions with many more subjects (e.g. dozens of participants) and in different settings (at rest, laid down, seated, standing, after moderate and intense exercises) are required to attain statistical significance. Still, the results reveal that it is possible to effectively acquire the respiratory rate via skeletal data from Kinect v2. In addition, more skeletal points need to be processed to properly map which points and point coordinate contains the most representative physiological signal.

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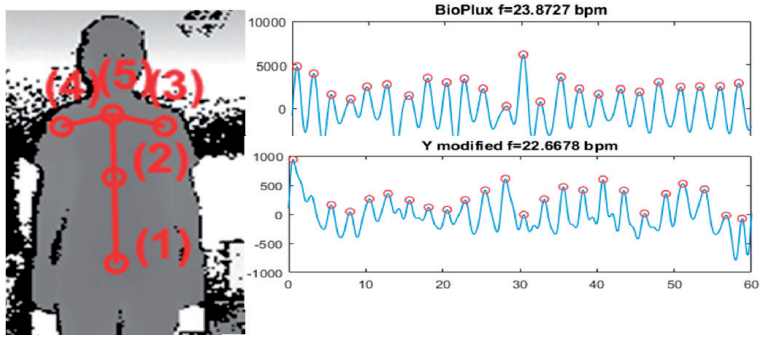

Figure 1. (Left) Skeletal data with 5 joints superimposed to the depth image. (Center) Plots of a normal breath cycle acquired with BioSignalsPlux (top) and with the Kinect v2 (bottom). (Right) Frequency breath difference between BioSignalsPlux and the closest result from the most representative point coordinate within the skeletal joints.
*Unsatisfactory BioSignalsPlux results; bpm – breaths per minute.

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Cross-validation and quality assessment of a prediction model based on salivary glucose for early screening of type 2 diabetes mellitus

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Introduction: Early screening of type 2 *diabetes mellitus* (DM) is essential for preventive treatment and effective delay of DM clinical complications [1]. However, testing for hyperglycemic status requires invasive and painful blood glucose (BG) testing, limiting its large-scale usefulness. To overcome this constraint salivary glucose assays have recently being studied as hyperglycemia (100–126 mg/dl BG) and type 2 DM (>126 mg/dl BG) screening tools [2]. Despite some promising results, to the best of our knowledge, no validation has been done on such salivary glucose assays for hyperglycemia/type 2 DM status detection. Consequently, we will fit, cross-validate and quality assess salivary glucose based prediction models for early screening of hyperglycemia/type 2 DM.
Materials and methods: Blood and salivary glucose data ($N_{total} = 84$) from Portuguese type 2 diabetics ($N = 29$) and healthy controls ($N = 55$) were randomly split in training (2/3) and validating (1/3) sample sets. The training sample set was used to fit hyperglycemia and type 2 DM prediction models, which were then cross-validated against the validating sample set as well as against foreign data (Brazil: $N_{DM} = 29$, $N_{Controls}=30$; India: $N_{DM} = 90$, $N_{Controls} = 80$). Prediction models accuracy for both hyperglycemia and type 2 DM screening was evaluated through ROC analysis.
Results: The salivary glucose based predicting models showed good accuracy predicting well both hyperglycemia (81.0%) and type 2 DM (84.5%) on our Portuguese validating sample set, while in the foreign data cross-validation accuracy was significantly lower (65.5% and 69.0%, respectively).
Discussion and conclusions: While our prediction models are well fitted for our Portuguese sample, cross-validation results using both Brazilian and Indian data showed a significant decrease on accuracy, suggesting that hyperglycemia status/type 2 DM prediction from salivary glucose should only be based on region specific models. Even if at present, due to several limitations, salivary glucose *per se* may not show enough cross-validation performance to be used as a global and autonomous early type 2 DM screening tool, our results suggest that it provides valuable information, and in the future may be combined with other salivary biomarkers to create an effective high sensitivity/specificity large-scale type 2 *diabetes mellitus* early screening system.

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NUTRITION

A novel quantitative approach for fish detection based on the use of mitochondrial gene markers

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Introduction: As a consequence of the growing seafood trade, more frequent cases of fish allergies have been recently reported, becoming an important public health issue nowadays. Fish allergy is estimated to affect about 0.1–0.4% of the general population. Through ingestion, direct contact or inhalation of their odors or fumes created during cooking, fish is able to trigger a hypersensitivity reaction mediated by immunoglobulin (Ig) E. Even when following a daily diet with total avoidance of the offending foods, the risk of allergic individuals of suffering from an abnormal immune episode remains a reality, mainly due to cross-contamination issues [1]. This fact emphasizes the need for accurate, fast and highly sensitive methods in order to manage trace levels of food allergens and protect fish-allergic consumers, while ensuring the accurate labelling of products. The main goal of this study was to develop a real-time PCR system using specific fluorescent hydrolysis probes to detect and quantify trace levels of fish in food matrices.
Materials and methods: Specimens of commercially relevant fish species available on the Portuguese market ($n = 25$) were acquired, including *Gadus morhua*, *Merluccius merluccius*, *Solea solea*, *Sardina pilchardus*, among others. The preparation of binary model mixtures was attempted to simulate the stuffing/filling of processed foods, such as patties and pies. The DNA was extracted using the Nucleospin® Food kit (Macherey-Nagel, Düren, Germany) with minor modifications. *In silico* analysis allowed the design of universal primers and probe targeting the 16S rRNA region of the mitochondrial genome of 25 fish species for the development of a fish-specific real-time PCR method.
Results: Specificity of primers was confirmed for most of the tested fish species, without reactivity with non-target species of plants ($n = 11$), avian and mammal meats ($n = 8$), crustaceans ($n = 16$) and mollusks ($n = 3$). A quantitative real-time PCR system was developed, with efficiencies close to 100% and coefficients of correlation (R^2) of 0.99. The method was able to detect and quantify down to 0.01 pg of fish DNA and 1 mg/kg of cooked fish muscle in a model food, with a dynamic range of 0.0001–50%. It was successfully validated with blind samples since bias ranged from 4 to 20% and relative standard deviations oscillated between 4.8 and 15.5%.
Discussion and conclusions: The present work allowed the development of a highly reliable DNA-based method targeting a 16S rRNA locus for the detection and quantification of an important allergenic food (fish) in processed matrices, being also important to control and manage allergen cross-contaminations in the food industry and, in this sense, to protect fish allergic individuals.

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Development of DNA-based methods for the identification of pistachio nut in foods

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Introduction: The consumption of tree nuts has been increasing in the past years, mostly due to their pleasant taste/aroma and to their association with several health benefits. Among the tree nuts, the pistachio nut (*Pistacia vera*) is widely appreciated, being consumed as raw/roasted in snacks or as an ingredient in a variety of food products, namely chocolates, ice creams, or cakes. Despite the alleged benefits, pistachio nuts pose a real health risk for a significant part of the population, namely the pistachio/cashew sensitised/allergic individuals. So far, five allergenic proteins have been identified and characterised in pistachio nut, being responsible for causing moderate to life-threatening immunological reactions (e.g. anaphylaxis) in allergic individuals [1,2]. The main objective of this work regards screening different DNA sequences coding for allergenic proteins, namely the Pis v 1, Pis v 2, Pis v 3, Pis v 4 and Pis v 5 in pistachio nut as potential markers for species identification in foods.
Materials and methods: Accordingly, genes coding for pistachio allergens were extensively *in silico* analysed, using the available nucleotide sequences at GenBank database. Considering the close genetic proximity of pistachio nut with mango and cashew nut species, only 3 DNA regions coding for Pis v 1, Pis v 2 and Pis v 5 allergens were used to design species-specific primers. Model mixtures of pasta spiked with known amounts of pistachio nut (50–0.0001% w/w, $n = 13$) were prepared. The DNA extraction was performed using the NucleoSpin® Food kit. Yield, purity and integrity of the DNA extracts were assessed by UV/Vis spectrophotometry and agarose gel electrophoresis. Qualitative PCR assays were optimised targeting each allergen-encoding gene (Pis v 1, Pis v 2 and Pis v 5). PCR fragments amplified with the best system were sequenced.
Results: Three PCR systems were successfully developed to enable absolute limits of detection (LOD) in a range of 1–10 pg of pistachio nut DNA and relative sensitivities of 0.05–0.005% of pistachio nut in pasta, depending on the target region (Pis v 1, Pis v 2 or Pis v 5). However, the three assays showed reactivity with mango and cashew nut.
Discussion and conclusions: The PCR assay targeting the Pis v 2 gene revealed the highest absolute and relative sensitivities, allowing detecting down to 1 pg of pistachio nut DNA and 50 mg/kg (0.005% w/w) of pistachio nut in pasta, respectively. Sequencing analysis of this region allowed identifying several nucleotide differences between pistachio nut and mango and cashew nut (9 and 11, respectively). This finding enables increasing the specificity of the assay based on the design of a hydrolysis probe for further development of a quantitative single-tube nested real-time PCR method.

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Nutritional and antioxidant screening of *Gracilaria vermiculophylla*

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Introduction: The nutritional composition of macroalgae, combined with their health beneficial properties, highlight them as interesting natural resources to be further explored. Within Rodophytas, *Gracilaria* is one of the species that has been used for animal feed supplement or replacement [1], but it can also be a good source of essential nutrients for human nutrition, with possible value for inclusion in diet or as an ingredient for new food formulations. This study aimed to evaluate *G. vermiculophylla's* nutritional profile and the influence of the extraction solvent in the total phenolic content and in the scavenging ability against hypochlorous acid (HOCl).
Materials and methods: Dried samples were acquired from a national specialty store (Porto), being thoroughly homogenized and ground before analysis. The screening comprised the evaluation of the following parameters: moisture, by thermogravimetric determination (SMO01, Scaltec); ash (incineration at 550 °C); protein, by the Kjeldahl method; total fat, by the Soxhlet procedure; and total dietary fibre (TDF), which were performed according to official methods [2]. Carbohydrates were calculated indirectly. The total phenolic compounds (TPC) and the scavenging capacity against HOCl, one of the most important reactive oxygen species, were both determined in aqueous, hydroalcoholic and ethanolic extracts [3].
Results: Overall, *G. vermiculophylla* presented a very high ash content (~30% on a dry weight basis, d.w.), total dietary fibre (~35% d.w.), high amounts of protein (~15% d.w.), and a very low fat level (<0.3% d.w.). The highest phenolic contents were found in both aqueous and hydroalcoholic extracts (45.9 ± 0.5 and 42.3 ± 0.8 mg/L of gallic acid equivalents, respectively, $p > .05$). In accordance, the highest scavenging efficiency against HOCl was observed for the same extracts ($IC_{50} = 8.0 \pm 0.6$ and 6.6 ± 0.3 μg/ml, $p > .05$).
Discussion and conclusions: In comparison with other red macroalgae available in the Portuguese market (e.g. *Palmaria palmata*), this macroalgae presents similar amounts of protein, fat, and TDF but is richer in minerals (19% d.w. in *P. palmata*) [4. G. *vermiculophylla* has great potential to be included in a healthy diet due to its high nutritional profile together with the promising antioxidant properties shown by both aqueous and hydroalcoholic extracts, which can be safely used for food pharmaceutical and cosmetic purposes.

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Nutritional composition and antioxidant activity of coffee silverskin (*Coffea canephora*) from different geographical origins

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Introduction: Coffee silverskin, a by-product of coffee roasting, emerges as natural source of several bioactive compounds [1,2]. Along the years, an increase of silverskin production has been noticed, due to the rise of coffee consumption worldwide [3]. The aim of this work was to evaluate, for the first time, the influence of different geographical origins on the nutritional composition and antioxidant activity of silverskin from *Coffea canephora* beans.
Materials and methods: Silverskin from six different geographical origins (Brazil, Uganda, Vietnam, Cameroon, Indonesia, and India), obtained by the same roasting procedure were provided by BICAFÉ (Portugal). Moisture content was determined using an infrared balance and the nutritional analyses were performed using AOAC methods. The results were expressed as g per 100 g of fresh weight. Hydroethanolic extracts (1:1) were prepared to evaluate antioxidant activity. Total phenolic content and ferric reducing antioxidant power (FRAP) of diluted extracts (1:10) were determined. Calibration curves with gallic acid (5–100mg/L; $r = 0.9984$) and ferrous sulphate (25–500 μmol/L; $r = 0.9996$) were

prepared, respectively, and results were expressed as mg of gallic acid equivalents (GAE)/L and μmol of ferrous sulphate equivalents (FSE)/L of extract. The radical scavenging activity ($DPPH^{\cdot}$ inhibition) was also studied and expressed as percentage (%) of $DPPH^{\cdot}$ inhibition. All experiments were performed in triplicate.
Results: Carbohydrates (including fibre) were the major macronutrient found (59–61%). The protein content of the evaluated samples was very high (17–20%), especially in Cameroon origin. Higher fat contents were found in silverskin from Brazil (3.15%). Total phenolics in extracts ranged from 104 to 406 mg GAE/L. The highest values were found in Brazilian and Indonesian silverskin extracts (406 mg GAE/L and 367 mg GAE/L, respectively). In accordance, higher antioxidant activities were observed for these two samples (41 and 39% of $DPPH^{\cdot}$ inhibition; 21 and 16 μmol FSE/L).
Discussion and conclusions: Significant differences ($p < .05$) were found between samples from different geographical origins, highlighting the relevance of chemical assessment. Overall, coffee silverskin is a by-product with a high potential for food applications, based on its nutritional composition and high antioxidant activity.

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Nutritional profile of green seeds from different species of legumes

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Introduction: Legumes represent an important component of the human diet, complementing the lack of protein/essential amino acids of cereals, roots, and tubers. In some regions of the world, legume seeds are the main protein supply in the diet [1]. Legumes are vital in agriculture as they form associations with bacteria that 'fix-nitrogen' from the air. Effectively, this contributes to soil fertilization and is the main reason why legumes are richer in proteins compared to other plants [2].
In this work, green (in *natura*) seeds of three different legume species (*Vigna unguiculata* L., *Phaseolus lunatus* L., and *Cajanus cajan* (L.) Millsp), from Brazil, were compared regarding their nutritional composition.
Materials and methods: Chemical analyses (ash, protein, fat and dietary fiber) were performed according to AOAC methods and moisture content was determined by a moisture analyser at 105 °C. The carbohydrates content was determined by difference.
Results: The results show that the nutritional profile of the different seeds is similar, although some significant differences were found for specific parameters. In a fresh weight basis, *V. unguiculata* seeds contained higher amounts of ash (1.8%), protein (9.9%), fat (0.7%) and carbohydrates (17.9%). In turn, *P. lunatus* presented the lowest contents of ash (1.5%), fat (0.3%) and carbohydrates (9.3%). Among the different species, no significant differences ($p > .05$) were found for dietary fiber levels (~11%) per 100 g of fresh sample. However, in a dry weight basis, *V. unguiculata* showed the lowest amount of fiber (26%, against 33–35%, for the remaining species).
Discussion and conclusions: The results show that in spite of the slight differences found on the nutritional profile, all the *natura* seeds analysed in this study are good sources of protein, carbohydrates and dietary fiber, containing simultaneously low levels of fat, and thus their consumption should be encouraged as part of a healthy diet.

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Physicochemical and bioactive characterization of honeys from different geographical origins: a comparison study

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Introduction: Nowadays consumers reveal more concerns with the food effects on their health and well-being. As a natural substance produced by honey bees, from the nectar or secretions of living parts of plants, honey is a reliable source of antioxidants (such as flavonoids and other phenolic compounds), with therapeutic potential. The honey composition and properties are mainly dependent on (a) botanical and geographical origin of the nectar; (b) honey bee species involved in its production; and (c) seasonal and environmental factors. Additionally, the processing, manipulation, packaging and storage time can also contribute to honey properties alteration [1]. The aim of this study is to characterize the physicochemical and bioactive composition of Portuguese honey samples and compare them to other world samples.
Materials and methods: Honey samples ($n = 27$) from different geographical origins were selected in supermarkets or directly from the producers. Antioxidant activity (DPPH$^{\bullet}$ scavenging activity and ferric reduction antioxidant power (FRAP)), total phenolic and flavonoid contents (TPC and TFC, respectively) as well as color parameters were analyzed. In addition, hydroxymethylfurfural (HMF) quantification and diastase activity (DA) were performed to estimate the shelf life and quality of honey.
Results: Significant differences were found between the antioxidant activity of the analyzed honeys, ranging from 6.1 to 36.5% and from 331.7 to 1845.6 μM Fe^{2+}, for DPPH and FRAP methods, respectively. TPC and TFC ranged between 6.6 – 29.9 mg of gallic acid equivalents/kg and 0.8–28.3 mg of epicatechin equivalents/kg, respectively. The HMF contents and DA values were in agreement with European legislation [2].
Discussion and conclusions: The influence of the geographical origin on the antioxidant properties was not significant. However, the honeys from foreign countries showed a lower antioxidant activity as well as TPC and TFC, in comparison with Portuguese samples. Color was correlated with the antioxidant activity. The values obtained for HMF and DA were typical of fresh and unprocessed honeys.

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Traditional sweet rice recipe – nutritional and sensory evaluation of friendly recipes for diabetics and lactose intolerants

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Introduction: Traditional food recipes are part of a country's heritage, frequently associated with traditional celebrations and events. Typically, they make use of regional ingredients and food preparation processes, being strongly influenced by the local geographic characteristics [1]. The nutritional content of some of these foods however, makes them not adequate to consumers with some pathological conditions, such as diabetes or lactose intolerance, which strongly restricts its consumption. With the traditional recipe sweet rice "Saloio" from Estremadura [2] as a benchmark, we aimed to introduce specific modifications, namely sugar and milk replacement, in order to make them appropriate to diabetics or lactose intolerants. The original and the innovated recipes were then analysed and compared concerning nutritionally and sensory properties.
Materials and methods: The traditional sweet rice was cooked according to the recipe. Afterwards, two modified recipes were developed, one using fructose instead of sucrose, and the other one replacing milk by lactose-free milk. The following parameters were determined for the nutritional composition: moisture, ash, nitrogen (protein), total fat, individual fatty acids, sugars and dietary fibre. The available carbohydrates and the energy value were obtained by calculation. Additionally, the traditional recipe of sweet rice, as well as the modified recipes, was used for the sensory analysis where a triangle test and a hedonic test were applied.
Results: For the nutritional analysis (per 100 g), of sweet rice recipes, the following results were obtained: an energy value between 94 kcal in the lactose-free recipe (LFR) and 110 kcal in the traditional recipe (TR); a protein content ranging from 1.89 g in LFR to 2.36 g in TR; a fat content from 1.27 g in LFR to 1.75 in the TR. The sensory analyses carried out with a trained sensory panel, highlighted differences between both modified recipes and the traditional one.
Discussion and conclusions: Results indicated that the major nutritional differences were the absence of lactose or sucrose in the modified recipes, as expected. Nevertheless, minor differences in the energetic value and in the protein, lipid and carbohydrates contents were detected, which can be attributed to the inherent differences among the ingredients. The sensory attributes of the modified recipes were, in general, considered satisfactory.

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Is the nutritional quality of Portuguese processed foods a cause of concern from a public health point of view?

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Introduction: Family lifestyle has changed greatly over the years, and nowadays there is a lack of time to prepare meals amongst other factors. Therefore, processed foods are a convenient alternative for several consumers. However, this type of foods is usually recognized as a source of salt, saturated and *trans* fatty acids, which are linked with the presence of

several chronic diseases, namely obesity, diabetes, cancer and cardiovascular diseases. This work was performed under the project PTranSALT which aims to evaluate the salt, total fat, and individual fatty acids profile of processed foods widely available in the Portuguese market in order to address further challenges in this area of research, as well as to update the current situation in Portugal.
Materials and methods: Between 2013 and 2016, 260 foods from different food categories (cookies, biscuits and wafers; potato and potato-products; ready-to-eat meals; bakery products; nuts and oilseeds; fast-food; snacks; cereal products; and sauces) were acquired in retail stores, supermarkets, food chains, restaurants and take away in Portugal. Total fat content was determined by acid hydrolysis and Soxhlet extraction with petroleum ether. Fatty acids methyl esters were obtained by cold transesterification and analysed by gas chromatography with flame ionization detection and the salt content was determined by Charpentier-Volhard's method. Besides the determination of these nutrients, different approaches were conducted: (a) comparisons among supermarket and commercial brands, but also among similar products from different commercial brands; (b) comparisons among similar foods with and without gluten and/or sugar and sugar-free; (c) accuracy of labeled nutrition declaration; (d) analysis of the contribution of processed foods for the daily intake of the analyzed nutrients; (e) effect of cooking methods on the nutritional quality of foods; and (f) establishment of further priority intervention areas.
Results: Regarding the salt content, the snacks, the fast-food, the sauces and the ready-to-eat meals had a significant higher amount than the other categories. The saturated fatty acids content was higher in bakery products (mean 7.07 g/100 g), cookies, biscuits and wafers (mean 9.11 g/100 g), and in the snacks group (mean 7.83 g/100 g). The highest levels of *trans* fatty acids were found in the fast-food group (mean 0.19 g/100 g), followed by the snacks, potato and potato-products (mean 0.07 g/100 g) and bakery products categories. Gluten-free cookies have higher fat content than cookies with gluten, but for salt the opposite was observed. Concerning the contribution for the daily intake, one of the obtained results was for curd cheese pie (one portion =193 g) which can reach 45% of the recommended value for salt intake.
Discussion and conclusions: Up to now food industry has developed efforts to decrease the salt and fat content of some food products. However, there are still foods with high salt and saturated fat content, and from a nutritional point of view this should be a priority area of intervention. Regarding *trans* fatty acids a significant decrease has been observed for the foods in which it is possible to perform an evolution of the last 30 years. The obtained results within our study are an effective assessment of the current status of the nutritional quality of Portuguese processed foods which will be important for further reformulation strategies and to monitor progress in the next years.

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Are detox juices a good source of nutrients?

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Introduction: Detox juices consist in a mixture of fruits and vegetables, and are very popular among consumers. They are considered as a good source to eliminate toxins, promote health, help with weight loss and are often used as a replacement for the main meals [1]. However, the scientific evidences which support their benefits are very limited and their suitability to replace main meals is not clear. The main objective of this study was to determine the nutritional contribution of two detox juices and to evaluate their appropriateness/impact as a meal replacer.
Materials and methods: In 2016, five types of fruits (orange, pear, banana, strawberry, kiwi), four types of vegetables (spinach, carrot, pumpkin, beetroot) and oilseeds were acquired in supermarkets, and were combined to prepare two recipes of detox juices. Samples were manually separated between edible and non-edible portion. The nutritional characterization of the detox juices was carried out by determining the following parameters: moisture, ash, protein, total fat and dietary fibre. The energy value (kcal/kJ) and the available carbohydrates were determined by calculation [2,3]. The moisture content was determined by gravimetric method (AOAC 952.08, 2000), using a dry air oven (Memmert, Germany) and total ash content by a process of incineration at 525 °C, according to AOAC 923.03. For total protein content, the Kjeldahl method was used, according to AOAC 991.20 [4]. For total fat content, acid hydrolysis followed by extraction with petroleum ether using a Soxhlet apparatus (Soxtec™ 2050, Foss, Hilleroed, Denmark) was used, and for total dietary fibre an enzymatic-gravimetric method (AOAC, 2000) was applied [4]. The nutritional contribution of the juices for adults was carried out using the reference intakes for energy and nutrients reported in Regulation (EU) n.° 1169/2011 [2].
Results: The analyzed juices had an energy value that varied between 26.3 and 64.6 kcal/100 g of edible portion, and contributed with 1% and 3%, respectively, for the energy reference intake. Water was the major constituent (varying from

83.4 ± 0.01 to 92.7 ± 0.04 g/100 g of edible portion) in the analysed samples, followed by carbohydrates (ranging between 5 ± 0.05 and 13.2 ± 0.06 g/100 g of edible portion). Their content in protein, total fat and dietary fibre was also low.
Discussion and conclusions: According to the results obtained, we can conclude that these detox juices should not be used as a substitute of main meals. They present a total energetic value and a macronutrients composition lower than the recommendations.

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Are yogurts and fermented milks that bear nutrition and health claims healthier than those that do not?

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Introduction: Non-communicable diseases are the leading cause of death in Europe and in Portugal [1,2]. Diets rich in vegetable, fruits and whole grain products and poor in foods high in energy, saturated fats, *trans* fats, free sugars and/or salt are recognized as an important factor that can contribute to reverse this situation [1]. An increasing number of foodstuffs put up for sale in Portugal bear nutrition and health claims. Since 2007, these claims are preauthorized volunteer statements on labels, advertising or other marketing products, listed in the EU register of nutrition and health claim [3]. In order to bear health-related claims the food should take into account the quantities of certain ingredients, such as fat, saturated fatty acids, trans-fatty acids, sugars and salt/sodium [3]. Nutrition and health claims may help the consumers identify healthier foods but recent research has also suggested these claims might be of negligible assistance or even hinder consumers in their decision-making for a variety of reasons [4]. Yogurts and fermented milks are seen as healthy foods. This study aimed to measure the prevalence of different types of nutrition and health claims on yogurts and fermented milks in a national sample and to assess the nutritional quality of the products bearing nutrition and health claims.
Materials and methods: A survey of pre-packaged yogurts and fermented milks available in national food retailers (online platforms of the hypermarkets Continente and Jumbo and of supermarket El Corte Inglés), excluding children yogurts, was performed in May 2017. R Project for Statistical Computing was used to design the study and data frame and to perform statistical data analysis. The data was explored via numerical and categorical explanatory variables. Nutrition information was taken from the nutrient declaration present on the online platforms and assessed through a comparison of median levels.
Results: Two hundred and thirty-six different references of yogurts and fermented milks (yogurts: 78%; fermented milks: 22%) were retrieved from those put for sale in the three online platforms. About 47% of these milk products carried nutrition and/or health claims. Ten different nutrition claims, mainly '0% fat' and 'no added sugar' were found in these products. Some fermented milks (23% of the total fermented milks) carried health claims. Yogurts carrying nutrition and/or health claims had statistical different levels of energy (kcal/100 g), protein (%), carbohydrates(%) and sugars(%) than those that had not; fermented milks bearing nutrition and health claims had only statistical different levels of energy (kcal/100 g) (Mann–Whitney test; $p < .05$). The results showed a very high prevalence of products with more than 10 g of sugars/100 g.
Discussion and conclusions: In general, yogurts and fermented milks carrying nutrition and health claims have better nutrition profiles than those that do not bear claims. The high levels of sugars and energy are of great public health concern.

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Effect of a tomato juice intake on resting heart rate, resting blood pressure, and perception level of recovery in elite swimmers

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Introduction: Sports recovery is an important inter- and intra-individual multilevel process for the re-establishment of athlete performance [1]. Sport drinks arose in order to complement the athletes' nutritional needs when their homoeostatic balance is affected [2]. Strenuous exercise affects this balance, leading to cell's oxidative stress that, without an appropriate recovery, will conduct to fatigue, inflammation reactions and muscular damage. Several alternatives have been developed to prevent these consequences [3]. Nowadays, tomato juice, which is rich in antioxidants, is studied as an enabler of the recovery process at sports level [4]. The aim of this work was to investigate the effect of the intake of a tomato juice on the resting heart rate, resting blood pressure, and perception level of recovery in elite swimmers over 2 months.
Materials and methods: Chemical analysis of Hydrophilic (HF) fraction of the tomato juice was performed, in order to characterize the antioxidant activity (DPPH and FRAP method) and total phenol content (TPC). Following ethical committee of *Cooperativa de Ensino Superior Egas Moniz* approval, the study was conducted after the recruitment of elite swimmers of *Sporting Clube de Portugal* (*SCP*) and acquirement of the informed consent of the subjects. A convenience sample was constituted and divided into two groups: control group (CG) and experimental group (EG). EG ingested 150 mL of the tomato juice on a daily basis after training over 2 months, while CG only served as comparison group, maintaining, all the athletes, their eating routine. Both physiologic and psychometric parameters were evaluated: the first ones through cardiovascular measurements while the others by the questionnaire RESTQP-Sport, in T_0, T_1 and T_2 (in the beginning, after 1 month and after 2 months of study, respectively). A food inquiry was also applied on a weekly basis in order to control the occurrence of biases on the study. Anonymized data was analyzed.
Results: For HF a TPC of 420.2 ± 8.7 mg/L GAE was obtained, and an antioxidant activity of 1400 ± 70.6 and 2326 ± 173.5 μg TE/L for FRAP and DPPH methods, respectively. At intervention level, with a sample of 19 athletes (CG, $n = 5$ and EG, $n = 14$) of *SCP*, it was only verified a significant difference for the resting heart rate (p=.015) and diastolic resting blood pressure (p=.021) over the time.
Discussion and conclusions: It can be concluded that the results show the high antioxidant activity and high total phenol content of the tomato juice. At intervention level, the results suggest a significant decrease on the resting heart rate and diastolic resting blood pressure over the time. Hereafter, will be required more similar studies in order to confirm the beneficial effects of a tomato juice intake in elite swimmers.

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Effect of *Cinnamomum burmannii* tea on postprandial glycaemia in type 2 diabetic adults

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Introduction: The type 2 diabetes *mellitus* is a chronic disease that affects 11.7% of the Portuguese population [1]. Cinnamon is a spicy used in the Portuguese cuisine, which has been associated with beneficial effects on postprandial blood glucose levels (BGL) [2]. The aim of the present study was to investigate the effect of cinnamon tea (6g C. *burmannii*/100 mL) on postprandial glycaemia in type 2 diabetic adults.

Materials and methods: Following ethical committee approval, 36 subjects were selected and randomly allocated in two groups ($n = 18$): cinnamon group, which was administrated OGTT (oral glucose tolerance test) followed by cinnamon tea; control group, which was administrated only OGTT. At baseline, anthropometric data, medical condition and pharmacological therapy were collected. A 24-h dietary recall was taken preceding each intervention. Food Processor SQL (version 10.5.9) program was used to analyze the food nutritional composition. Chemical analysis was performed for total phenols determinations (adapted from Prabha et al. [3]) and antioxidant activity for FRAP and for DPPH tests (adapted from Thaipong et al. [4]). Statistical analysis was performed using SPSS Statistics. Data are mean ± SEM.

Results: Results revealed that there is no interaction between the independent and repeated measures factors ($p = .870$), which means that it is not possible to infer about BGL differences in different moments. However, data suggest that cinnamon intake did not alter the area under the curve (AUC) ($p=.710$), maximum concentration ($C_{máx}$) ($p=.626$) and variation of maximum concentration ($\Delta C_{máx}$) ($p=.626$). Chemical analysis revealed that cinnamon tea, used in this study, has a high total phenolic content (1554.9 ± 72.8 mg/L of gallic acid) and a strong antioxidant activity according with FRAP (3658.8 ± 16.7 μmol Trolox Equivalent/L) and DPPH (5125.0 ± 74.3 μmol Trolox Equivalent/L) tests.

Discussion and conclusions: In spite of the high total phenolic content and antioxidant activity, determined for the cinnamon tea, the present study suggests that there are no differences in capillary glycaemia response between the two groups. These results are in agreement with previous findings in type 2 diabetic adults, where cinnamon administration did not significantly improve postprandial BGL [2]. This outcome could be explained considering the bioactive compounds composition of cinnamon extract, which can be different from other studies due to the extraction process (aqueous extract or powder) and the administrated doses [5].

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Hypocholesterolemic effect of Chaves hyperthermal spring water: a pilot study

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Introduction: Hypercholesterolemia is recognized as a major risk factor of atherosclerosis, one of the leading causes of morbidity and mortality worldwide [1]. Bicarbonated waters have shown several digestive properties and hypocholesterolemic potential [2,3]. Despite the richness and diversity of the Portuguese thermal spring waters, studies of this type carried out in Portugal are scarce. Chaves mineral-medicinal water is an hyperthermal water (76 °C), bicarbonated and rich in sodium, silica and fluoride. This study intended to explore the possible effect of the intake of Chaves hyperthermal water on the reduction of total cholesterol values among individuals undergoing treatment at Chaves Thermal spa.
Materials and methods: The study involved 34 individuals (27 women, 7 men) doing thermal treatment at Chaves Thermal spa for ≥10 d. Total cholesterol levels (measured in capillary blood) obtained at admission and on the last day of treatment were compared. Participants had the following cumulative inclusion criteria: age ≥18 years; no medical contraindication for drinking Chaves hyperthermal water; total cholesterol ≥200 mg/dL (at time of admission); hypocholesterolemic therapy initiated ≥6 months ago or no hypocholesterolemic therapy. Subjects which initiated hypocholesterolemic therapy or functional foods rich in phytosterols or omega-3 during the study period or presented intestinal transit disorders (diarrhea) were excluded. The participants consumed the hyperthermal water exclusively at the prescribed frequency and volume, and the time and volume of water intake were registered. Additionally, subjects were instructed not to deviate from their regular habits during the study (diet and exercise). Each participant gave an informed written consent before the study and the protocol was approved by the Ethical Committee of Egas Moniz C.R.L. Data were analyzed by using descriptive and inferential statistics methods. A significance level of 5% was considered for the later.
Results: Subjects ingested an average volume per dose of 97.5 ± 7.2 mL of hyperthermal water, 4.15 ± 0.7 times a day and the duration of treatment was 11.3 ± 1.8 days. Results showed a significant decrease in average total cholesterol levels, when comparing initial and final values: 235.6 ± 26.1 to 223.9 ± 38.4mg/dL ($p = .034$).
Discussion and conclusions: These promising preliminary results highlight the hypocholesterolemic potential effect of Chaves mineral-medicinal water in dyslipidemic adults, although further studies are needed.

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Melon seeds flour: a comparison of hydraulic and screw methods

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Introduction: Fruit industry generates high amounts of by-products. The valorization of these wastes is of utmost importance, not only for environmental sustainability but also in an economic and social point of view. An example is melon (*Cucumis melo L.*) used in industry that generates considerable amounts of seeds. According to FAO, the world melon cultivated area is approximately 900,000 ha, generating a production of around 800,000 tons of seeds [1]. These melon seeds can be used as alternative vegetable oil source, using different methodologies. After that the remaining product is a flour, a by-product with a potential for different applications. The flour could be a source of antioxidants, protein or fat, but their composition can be influenced by the used extraction methodology. To the best of our knowledge, few are described about this by-product as well as for the extraction techniques used. The aim of this study is to evaluate and compare the nutritional composition, as well as the antioxidant activity (through DPPH and FRAP assays) and total phenolic and flavonoid contents (TPC and TFC, respectively) of melon seeds flours, resulting from oil extraction by hydraulic and screw methods.

Materials and methods: Seeds of *Cucumis melo* L., Amarillo Canario cultivar, were submitted to a hydraulic and a screw press in October 2016 in Albacete (Spain), allowing obtain oil and flours. The flours were evaluated in order to determine and compare their nutritional composition (moisture, ash, fat, protein and carbohydrates), as well as TPC, TFC and the antioxidant activity (scavenging activity on DPPH radicals and ferric reducing antioxidant power, FRAP).
Results: In what concerns nutritional composition, flours obtained with hydraulic method presented the highest values of fat (18%), comparing to 5% in the screw method. Conversely, carbohydrates and protein are high in flours obtained by screw procedure. The TPC were 2403.8 and 1703.2 mg gallic acid equivalents per gram of dry basis sample (mg GAE/g db), respectively, for hydraulic and screw samples. Regarding TFC, the values obtained for hydraulic samples were higher (1682.8 mg of catechin equivalents per gram db sample (mg CEQ/g db)). Significant differences were found between the antioxidant activity of the analyzed seed flours for both methods used, being higher in flours obtained by screw press.
Discussion and conclusions: Seed flours from melon, obtained by the screw process of extraction presented higher antioxidant capacity and protein and carbohydrate contents. However, the hydraulic seed flours were richer in fat. The TPC and TFC content are higher in flours obtained by hydraulic process. Thus, the method used to obtain seed flours should be select according to the final use intended. Melon seeds flour could be used in food supplementation due to their ability to decrease the risk of oxidative stress-related diseases.

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Preliminary studies on the extraction of phenolic compounds from olive leaves of Portuguese cultivars

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Introduction: Phenolic compounds of olive tree (*Olea europaea*) are considered one of the responsibles of the health-promoting of Mediterranean diet [1]. Recent studies on oleuropein have shown several potential applications in human health like antidiabetic effect [2] and prevention of intraocular pressure elevation [3], among others [4]. In the present work, started in November of 2015, the optimization of extraction and analysis of phenolic components of leaves has been studied.
Materials and methods: Olive leaves of Galega Vulgar (G) and Cobrançosa (C) varieties with 8 years old were collected in November (N), March (M) and July (J). After drying at 50 °C and scrunching with an electric mill, extractions were performed with ethanol/water. After removing the solvent, the yield was calculated as % mass extracted per mass of dried leaf. Thin layer chromatography (TLC) of the ethanolic extracts was performed on a silica gel plate; spots were detected by iodine vapor (Figure 1).
Results: The yield of ethanolic fraction of Galega and Cobrançosa were different ($p < .05$) in November and July (Galega > Cobrançosa) but did not differ in March. The yields in November were lower than those of March and July, for both varieties. The results by TLC suggest that the general composition is not very different between samples, but these preliminary results need to be confirmed. It is interesting to note that although some hydroxytyrosol (HT) was detected, most of the oleuropein (O) remained intact (Figure 1). This fact showed that the entire procedure used, from the collection, drying, storage, extraction, evaporation and re-storage, did not destroy the larger secoiridoid of the leaf. If desired, a subsequent hydrolysis, by chemical or enzymatic or microbiological methods, may be performed in order to produce derivatives of lower molecular weight such as hydroxytyrosol.
Conclusion: Further assays to improve the purification and isolation of oleuropein from both olive varieties are now in development.

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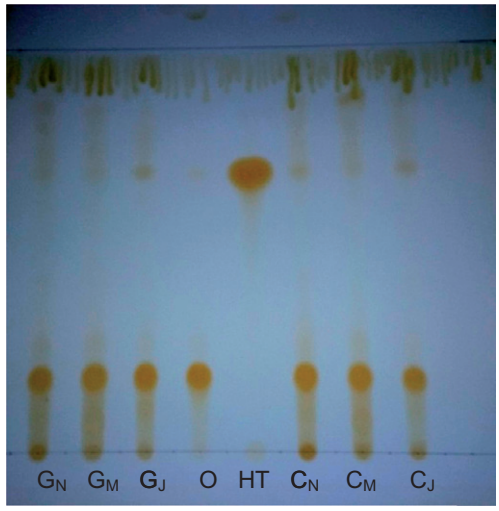

Figure 1. TLC of ethanolic extracts from Galega and Cobrançosa leaves, collected in November, March and July. Comparison with oleuropein (O) and hydroxytyrosol (HT) standards.

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Preliminary studies on the lipidic compounds in the gonads of *Catostylus tagi*

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Introduction: Marine invertebrates are among the most promising organisms as a source of new pharmaceutical drugs. The most promising chemical classes of new drugs are found in lipids, especially terpenoids, steroids and eicosanoids [1]. The objective of this study was to initiate the chemical characterization of the lipid compounds of the gonads of *Catostylus tagi*, a jellyfish native of the Tagus and Sado estuaries that can be eaten by humans allergic to seafood [2].
Materials and methods: Chloroform soluble compounds were obtained by soxhlet extraction from the lyophilized gonads of *C. tagi*. Chromatographic conditions: Samples were injected as trimethylsilyl derivatives. T_i: 250 °C; Split ratio: 1:20; gas rate (He): 1 mL/min. Column: VF5-ms, 30 m, 0.25 nm ID, 0.25 mm film; T_c: 60 °C (1 min), 2 °C/min until 90 °C (0 min), 3 °C/min until 280 °C (40.67 min). Equipment GC/MS: GC Agilent 6890N – MS Thermo DSQ.
Results: Stearic, palmitic and oleic, respectively, were the three most abundant fatty acids in both sexes. Considering sterols, cholesterol, 24-methylenecholesterol, campesterol and β-sitosterol were unequivocally detected both in male and female. The [(3β)-cholesta-5,24-dien-3-yloxy], RT 78.094, was confirmed only in male (Figure 1). Isofucosterol

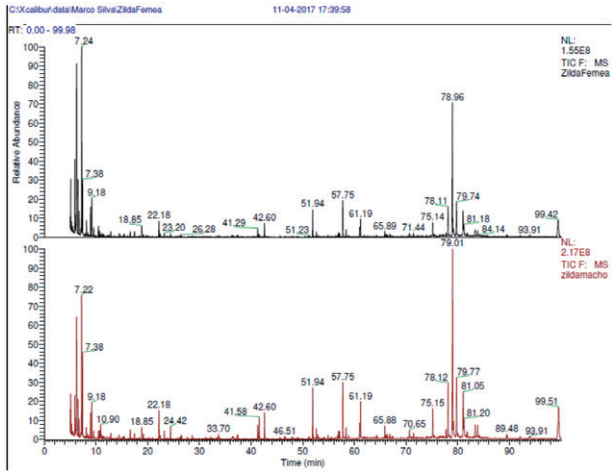

Figure 1. GC-MS of chloroform extracts of gonads of *C. tagi*.

(Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)) reported for a jellyfish [3] was not detected. These findings are in agreement with lipids reported for another cnidarian [4].
Conclusion: Studies focused at unambiguous chemical differentiation of each sex are now being planned.

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Vitamin C content in fruits: comparison with food composition databases

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Introduction: Vitamin C, which is naturally present in fruits and vegetables, is a water-soluble vitamin, with crucial functions in humans. Several studies have shown an inverse correlation between the regular consumption of fruits and vegetables and the development of chronic diseases [1]. Their beneficial features are linked to their nutritional and bioactive composition, where dietary fiber, vitamins, and minerals content are included. According to Costa et al. [2] Food Composition Databases (FCDBs) are sources of information which provide detailed information on nutrients and other components in foods [2]. However, for some foods, there is a need to update the vitamin C content in some FCDBs. The aim of this study was to measure vitamin C content of 14 types of fruits and to compare the analytical results with the Portuguese and American FCDBs [3,4].
Materials and methods: In 2016, 14 types of fruits were purchased on commercial areas in Lisbon (pineapple, kiwi, papaya, orange, lemon, raspberries, strawberries, cantaloupe, mango, banana, blueberries, pear, avocado and peach).

Samples were manually separated between edible portion and non-edible portion. The content of vitamin C (L-ascorbic acid and dehydroascorbic acid) was determined by a previously validated HPLC method [1,5].
Results: The vitamin C content in the analysed samples ranged from 3.8 to 59.4 ± 1.1 mg/100 g of edible portion for peach and pineapple, respectively. The vitamin C content (mg/100 g) of some analysed samples, such as banana (11.4 ± 0.4 mg/100 g), avocado (4.2 ± 0.3 mg/100 g) and peach (3.8 ± 0.02 mg/100 g) were more similar to the ones reported in the Portuguese FCDB (10, 3 and 4 mg/100 g of edible portion, respectively) than to the values reported in the American FCDB (9, 10, 7 mg/100 g of edible portion, respectively) (3, 4). However, other fruits, such as kiwi, papaya and lemon, showed significant differences between the analysed samples and the reported amounts in the Portuguese FCDB.
Discussion and conclusions: Vitamin C content of the analysed samples differ more from the results reported in USDA Nutrient Database than from the Portuguese FCDB, which enhances the importance of analysing the fruits consumed in our country. Moreover, regarding the comparison with the data reported in the Portuguese FCDB, considerable differences were found, indicating that it is crucial to continuously update the existing information, since this data is fundamental for the assessment of dietary intake and health impact.

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Food insecurity and its social and health determinants among a Portuguese sample of senior center attenders

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Introduction: Food insecurity (FI) is a social and public health problem [1], that affects older adults the most [2]. Being an indicator of individual health [3], Food Security appears, by definition, "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" [4]. The present study aims to identify determinants that are related to higher risks of Food Insecurity status among a sample of senior center (SC) attenders.
Materials and methods: This cross-sectional study was carried out among elderly participants in the municipality of Lisbon and was approved by a local ethics committee. Data was collected through face-to-face interviews and the informed consents were obtained. FI was assessed using FIES (Food Insecurity Experience Scale) instrument, measured with 3 categories: 1 = Food Secure; 2 = mild FI; 3 = Severe FI, and social risk using Gijon's social-familiar evaluation scale

(SFES). Multinomial regression models were used to assess the significance of social and health determinants predicting FI. To determine which variables should be present in the model, initially a set of 27 variables were considered. Based on a first phase of unifactorial tests, 11 variables were retained in the final model. For the model testing, the reference category was "Secure".

Results: The risk of having *"mild FI"* among the elderly of this sample is significantly higher for those who requested SC support because of their Lack of Money (OR = 5.562, $CI_{95\%}$ [2.028–15.249]). To declare Convivial as a reason for attending SC (OR = 0.068, $CI_{95\%}$ [.024-.199]) is related to lower chances of mild FI. Having *"Severe FI"* is significantly higher for those who requested SC support because of their Lack of Money (OR = 12.737, $CI_{95\%}$ [3.693–43.931]), and having higher levels of Social Risk (OR = 1.233, $CI_{95\%}$ [1.006–1.512]). Wishing for SC Convivial (OR = .127, $CI_{95\%}$ [.042–.387]) and having Diabetes (OR = 0.310, $CI_{95\%}$ [0.130–0.740]) is related to lower chances of Severe Food Insecurity.

Discussion and conclusions: This research shed some light on the complexity of FI among attenders of SC and may also highlight the importance of developing preventive intervention and programs focused on this burden, and consequently assuring food security to one of the most vulnerable populations, the older people.

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Food insecurity and obesity: revealing poor food choices among the unemployed

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Objective: Financial and economic constrains have been associated with poor food choices and less healthy lifestyle habits [1]. This association has been recently referred to as the obesity–poverty paradox [2,3]. The present study aimed to evaluate the prevalence of obesity and food insecurity (FI) and characterize food intake in a sample of unemployed participants.

Material and methods: This cross-sectional study was conducted in a support recruitment centre. The proceedings of the study included anthropometry and body composition analysis, socio-demographic survey, FI scale, and food intake analysis using a food frequency questionnaire (FFQ). The data from the food intake analysis was converted into nutrient intake values using the Portuguese nutritional table.

Results: In total, 65% of unemployed participants were overweight, and 30% were obese. The prevalence of FI among this socially vulnerable group was 51%. The food intake assessment revealed high sugar and fat intakes, low fiber intake, and insufficient micronutrient supply.

Conclusions: Our results expose the nutritional vulnerability of the unemployed participants and the relationship between unemployment, obesity, and FI. These findings also reinforce the need for planning and implementing effective community nutrition programs that target the unemployed individuals.

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Obesity and dependency among elderly receiving home care services: revealing the need for nutritional and home environment assessment

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Objective: Over the past few decades, ageing of the population has been a major concern for public health. Dependent seniors frequently receive home care support services, especially those related to hygiene and food assistance [1,2]. As dependency has been considered a risk both for malnutrition and obesity [3], our aim was to study assess the nutritional status and to evaluate home facilities and environment of seniors receiving home care support services (HCS) in Almada, Portugal.
Material and methods: Cross-sectional study enrolling a convenience sample of 51 old adults living in their homes and receiving HCS. Data were collected through personal interview and observation. Anthropometric and social-demographic data were collected. Mini nutritional assessment (MNA) was performed. Independency for Activities of Daily Living (ADL) were assessed through the Katz index and the Lawton scale. Environmental and behavioral characteristics were also assessed during home care service.
Results: Fifty-one old adults (34 females) with a mean age of 83.71 ± 7.46 years. The majority of the sample was considered dependent for ADL according to both the Katz index (61% of females and 70.6% of males) and the Lawton scale (91.2% of females and 70.6% of males). Mean BMI was 27.02 ± 4.97 kg m^{-2}, with 66.67% of the sample above 25 kg m $^{-2}$. Overweight was present in 39.22% and overall obesity was 27.45%. Only 8.8% of females and 11.8% of males were considered malnourished by MNA assessment. The main reasons for HCS were dependency for ADL (41.5%) and food assistance requirement (34.1%). Food assistance by HCS was negatively correlated with Katz index ($r = -0.366$, $p = .019$) and Lawton scale ($r = -0.394$, $p = .011$). About 97.6% of the sample lived in a house with proper conditions, and 24.4% lived alone.
Conclusion: We demonstrated the presence of overweight and obesity among seniors receiving HCS, along with a with prevalence of dependency. We also demonstrate that food assistance is one of the main reasons for require HCS. Emergent data from epidemiological studies have demonstrated that home care support should not be regarded through a quantitative point of view; but more importantly, a special attention should be given to the quality of support. This is particular relevant when food quality is involved. Our results highlight the need for the integration of nutritionists in the HCS teams.

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Social and health determinants for malnutrition and malnutrition risk among elderly senior center attenders in the city of Lisbon

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Introduction: In recent decades, population pyramids have dramatically change worldwide [1] and Portugal has not been an exception [2]. Nutritional status is important to older adults to live without disabilities. In fact, the elderly are more susceptible than the younger to the impact of nutrition and other health-related factors, such as food insecurity (FI) [1]. Elders' susceptibility has been related to frailty, presence of chronic conditions, poor incomes and need for assistance in activities of daily life [3]. The present study aimed to identify determinants that are related to higher risk of Malnutrition status among a sample of senior center attenders.

Materials and methods: This cross-sectional study was carried out from September 2015 to February 2016 and approved by a local ethics Committee. Data were collected through structured face-to-face interview and the informed consent of the subjects has been obtained. Malnutrition was determined using the Mini Nutritional Assessment (MNA) instrument, measured with 3 categories: 1 = Malnutrition; 2= Risk of Malnutrition; 3 = Nourished. Multinomial regression models were used to assess the significance of social and health determinants predicting malnutrition. To determine which variables should be present in the model, initially a set of 27 variables were considered. Based on a first phase of unifactorial tests, 11 variables were retained in the final model. For the model testing, the reference category was Nourished, from which the other two categories were compared.

Results: The risk of "*Malnutrition*" among the elderly of the present sample is significantly higher for Women (OR = 7.87, $CI_{95\%}$ [1.325–46.724]), also among participants with higher scores of Food Insecurity (OR = 1.728, $CI_{95\%}$ [1.20–2.475]), and for elderly who have Dementia – light or severe – (OR = 37.41, $CI_{95\%}$ [2.06–679.55]). Having Diabetes (OR = 0.105, $CI_{95\%}$ [0.010–1.056]) and being part of the age group between 74 and 85 years old (OR = 0.102, $CI_{95\%}$ [0.018-.570]) reduces the chances for these seniors to have a Malnutrition diagnostic. Also, the results indicated that seniors who have reported Loneliness (OR = 2.0009, $CI_{95\%}$ [1.059–3.813]) – as a motive for attending the senior center- were more likely to be at "*Risk of Malnutrition*", as well as seniors who have had Acute Myocardial Infarction (OR = 2.119, $CI_{95\%}$ [0.951–4.718]), and Cerebrovascular Accident (OR = 4.036, $CI_{95\%}$ [1.185–13.744]). Elders who reported having better Health status (OR= 0.539, $CI_{95\%}$ [0.367–0.792]) and attending the senior center for less than 5 years (OR = 0.408, $CI_{95\%}$ [0.160–1.037]) have lower chances of been at *Risk of Malnutrition*.

Discussion and conclusions: The present findings suggested that nutritional status of the elderly is very much related to their health status, but also with their social conditions, where loneliness and food insecurity are contributors to malnutrition.

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First acceptability trial of a *Catostylus tagi* snack in healthy untrained panellists

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Introduction: International food monitoring agencies for sustainability have proposed that responsible consumption of seafood should not only respect the abundance and seasonality of species, but should also focus on local products that

Figure 1. *C. tagi* snack.

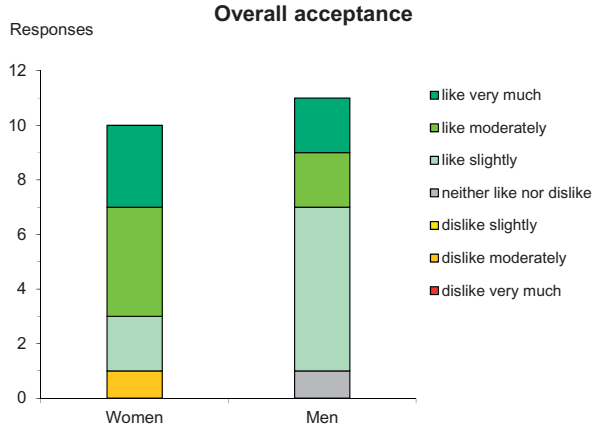

Figure 2. Overall acceptance of *C. tagi* snack.

are underutilized [1]. In this sense, the culinary use of the edible *C. tagi* medusa, native of the Tagus and Sado estuaries, could be an effective and innovative contribution. After discarding the allergenicity [2] and defined the sensory profile [3], a sensory assay of general acceptance, to initiate acceptability studies in which *C. tagi* is one of the main components was carried out. The results are described in this abstract.

Materials and methods: *Sample preparation*: the jellyfish was treated according to Morais & Raposo [4], prepared as mayonnaise pâté, 15% w/w, and spread on wheat toast or cracker, according to the taster's option. The ratio pâté:toast was approximately 50% w/w (Figure 1). *Sensorial analysis, acceptance test*: a panel of 21 marine researchers, 10 women and 11 men, mostly in the age group 22–40 years old and a hedonic rating scale, ranged from dislike very much to like very much, was used to assess the overall acceptance of *C. tagi* snack [5]. The experiment was held in Vasco da Gama Aquarium in June 8th 2016.

Results and discussion:

The responses were compiled in Figure 2. In summary, 90% of the responses were distributed in positive acceptance categories ranged from like slightly to like very much. The overall acceptance reported for men was slightly different comparing to women: 55% of men's responses were reported for the category *like slightly* whereas 70% of the women responses were distributed between the categories *like moderately* and *like very much*. The light marine flavour was the most appreciated attribute.

Conclusions: Further assays to improve the snack preparation are now in development.

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Accuracy of labeled nutrition declaration among cereal products

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Introduction: In the last years, consumers are paying increasing attention to the nutritional composition of foods, in order to make healthier food choices, and therefore to prevent chronic diseases. The European Union has faced the challenge of standardizing the nutritional declaration of prepacked foods. Therefore, in 2011, Regulation (EU) No. 1169/2011 on the provision of food information to consumers was published [1]. Accordingly, and concerning the declared values on the nutrition declaration, they should be an average and can be obtained by: (a) the manufacturer's analysis of the food; (b) a calculation from the known or actual average values of the ingredients used; and (c) a calculation from generally established and accepted data. However, the food nutrient content should not deviate markedly from labeled values, otherwise consumers can be misled. Therefore, a tolerance guide regarding the deviations of labeled values for the different nutrients for analytical control purposes has been set up [2]. In our study, an analysis regarding the accuracy of labeled nutrition declaration in cereal products was performed.
Materials and methods: In 2015, twelve samples of cereal products (cereal bars and breakfast cereals) were selected from the major supermarket chains in Portugal. An Excel® form was filled out with the labeled information for each one of the selected foods. Afterwards, the same foods were analysed concerning their salt, total fat and fatty acids composition, according to the methods described by Albuquerque et al. [3]. Then, all the information was used to perform an analysis of the accuracy of the declared values, considering the tolerance limits defined for salt, total fat and saturated fatty acids (SFA).
Results: Considering salt, in two (17%) of the selected foods, the declared values were not in compliance with the tolerance limits, being the analytical values lower than the lower tolerance value. The comparison between declared and analytical values for total fat shows that all the selected foods were within the tolerance limits fixed for this nutrient. On the other hand, for SFA, 33% of the samples were out of the tolerance limits, but from those samples, only one had an analytical value (10.3 g/100 g) higher than the upper tolerance value (4.7 g/100 g).
Discussion and conclusions: Food labels, namely nutrition declaration, are the most direct source of information for consumers. Therefore, it is very important that these labels provide accurate information in order to allow consumers to make informed and healthier dietary choices. In our study, SFA values were the ones for which a higher number of samples was out of the tolerance limits.

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FORENSICS AND FORENSIC PSYCHOLOGY

Development of a novel methodology to produce synthetic cannabinoids' combusted products for cellular toxicity assays

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Introduction: Synthetic cannabinoids (SC) have become a public health concern due to the poorly understood toxicological mechanisms and the easy access on internet [1,2]. Recently, some countries have banned the novel psychoactive substances (NPS) to avoid "an imminent hazard to the public safety" [1]. Toxicological data must be gathered to understand the impact of these substances and ban NPS as a public health threat. Current approach to address SC toxicological impact uses standards. However, SC are mostly smoked which hides the real impact of these NPS. To overcome this issue, we have developed a burning device for synthetic cannabinoids and other smoked substances of abuse. The in-house built burning device was designed to trap combustion products of synthetic cannabinoids, mimicking some physiological and biochemical conditions in humans (Figure 1).

Materials and methods: The burning device incorporates a burning chamber and two solvent traps to mimic aqueous cellular mediums and membranes. The presence of combustion products from burned nicotine and JWH-018 was confirmed by GC/MS and its toxic impact was tested on yeast cells.

Results: Figure 2 shows GC/MS chromatograms combustion products of nicotine and JWH-018. To identify the emerging substances from the burning procedure, mass spectra were analysed using NIST 2014 database. Burned nicotine chromatogram shows that three main new peaks were found (Figure 2(A)) corresponding to myosmine, nicotyrine and cotinine. A chromatographic peak at 28.4 min was identified as a combustion product of JWH-018 not present in the NIST 2014 database and was named J1 (Figure 2(B)). Product J1 (MW 177) have an *m/z* 160 fragment that corresponds to the loss of hydroxyl group present in the end of alkyl group (-OH), and consequently the *m/z* 146 and 133 fragments by the loss of a methyl (-CH3) or an ethyl (-CH2CH3), respectively. The benzene group is also represented by the *m/z* 77 fragment. This experiment was performed in triplicate and results were consistent for the major new peaks. JWH-018 and its combustion products were tested on yeast cells and the results show that combustion products decrease the growth rate of yeast cells when compared with parent drug.

Discussion and conclusions: Our results show that our burning device generates combustion products of drugs. As far as we know, it is the first approach to trap combustion products from synthetic cannabinoids, as they are generated during their smoking, and to use it in toxicological studies.

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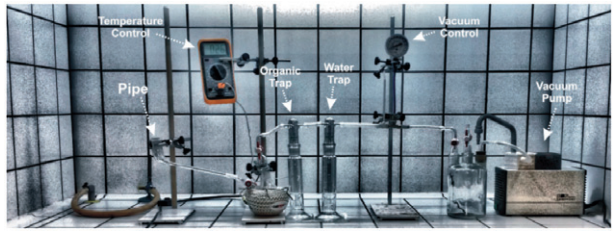

Figure 1. Art-work photography of the in-house built burning device previously described.

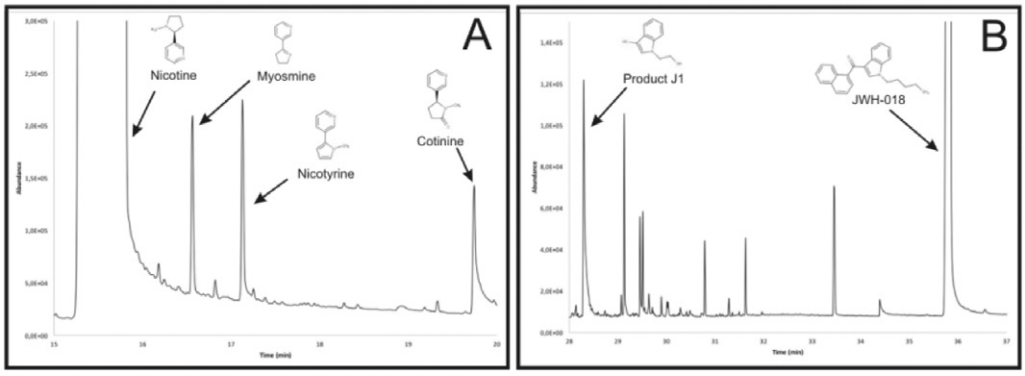

Figure 2. GC/MS Chromatograms of nicotine (A) and JWH-018 (B) burned in our in-house built burning device.

Towards understanding of synthetic cannabinoids evolvement pathways through a molecular docking approach

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Introduction: Synthetic cannabinoids (SC) were initially developed with research purposes for the study of the endocannabinoids pathways and associated pharmacology [1], however, it has rapidly become one of the largest and most dynamic groups of novel psychoactive substances. The European Monitoring Centre for Drugs and Drugs addiction (EMCDDA) has a monitoring list of 160 SCs that have been seized from 2008 till 2015. Currently, *in vivo* and *in vitro* approaches are used to understand the effect of the drugs. However, *in silico* approach offers fast and low cost methods for studying these SCs. The present study models the binding of several reported synthetic cannabinoids into the cannabinoid receptor CB1 which is responsible for inducing the psychoactive effects of the synthetic cannabinoids. This was achieved through a homology modelling [2] and flexible docking approach that allows the identification and evaluation of potential pharmacophores [3] that can help predict the directions synthetic cannabinoids may take in the future.
Materials and methods: Autodock vina was used to evaluate the binding position, conformation and associated binding energy of several synthetic cannabinoids and the human cannabinoid receptor CB1 (PDB:5U09). Docking was performed with the default autodock vina parameters, and the search region was defined by a grid box that encompassed solely the C-terminal and extracellular region of the receptor, having all residues within the binding region been defined as flexible. The SCs chosen were those present in the EMCDDA monitoring list, having their 3D structures been retrieved from the PubChem and ChemSpider chemical databases.
Results: The binding conformation corresponding to the lowest binding affinity from each docking procedure was used to identify both the SCs groups and the receptor amino acid residues required for binding. Preliminary results show that there are common chemical groups to all SCs families involved in binding, sharing amino acid residues of the receptor as showed in Figure 1.
Discussion and conclusion: Analysis of the interactions between the ligands and the cannabinoid receptor shows a higher number of amino acid residues involved in the binding of the SCs when compared to Δ-9 THC. This result points a molecular base for the differences in affinities observed for these molecules. Moreover, identification of both chemical groups and the amino acids residues involved in the binding, may help the prediction of the direction future SCs could take.

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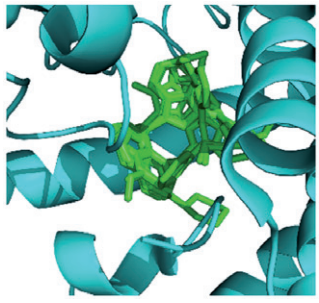

Figure 1. Superimposed image of 10 Naphthoylindoles molecules at the binding site.

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Novel strategies to address the challenge of synthetic cannabinoids: first developments

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Introduction: The use of novel psychoactive substances (NPS) has been increasing and has become a major public health concern [1]. These substances are legal until specifically scheduled by legislation, therefore they are widely distributed on the internet and headshops as harmless products commercially classed as herbal blends or incense [2]. Synthetic cannabinoids (SC) are the most diversified and fastest growing category of NPS [1]. SC are mimetic of Δ^9-THC, the primary active principle found in cannabis, and they are full agonists of CB1 receptor. Consequently, SC have a cannabis-like effect. However, they appear to present higher risks to users than those observed with Δ^9-THC [3]. Previously we put forward that SC phase I metabolites present higher levels of toxicity than the parent drug [4]. Here we propose that combustion products are as toxic as SC phase I metabolites.
Materials and methods: THJ-018 was acquired from the internet with a purity degree of 98.5%. This SC was burned in a house-built burning device to produce its combustion products. The presence of these compounds was confirmed by GC/MS analysis. The cytotoxic properties of THJ-018 and its combustion products were assessed through MTT assay in human neuroblastoma cell line SH-SY5Y.
Results: Figure 1 shows the MTT results for SH-SY5Y viability in the presence of THJ-018 and its combustion products. SH-SY5Y cell viability does not decrease in the presence of THJ-018 at the range of concentrations used. However, when the same cell line is exposed to THJ-018's combustion products there is a decrease in cell viability at the highest concentrations used.
Discussion and conclusions: The results established that combustion products of THJ-018 are more toxic to the neuroblastoma cells than the parent drug. This is not completely unexpected since combustion products are oxidized compounds of the parent drug, involving hydroxylation among others modifications, and our last work shows that hydroxylated phase I metabolites of SC presents a higher level of toxicity when compared to the parent drug [4]. Our results suggest that the toxicological profile of combustion products of SC might be a key to study long term and chronic adverse effects of these psychoactive substances.

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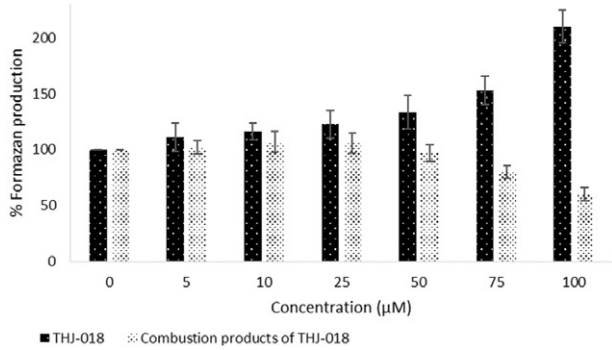

Figure 1. Cytotoxic evaluation by MTT assay of THJ-018 and combustion products of THJ-018 in human neuroblastoma cells SH-SY5Y. Data are expressed as the mean ± SD ($n = 3$).

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An investigation of digoxin by cyclic voltammetry using gold and silver solid electrodes and chemometric analysis

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Introduction: Digoxin, from the foxglove genus *Digitalis*, has been used for many years as one of the main medications for congestive heart failure. Since it made its entrance into common medical practice, over 200 years ago [1], there have been continuous writings with regard to its toxicity [2]. Instances of associated illness have been well documented, demonstrating that the timeline of *Digitalis*' toxicity continues and medical practitioners should be heedful of the poisonous potential that can result from its ignorant prescription [3]. Studies as far back as 1329 [4] have reported the use of *Digitalis* as the cause of death, proving that accidental overdose as well as cases of suicide and homicidal intent are just some of the threats that this medication poses. Charles Cullen, one of the most prolific serial killers in American history, took advantage of this and used Digoxin as his poisonous weapon of choice [5]. It is believed that he murdered over 200 people using this method of lethal injection, which went completely undetected for over 15 years. The drug poses an analytical challenge too, however, it has been shown to have a strong analytical response when under investigation by voltammetric techniques. In this study we investigated the suitability of gold and silver electrodes in voltammetric analysis of this compound, in order to present a simple, time and cost effective method for the analysis of Digoxin.
Materials and methods: Cyclic voltammograms were obtained using a 757 VA Computrace Metrohm connected to a Compaq deskpro personal computer. Principal Component Analysis (PCA) was performed on voltammogram data using Tanagra data mining software. Medusa graph analysis software has been used to analyse and visualise the concentration of the different species in solution versus pH.
Results: Analytical determination of digoxin in solution was successful at concentrations between 3.92 and 14.81 μg/ml. The limit of detection when using a gold electrode at pH 11 was 1.7 μg/mL with a corresponding coefficient of variation of 2.07% and 4.13%, respectively, for both RED-OX and OX-RED sweeps. Analysis with the silver electrode shows the limit of detection is best at pH 11 and pH 5 with 0.9 μg/mL, with a coefficient of variation of −3.90% and −1.61% at pH 5 and pH 11, respectively.
Discussion and conclusions: The voltammetric data and subsequent chemometric analysis demonstrated the best analytical conditions for both electrodes by the calculated limit of detection and linearity measurements. Both gold and silver electrodes were good candidates in the voltammetric analysis of Digoxin for identification and quantification purposes, presenting a novel time and cost effective analytical approach. The use of solid electrodes here facilitates the use of portable electrochemical systems.

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Mitochondrial DNA: approach of a genetic marker with population, forensic and medico-legal applications

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Introduction: There are at least three main distinct areas of mtDNA research. Population genetics/molecular anthropology, study's the differences between human populations. Forensic genetics, evaluates the chances for matches between samples. Medical genetics, hunts pathogenic mutations. In this letter, we will not deal with medical genetics.
Materials and methods: Review of mitochondrial DNA literature using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/).
Results, discussion and conclusions: Population geneticists have been interested in the analysis of mtDNA sequences from the beginning of the 1980s. However, it was the medical field that took the lead in sequencing mitochondrial coding region [1,2]. In the last years, mtDNA data have been stored and made publicly available via the Internet in a growing number of databases including MITOMAP (www.mitomap.org), the Human DNA polymerase Gamma Mutation Database (http://tools.niehs.nih.gov/polg/), PhyloTree (www.phylotree.org) and the EDNAP mtDNA Population Database, short EMPOP (http://www.empop.org). Mitochondrial DNA analysis has become a routine target in forensic casework where standard nuclear markers cannot be applied. Mitochondrial DNA databases stand as the basis for frequency estimations of mtDNA sequences that became relevant in forensic cases. Among all databases, EMPOP provides the leading mtDNA database of control-region sequences for comparison with partial mtDNA sequences from forensic traces [3]. Online release 11, dated from 2013, currently offers 34,617 entire control-region sequences. Comparison of a trace sequence with the sequences from databases can provide rough estimates of match probabilities in certain human population groups.
At the beginning of the 1990s, forensic geneticists also realized the importance of mtDNA in human identification cases and also for the study of evidential material with very little nuclear DNA (for example highly degraded biological material and hair shafts) [4].

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Forms of violence prior to femicide in abusive intimate relationships

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Introduction: Homicide is one of the most significant causes of premature death and it is estimated that more than a third of female homicides globally are perpetrated by an intimate partner [1]. In Portugal, it is stated that around 85% of all female homicides are committed by a current or past intimate partner [2], a far higher figure than the one recorded at the international level. This paper is a retrospective and exploratory study on the various forms of violence prior to femicide – the homicide of women in the context of intimate relationships – in cases with previous intimate partner violence (IPV).
Materials and methods: Judicial records of 25 femicide cases with previous IPV, committed between 2010 and 2015, in Greater Lisbon, were selected and analyzed (71.4% of the total case files reviewed) quantitatively and qualitatively, through the content analysis of the data regarding the various forms of violence prior to femicide. Only the data strictly necessary to carry out the research were collected and were subsequently transformed into numerical information, making it impossible to identify the participants, thus ensuring their anonymity. The study protocol was submitted to the validation of the Ethics Committee of Universidade Fernando Pessoa and obtained a favorable opinion.
Results: Results indicate that 84% of the victims have suffered from psychological violence, including threats to kill (68%), threats with weapons (28%) and suicide threats (28%), 60% physical violence (non-fatal strangulation in 16%), 48% stalking, 20% controlling behaviors and 4% sexual violence. The severity, the injuries caused and the frequency of the abuses vary drastically between cases, although 80% of victims suffered more than one type of violence. About half of the victims (48%) had already lodged a complaint for domestic violence to the authorities and an increase in severity and/or frequency of the violence was identified in 34% of the cases. 60% of the couples were in the process of separation and, in 64% of these cases, the femicide occurred within the first 2 months following the separation. Most offenders (56%) had a history of substance abuse, 36% had previous convictions and 36% had access to a firearm.
Discussion and conclusions: We conclude that there are opportunities for prevention, identification of at-risk women and appropriate intervention, that the detection of previous IPV is not enough and that it is essential to take measures that effectively protect victims. The first months after separation constitute a period of high risk [3] and measures such as substance abuse prevention and restricting the perpetrator's access to firearms can positively contribute to the reduction of cases of femicide. Risk assessment practices should integrate and analyze the various forms of violence in detail. This article emphasizes the need to reinforce qualitative research on the subject and adopt a holistic approach regarding the data collection methods, complementing case file reviews with interviews with proxies and perpetrators, thus allowing a more complete view of the phenomenon.

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Female-perpetrated homicides against intimate male partners

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Introduction: The aim of the present investigation is to characterize empirically and theoretically the population of females who commit murder against their intimate partners. These types of occurrences are rare comparing with male counterparts, and few studies have been carried out, particularly in Portugal. According with statistics in Portugal, in 2016 were committed 76 homicides in witch 10.4% of defendants were females [1]. Considering intimate partner homicides, in 2015, 7.7% of the defendants convicted were females [2].
Materials and methods: At theoretical level it was used the systematic review of literature method. This review included academic sources from databases using the following keywords equation: (female-perpetrated homicide) AND (risk) AND (prevalence OR trends). At empirical level and with the purpose of characterize the Portuguese women who kill their intimate partners it was identified 8 homicide cases between 2008 and 2015 in metropolitan area of Lisbon. The collected information was assessed qualitatively and the case study approach was carried out.
Results: The results of international investigations reveal that these women tend to have a pattern of disruptive family history [4–7]. In Portugal the pattern tend to be the same in which two females lost their parents in early ages, at least one grew on a disadvantage family environment and one was victim of sexual abuse during childhood. The presence of

violence against family members, violence against acquaintances or strangers and psychiatric antecedents are the risk factors more frequently observed from our sample of women who kill their intimate partners. Episodes of violence perpetrated by the male victim during the relationship were frequent and were mainly verbal abuse, slaps/pushes, punches/kicks and beatings/strangulations. Theoretically some authors defend that this perpetration of violence against female offenders are the most important factor that motivates the homicides [3,5,8]. Regarding to motivations for the homicide four occur immediately after aggressions committed by the male victim against the female offender, one during mutual aggressions, one after a quarrel between the victim and the offender, one during a psychological decompensating and one related to jealousy about a new relationship of the victim.
Discussion and conclusions: Our results allow us to conclude that these women tend to live in contexts of socioeconomic disadvantage during their life course and the majority of the homicides committed occur in contexts of violence perpetrated by the male victim being this risk factor the one that stands out.

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Homicide in intimacy: a meta-analysis on risk factors

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Introduction: Homicide in intimate relationships is one of the most prevalent causes of death for women worldwide. This meta-analysis aims to identify and to integrated, through analytical and statistical methodologies, the risk factors associated with homicide in intimate relationships.
Materials and Methods: The studies analyzed were selected from eight databases (PsycInfo, PsyArticles, Scopus, MedLine, Web of Science, SAGE, Pubmed and Science Direct) published between 1998 and the present, with most studies being published in the 2000s. All the studies analyzed are retrospective, with control group, performed mostly in North America, whose main purpose was to study the femicide.
Results: Risk factors were identified across five categories: structural (e.g. ethnicity), individual (e.g. history of domestic violence), situational (e.g. separation), criminal behavior (e.g. stalking) and victim-homicidal dyad (e.g. formal relationship). The most studied risk factor was criminal behavior.
Discussion and conclusions: Femicide was the target of greater scientific attention compared to intimate partner homicide in general. Nevertheless, a more extensive approach is necessary to understand this subject, using more heterogeneous samples: female, male and mixed samples, as well as including homosexuals and heterosexuals relationships. The results evidenced that it is necessary to conduct studies that allow a better understanding of the factors associated with this phenomenon, in order to improve the efficiency of the investigative action of intimate partner homicides, making crime repression more effective and the victims protection more effective.

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Forensic psychology assessment in cases of intimate partner violence: the relationship between neuroticism and psychopathology

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Introduction: The role of mental health in judicial ruling has fostered forensic psychology to gain special prominence in the Criminal Justice System [1]. Currently, through psychological assessment protocols defined for this purpose, the Egas Moniz Institute has contributed to the identification of risk factors for intimate partner violence (IPV). The assessment of psychopathology and personality traits of offenders constitutes a central element to an intelligible dimension of the individual's global functioning structure. Specifically in Neuroticism (N), offenders tend to be emotionally insecure, show difficulties in controlling impulses and a high vulnerability to stress [2].
Materials and methods: The sample ($n = 83$) is derived from the IPV risk assessments of Egas Moniz Assessment Offices (GIAV & GPF) (2011–2016). The sample is composed by a majority of Portuguese individuals (89.2%), men (98.2%) with basic education (30.1%), unemployed (27.7%) and aged between 22 and 80 years old ($M = 46.6$, $sd = 11.76$). We use the Brief Symptom Inventory (BSI; Derogatis, 1993; Portuguese version Canavarro, 1995), a psychopathological inventory of self-response symptoms. And the Neo-Revised Personality Inventory (NEO-PI-R, Costa & McCrae, 1992; Portuguese adaptation Lima & Simões, 2000, 2009), a self-response instrument to assess personality traits based on the Big Five model.
Results: There were positive correlations between Neuroticism and the following BSI dimensions: Obsessions-compulsions ($r = .60$, $p = .023$); Depression ($r = .59$, $p = .028$); c) Hostility ($r = .54$, $p = .047$); Phobic anxiety ($r = .58$, $p = .030$); and Psychoticism ($r = .60$, $p = .024$). When decomposing the facets of Neuroticism, we demonstrate a positive correlation between self-consciousness and hostility ($r = .74$, $p = .003$).
Discussion and conclusions: The correlation with Obsessions-compulsions indicates a relationship between emotional vulnerabilities and impulse control. Depression suggests vulnerability to stress and emotional insecurity. In hostility, subjects tend to experience a higher number of thoughts and emotions associated with negative affective states of cholera; phobic anxiety characteristic shows an irrational/disproportionate behavior towards a stimulus; and Psychoticism refers to interpersonal isolation. Finally, a correlation between Self-consciousness and Hostility suggesting that social anxiety and inferiority feelings tend to relate to negative emotions. These results demonstrate a moderate-strong relationship between neurotic traits and vulnerability for psychopathology development in offenders of IPV [2] and support the trend for a new line of prevention and interventions in the field of Forensic Psychology Assessment.

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The forensic psychology role: the victims information and assistance office

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Introduction: The purpose of this paper is to demonstrate the work developed by the Victims Information and Assistance Office (GIAV), and specifically about intimate partner violence (IPV) risk assessments. GIAV was created in partnership with Egas Moniz Institute and it is located at the Combat Unit against Domestic Violence, 7th Section of the Lisbon Public Prosecutor's Office. GIAV's main goals are performing domestic violence risk assessments, assist victims, and perform crisis interventions as part of the domestic violence criminal proceedings, as well as scientific research. In its role

of technical advisor to the Public Prosecutor's Office, GIAV presents itself as the main response to cases with higher complexity and it provides guidance about the necessary measures to protect victims [3,4].
Materials and methods: Data were collected between November 2011 and March 2017 from lawsuits, semi-structured interviews of both the victim and the defendant [Psychopathy Checklist Screening Version, Hart, Cox, & Hare, 1995, Portuguese Version [2]], collateral information and clinical and forensic assessment tools [e.g. Brief Symptom Inventory, Derogatis, 1993; Portuguese Version Canavarro, 1995; Spousal Assault Risk Assessment, Kropp, Hart, Belfrage, Webster & Eaves, 2003, Portuguese Version [1]]. A total of 202 IPV risk assessments were performed by GIAV: 46.6% from victims, 43.5% defendants and 9.4% were simultaneously victims and defendants of intimate violence.
Results: Victims had more symptoms of depression, phobic anxiety, paranoid ideation (30%) and anxiety (31.3%) than the defendants (18.5% depression and paranoid ideation symptoms). Victim-defendants individuals showed symptoms associated with paranoid ideation (56.3%), interpersonal sensitivity and depression (50%). The defendant's personality assessments unveiled that 43% do not have any personality disorder. However, 15.1% had antisocial personality disorder. In the risk assessments, the majority of the cases presented a moderate risk (38.2%), especially factors associated with recent relationship problems (61.8%), former physical assault (60.8%) and extreme minimization or denial of spousal assault history (60.8%).
Discussion and conclusions: The data show that depression, phobic anxiety, paranoid ideation and anxiety are the most common psychiatric symptomatology indicators in victims, while paranoid ideation, interpersonal sensitivity and depression in victim-defendants. The main risk factors for IPV are the recent personal relationship problems and extreme minimization or denial. Moderated risk is the most common level in the cases assessed by this Office. GIAV is a perfect example of a good articulation among Forensic Psychology and Law [4], is an asset in technical reporting to the judicial system, it acts close to the victim and helps in the legal decision and serving the citizen.

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PSYCHOLOGY

"Mens Sana in Corpore Sano": the mental representation of the skin in tattooed individuals

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Introduction: The present qualitative and exploratory study aims at understanding the mental representation of the skin [1,2], in tattooed individuals and the role that tattoos play in body image [3,4].
Materials and methods: The sample consisted of 145 individuals from both genders, who were invited to produce two self-portraits, one before and one after they were tattooed. We then proceeded to the analysis of the content of the 292 collected pictorial drawings, using a grid featuring analytical categories, purposely conceived for this study. All participants answered a brief socio-demographic inquiry and provided a written answer on whether they have experienced any form of discrimination due to their tattooed body.
Results: The results reveal preferences on the location of the tattooed body part as well as a prolific symbology associated with the designs chosen. This symbology is frequently associated to relevant personal events or meaningful people (e.g. pets, objects). Superstition is also a frequent theme, with references to protection amulets.
Discussion and conclusions: The study analyzed the function of the tattooed skin as a vehicle for the projected identity of the *Self* [5,6]. The self-portraits before the subjects were tattooed show less investment than those after they were tattooed. However, the mental representation of the self-portraits drawn after the subjects were tattooed show a fragmented idea of the body – only the body part that was tattooed is pictorially represented [7]. Tattoos serve as anchors to the *Self* and narrate an individual's conception of his or her identity, helping to solidify a sense of *Self* that relates to culture in a coherent way.

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Childhood traumatic experiences and adult adjustment

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Introduction: There are many children who, throughout their lives, have gone through traumatic experiences [1]. Given these experiences, individuals tend to mobilize specific coping strategies, either adaptive (e.g. seeking social support) or maladaptive (e.g. denial) [2]. The main goals of this study are to characterize childhood traumatic experiences and to analyse the relation between childhood trauma and coping strategies in adults, as well as to verify the existence of differences in the childhood traumatic experiences and coping strategies in adulthood as regards sociodemographic variables.
Materials and methods: The current study has an observational, descriptive and cross-sectional design, with a non-probability convenience sample composed by 150 Portuguese adults. For data collection, we used the Childhood Trauma Questionnaire [3] (short version) and the Brief COPE [4].
Results: The results point to a relatively low mean of childhood traumatic experiences ($M = 45.46$; $SD = 7.29$). There is an association between childhood traumatic experiences and coping strategies used in adulthood, according to the type of victimization suffered. The participants show more adaptive coping strategies, centred around planning ($M = 6.18$; $SD = 1.30$) and active coping ($M = 6.11$; $SD = 1.29$) and less maladaptive coping strategies, centred around behavioural disinvestment ($M = 2.65$; SD=.98) and substance use ($M = 2.25$; SD=.80). There are statistically significant differences in the childhood traumatic experiences per age group – participants over 87 years old have higher means when it came to reporting physical neglect ($M = 8.13$; $SD = 4.22$). The older participants also show higher means in planning ($M = 7.00$; $SD = 1.00$), religion ($M = 4.60$; $SD = 2.03$) and acceptance ($M = 6.27$; $SD = 1.16$). Regarding sex, there are no statistically significant differences concerning the traumatic experiences, as opposed to the adopted coping strategies – women adopt adaptive coping strategies (e.g. expression of feelings – $M = 4.91$; $SD = 1.68$), while men have the higher mean in the substance abuse scale ($M = 2.41$; $SD = 1.05$). Regarding marital status, singles resort more to instrumental support ($M = 5.21$; $SD = 1.55$), while widows resort more to religion ($M = 5.05$; $SD = 2.09$).
Discussion and conclusions: It is possible to establish a relation between childhood trauma and maladaptive coping strategies in adulthood, although the literature about this phenomenon is still scarce. Faced with adversity, participants of this study reveal a tendency to use confrontational strategies, mostly centred around planning, active coping, positive reinterpretation and acceptance. In-depth knowledge of the relation between trauma and coping will allow the improvement of intervention programs.

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Cognitive distortions on sexual abuse of children and empathy: an exploratory approach of a normative sample

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Introduction: This paper intends to present the relationship between the cognitive distortions on sexual abuse of minors and the perspective of Marshall and Barbaree [1] that emphasize the possibility that the low levels of empathy can disinhibit deviant sexual behavior, justifying this fact with the incapacity of these subjects Recognize or feel compassion for the suffering of their victims, that is, empathy.
Materials and methods: This study consisted of $N = 1193$ individuals, 45.3% male and 54.6% female. The ages ranged from 18 to 74 years ($M = 27.25$, $sd = 9.62$). The following instruments were used:Abel & Becker Cognition Scale (EC; Abel, Becker, & Cunningham-Rathner, 1984; transated by Baúto & Cardoso, 2011) Self-report scale assessing the existence of cognitive distortions on child sexual abuse; Empathy Quocient-Short Version (EQ-SV; Muncer & Ling, 2006; translated by Baúto & Cardoso, 2011) self-report instrument that aims at assess the levels of empathy (how the individual perceives the emotions and feelings of others, their social competences, and their reaction and impact caused by perceiving damage in the other).
Results: When analyzing the association between subscales of the Abel & Becker Cognition Scale (EC) and the Empirical Quotient (EQ), we found statistically significant correlations between different subscales. Regarding the "Social Competences", it has correlations with the subscales of "Benefit in Sexual Practices between Adults and Children" ($r = -16$, $p = .000$), "Decision Ability and Child Initiative in Sexual Practices ($r = -.19$, $p = .000$), "Positive Perception of Sexual Practices with Children" ($r = -.13$, $p = .000$), "Child as Sexual Being" $= .000$) and "Banalization of Sexual Contacts" ($r = -.20$, $p = .000$). At the same level of analysis, we verified that the subscale "Emotional Reactivity" correlates with the same subscales of the Cognition Scale (EC), "benefit in sexual practices between adults and children" ($r = -.19$, $p = .000$), "Decision Ability and Child's Initiative in Sexual Practices" ($r = -15$, $p = .000$), "Positive Perception of Sexual Practices with Children" ($r = -16$, $p = .000$) Child as Sexual Being "($r = -.15$, $p = .000$) and" Banalization of Sexual Contacts "($r = -14$, $p = .000$).
Discussion and conclusions: We conclude that the more present the cognitive distortions on child sexual abuse, the less social skills and the ability to react to the suffering of others. This result may be related to the fact that the literature recognizes the possibility of empirical deficits, only as a way of maintaining the act or preserving the subject's self-image. Given the sample in question, these results suggest that empathy deficits may arise only in post-passage [2]. Since this is a normative sample, these results reinforce the need to analyze these variables in forensic populations.

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Effects of sleep difficulties on global quality of life

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Introduction: The relationship between sleep difficulties and quality of life (QoL) is multifaceted and extensive, and in the literature several studies have shown a negative association between sleep difficulties and QoL, i.e. there is a lower QoL in individuals with sleep disturbances (e.g. insomnia), with greater commitment of the daily activities and greater

loss of productivity at work. Also, health-related quality of life is almost always affected in individuals with sleep disturbance, namely excessive daytime sleep or difficulties in initiating or maintaining sleep. Besides the scientific evidence, some studies found no significant differences between the groups of participants with and without sleep difficulties, regarding the mental and physical component of QoL. The main objective of the current cross-sectional study was to assess the effects of sleep problems on quality of life of Portuguese normative population.
Materials and methods: The non-probability convenience sample included a total of 1119 adults from general Portuguese population. Participants were excluded if they did not answer affirmatively to one of the three DSM-V [1] criteria to clinically diagnose insomnia. Moreover, we assess these symptoms "during the last month" and consequently, the criteria C and criteria D ("The sleep difficulty is present for at least 3 months") were not considered. Thus, sleep disturbances and not insomnia are evaluated. Final sample was composed by 987 participants that completed a self-administered questionnaire with socio-demographic and sleep-related items. QoL was assessed with WHOQOL-Bref [2–4].
Results: Sleep Difficulties Index (SDI) has a significant and negative association with all QoL domains, being Physical Health the domain with the strongest correlation ($r = -.402$, $p < .001$). Index of Subjective Day Well-Being has a significant positive correlation with Physical Health ($r = .111$, $p < .001$) and Psychological ($r = .150$, $p < .001$) domain of QoL. Also, some items about sleep satisfaction are significantly correlated with QoL domains: the quality and depth of sleep and the appropriate number of sleep hours are positively associated with all domains of QoL, with exception of the Psychological domain that is not significantly associated with sleep depth. Physical domain has the strongest association with each variable, namely: sleep quality ($r = .379$, $p < .001$); sleep depth ($r = .396$, $p < .001$) and appropriate number of sleep hours ($r = .147$, $p < .001$). Contrariwise, the number of hours needed to feel good is not significantly correlated with QoL.
Discussion and conclusions: Perceived sleep difficulties are inversely related to QoL, i.e. greater sleep difficulties are associated with lower QoL in the psychological, physical, social relations and environmental factors domains. The physical domain is the one that is most affected by sleep satisfaction, reinforcing the importance of a satisfying sleep for a daily physical well-being.

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Sleep quality in the general Portuguese population

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Introduction: Epidemiological data from Portugal found that 10.1% of the sample obtained reported global sleep dissatisfaction and 28% showed one or more symptoms of insomnia at least 3 nights per week [1]. Sleep difficulties have a significant impact on individual functioning and quality of life (QoL), resulting in decreased ability to perform daily and professional activities [2]. In order to extend the study of sleep difficulties in Portuguese population, the main objective of the current study is to characterize sleep patterns and sleep difficulties and to identify possible psychosocial variables that may influence them.
Materials and methods: The sample is composed by 1119 Portuguese adults. The current study has an observational, descriptive and cross-sectional design. As part of a convenience sample, all participants signed informed consent and completed the questionnaire, which includes questions regarding socio-demographic features, sleep patterns, sleep difficulties, global functioning and sleep satisfaction.
Results: The majority of the participants are female ($n = 685$, 61.2%), with ages between 18 and 25 years old ($n = 260$, 23.4%), married or partnered ($n = 569$, 51.7%) and high school graduates ($n = 466$, 42.1%). The sleep-related questionnaire presented the following results: (a) sleep patterns: The majority of the participants usually have 6–8 h of sleep during the week ($n = 698$, 62.9%) and they take only 1–15 min to fall asleep ($n = 490$, 44%). The majority of participants reported that they wake up during the night ($n = 791$, 70.7%) and before the time they needed ($n = 741$, 66.2%); (b) sleep difficulties: 314 (28.2%) participants reported sleep disturbances, and from this group, 171 (46.8%) are reasonably concerned with the sleep disorder; (c) global functioning: most of participants, never or rarely perceived difficulties in staying awake while studying or working ($n = 668$, 60.5%) and frequently have energy to perform activities ($n = 519$, 47.1%); (d) sleep

satisfaction: 468 (41.9%) individuals considered 7–8 h a day as the appropriate sleep time, 504 (45.2%) perceived good quality of sleep and 546 (48.9%) participants reported that their number of sleep hours is, frequently or always, appropriated. The mean of total score of Sleep Difficulties Index (SDI) [3] is 1.97 ($SD = .56$; Min =0; Max =4), suggesting that most of participants have some (but not several) sleep difficulties. There are significant differences on SDI scores between gender [$F(1,1117) = 40.378$, $p < .001$] age groups [$F(7,1103) = 3.495$, $p = .001$] and physical activity [$F(1,1107) = 9.277$, $p = .002$]. Therefore, the score of SDI is likely to increase with age, and mostly after 57 years. Also, women ($M = 2.59$, $SD = .517$) and participants who do not engage in physical activity ($M = 2.55$, $SD = .529$) have higher scores of sleep difficulties. Men have significantly higher sleep difficulties and wake up during the night more often, in comparison with women.
Discussion and conclusions: The Portuguese population has a moderate rate of sleep difficulties. Besides the reported difficulties, participants perceived lower effects of sleep disturbances on daily life. Women, individuals with age after 57 years, and who do not engage in physical activity perceived higher sleep difficulties.

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The lived experience of the person in critical situation who was cared in an intensive care unit

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Introduction: Being in an ICU is an abrupt and unexpected situation, reflecting a unique event for the Person in Critical Condition; it changes temporarily their life projects. The aim of this study is, to explore the lived experience by the Person in Critical Condition who was cared in an ICU.
Materials and methods: Study within the qualitative paradigm using a phenomenological descriptive approach by Amadeo Giorgi [1,2]. We used the in depth unstructured interview as a source of access to the lived experience of 12 participants aged between 27 and 77 years. The data was collected between October 2012 and December 2013.
Results: It was possible to identify the essential structure that reflects the nature of the lived experience by the PSC as a process of health-illness transition marked by total despair, distress and disbelief in the future. This structure consisted of 3 components and each component consisted of four key elements. (1) Feeling trapped – the loss of control over the own body; 1.1 – Tremendous shock of waking up in ICU, 1.2 – the suffering of the body invaded by the technique, 1.3 – the body exposed to others, 1.4 – the threatened identity. (2) Between life and death, 2.1 – the fear in the announced death presence, 2.2 – the anguish of the night, 2.3 – transformation of the perception of the lived time, 2.4 – surrounded by noise and alarms. (3) The need for safety; 3.1 – the continuous presence of professionals, 3.2 – the family as a safe haven, 3.3 – available information about their situation, 3.4- hope and internal strength.
Discussion and conclusions: This process of health-illness transition experienced by the PSC during hospitalization in the ICU allows us to uncover the hospitalization violence marked by the distress and despair of the participants upon waking up in the ICU, one of the most aggressive, austere, and frightening hospitalization environments, surrounded by equipment and health care professionals. It is scary, exasperating for the participant. Depending on other people to maintain, one's existence described as living in a limit situation. This patient is vulnerable and fragile as his existence compromised and death becomes a possibility. Children are crucial to the preservation of their existence. In addition, lack of information about their clinical situation, the professionals talked about them but not to them, they were invisible, they tried to listen, they needed that information to help them to situate in the new lived reality. They feel that they have been a new opportunity to maintain their existence, eventually succeeding in regaining harmony with their suffering, giving it meaning in their existence and allowing them to continue with their normal course of being a being-in-the-world. The lived experience of the participants shows that caregivers need to look for the uniqueness, sense and meaning of the health situation in their existence and, together, be capable of the care they need.

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Understanding the psychological status in orthognathic patients

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Introduction: Orthognathic surgery (OS) is performed to correct a range of facial deformities affecting the jaws and teeth. The scope of OS is to harmonize the facial skeletal structure in order to ameliorate physical complaints. However, it is also believed to influence the psychological status of patients. First, reviewing the literature, we aim to characterize psychological and quality of life status of OS patients. Second, we also present a study, whose purpose is to screen for psychiatric symptoms and other variables related to personality and quality of life (QoL) of patients undergoing OS at Centro Hospitalar Lisboa Norte.
Material and methods: For the literature review, Internet databases indexed for MEDLINE were searched up to January 2017, using the following key-words: "orthognathic surgery", "depression", "anxiety", "body dysmorphic disorder", "quality of life" and "personality". Only articles written in English were included. In our study, several questionnaires, such as Brief Psychiatric Rating Scale, Montgomery-Asberg Depression Scale, Hamilton Depression Rating Scale, Quality of Life Scale, Hamilton Anxiety Rating Scale, Figure Rating Scale and Structured Clinical Interview for DSM-IV-TR Axis II Personality Disorders, are applied to a group of 30 patients. This data will allow assessment of depressive symptoms, anxiety (including social anxiety), QoL and personality. Patients will be assessed before surgery (T0), 3 months after surgery (T1) and 12 months after surgery (T2).
Results: Twelve significant studies were included: five studied psychological status, fivequality of life and two body dysmorphic disorder (BDD), accounting for a total of 1082 patients. The included studies showed large variation in sample size (range =26–194 patients), distinct psychological evaluation tools, and variable time elapsed between the assessment and the completion of treatment.
Discussion and conclusions: Orthognatic patients experience significantly higher levels of social anxiety when compared to the general population. Consequently, social anxiety disorder may be an important reason for seeking orthognathic treatment [1]. The negative impact on QoL is greater in individuals who present dentofacial deformity than in those who do not [2]. Brunault et al. found a significant improvement in QoL and in depression 12 months after OS, but not in anxiety [3]. Although OS has a positive effect on QoL, depression is a major predictor of poor QoL in these patients and therefore an additional treatment for depression could further improve QoL. Concerning BDD, its prevalence in OS settings is low [4,5]. Together, these results suggest that our study could be relevant to provide better characterization of these patients and, thus, improve their psychological status.

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Victimization and psychopathology in adolescents

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Introduction: Interpersonal victimization is characterized as an injury, committed by an individual, to another, as a consequence of behavior contrary to established social norms [1], constituting in itself a potentially negative victim's life experience [2]. The present study aimed to analyze the relationship between victimization throughout life and its impact on the level of manifestation of psychopathological symptomatology in a sample of adolescents.
Materials and methods: For the data collection, two psychometric instruments were used, the Juvenile Victimization Questionnaire (JVQ) [3], self-report version adapted for the Portuguese population by Vara [4], which evaluates the experiences of life-long victimization, and the Anxiety, Depression and Stress Scale 21 for Children (EADS-21 C) [5], which assesses the emotional state of the individual during the last week. The sample consisted of 629 adolescents, 341 girls and 288 boys, aged between 12 and 17 years ($M = 14.17$; SD $=1.597$), from 11 Portuguese schools included islands.
Results: The results indicate that victimization, in its different forms, was a significant predictor of psychopathological symptomatology ($t = -3.575$; $p < .05$) ($\beta = 0.745$). In turn, it is the combination of the variables total victimization, victimization by the caregiver and victimization by conventional crimes, that most contribute to the onset of depression, explaining 34% ($R^2 = 0.340$) of the variance.
Discussion and conclusions: The results suggest that life-long victimization contributes to the emergence of psychopathological symptomatology, with an association between these variables, specifically with the experience of different forms of victimization and the development of depressive symptomatology.

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What adolescent mothers most need to take care of themselves in postpartum

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Introduction: Adolescent pregnancy is a crisis that can overcome the crisis of adolescence itself [1], leading to the need for personal and social readjustment, with the redefinition of roles, articulated with the organic and psychic modifications [2]. Considering the logic of the co-construction of care practices, in this article we proposed to bring to the reflection the results, based on the self-perceived knowledge of the adolescent mothers most value to take care of themselves in the postpartum period, necessary for the construction and maintenance of a care more suited to their needs.
Materials and methods: The sample consisted of 251 healthy adolescent mothers. Inclusion criteria: mother between 14 and 21 years, in the immediate puerperium, with full term pregnancy and with children without malformations. It is a quantitative, exploratory and cross-sectional study. Data collection was done between 2011 and 2015, in the midwifery services of the Metropolitan Area of Lisbon, applying the Postpartum Learning Needs, with 23 items related to maternal needs, using a 4-point Likert scale. Statistical analysis was performed using the statistical software SPSS® Statistics 20.
Results: The 251 adolescent mothers who collaborated in the study had ages ranging from 13 to 19 years, with a mean age of 17.5 years with a standard deviation of 1.35. As a result of greater statistical significance, we can conclude that the majority of adolescent mothers refer to the signs and symptoms of complications (95.2%), perineal care (91.5%) and the

couple's relationship (98.8%), as the knowledge that most value to know in this period of postpartum.
Discussion and conclusions: Adolescent mothers were more interested in knowing the signs and symptoms of complications, the care and early prevention of signs and symptoms of complications. These results alert us to the existence between the greater will to know the questions of the area of sexuality and the adoption of healthy sexual behaviors. Witch are not corroborated by the literature, when referring to the fact that during the early stages of the relationship between adolescents, intimacy and maturity, are not enough to make decisions and adopt safe and satisfactory practices [3]. It will be important to promote the inclusion in the maternal and pediatric health surveillance programs, this areas [4].

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X-ray vision: mental representation of the inner body of a healthy and of a sick person

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Introduction: The present study aims to understand how the College students of Health Studies mentally represent the internal morphology of the human body of the "Healthy Person" and the "Sick Person" [1–3].
Materials and methods: The longitudinal study was carried out on the basis of drawings made by 123 students (30 males and 93 females) who attended a higher education institution in the metropolitan area of Lisbon and Tagus Valley, who were asked to draw the interior of the body of a healthy person and of a sick person [3–5], in two different moments: (M1) Before and (M2) After having attended the Curricular Unit of Anatomy. In all, 492 drawings were evaluated, and the analysis of the drawings was based on an analysis matrix designed for this purpose. A comparative analysis of the two moments, before and after the academic training, was also carried out.
Results: The data suggest that in most cases the age attributed to the healthy figure is inferior than the age attributed to the sick figure. Simultaneously, there is an absence of the contour of the body (the skin), which represents the largest organ of the human body. The drawings reveal an absence of the sexual organs, suggesting a desexualization of the portrayed human bodies. When represented, they are drawn by the female group that depicts them when representing the masculine gender. In contrast, females sketch reproductive organs in representation of the feminine body. Organ neoplasms are the most represented pathologies: in man, lung cancer associated with smoking is seldom illustrated whereas in women the choice goes for breast cancer. Interestingly, the damaged organs were highly invested pictorially, in contrast to a smaller investment in the quality and quantity of accessory organs represented in the healthy person.
Discussion and conclusions: The present study demonstrates the absence of significant differences in the representation of the interior of the body before and after the academic training [6]. The fact that the few female students who have depicted the sexual-reproductive system associate them with reproductive organs but not to sex organs suggests that female sexuality seems to have been unconsciously replaced and masked by the biological/reproductive function. This strengthens the idea that sexual identity is fundamentally emotional and psychosocial in character, and unconscious in its essence.

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SOCIOLOGY

Sociodemographic factors and risky behaviors for the acquisition of sexually transmitted infections by international exchange students in Portugal

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Introduction: Sexually transmitted infections (STI) are a worldwide public health problem, due to its reproductive complications, mostly observed in teenagers and young adults [1]. Those infections, despite of its associated morbidity and mortality are frequently overshadowed and neglected in favour of the human immunodeficiency virus infection [2]. The purpose of this study was to evaluate sociodemographic factors and risky behaviours associated with STI acquisition and to assess personal awareness of risky behaviour and the knowledge about *Chlamydia trachomatis* infection between international exchange students in Portugal.
Materials and methods: The main instrument for data collection was a questionnaire, applied to foreign students in university exchange in Portugal, both on-line and in person, during the years 2012/2013, 2013/2014 e 2014/2015.
Results: Four hundred and eighty-eight (488) questionnaires were evaluated, the participants sample being composed by 60.8% females students and 39.2% male students, with ages between 18 and 30 years old (a mean of 23.5 years old). The mean age for the beginning of the sexual life was 17.6 years old and the mean number of lifetime sexual partners was 7.1. Concerning the sexual practices, 88.6% assumed the practice of oral sex and 33.1% of anal sex. Of the participants who provided answers, 6.2% mentioned a sexual relationship exclusively with the same gender and 5.8% mentioned a sexual relationship with both genders. Also, 66.5% reported having had sex under the influence of alcohol and 18.7% under the influence of drugs. In the studied sample, the condom use in vaginal/anal sex was mentioned by 65.5%, while in oral sex, only 4.7% of the participants reported its use. Of all participants, 6.4% reported a background of STI, showing a higher number of sexual partners, relatively to the group without STI history. Despite the academic degree of the participants, 39.4% didn't recognize *C. trachomatis* as an STI agent and 18.5% did not know that STI can be transmitted through oral sex.
Discussion and conclusions: Although sexual transmitted infections can affect individuals of all ages, races and sexual orientation, various demographic, social and behavioral factors are known to influence in their prevalence rates [3]. The answers given suggested that the risky behaviours are important in this studied sample, mainly the early age for sexual activity, multiple sex partners, the absence of protection during sexual intercourse and the regular practice of sexual intercourse under the influence of alcohol. Our study reinforces the importance of ensuring a good prevention and increasing of the knowledge about STI, in order to reduce the infection rates and its sequelae, mainly in young people. It is also important to engage the health care professionals in the promotion of condoms use, since as in other studies, these are seldom used.

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The social management of sleep in old age: conceptions and practices regarding medication

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Introduction: In this paper, we discuss sleep as a social object, focusing on the older population (aged 65 years and over), through a sociological analysis of the social meanings given to sleep – and its disturbances – and the ways to manage it. We particularly intend to identify the different strategies (therapeutic and non-therapeutic) employed to manage sleep, as well as to grasp how these strategies are inscribed in different conceptions of sleep and different rationales favoring or resisting the use of sleep medication [1].

Materials and methods: This analysis is empirically based on data from a research project on the consumption of psychopharmaceuticals among the older population, in an urban context in Portugal. Our target population was individuals aged 65 years and over with physical and cognitive autonomy, living alone at home or in institutional settings. The research methodology followed a mixed methods approach: a survey ($n = 414$); and life history interviews with respondents ($n = 30$) from the previous survey. Both stages of the research were approved by the Ethics Commission of Egas Moniz-Higher Institute of Health Sciences, and an informed consent form was presented to and signed by all participants in the study.

Results: Results reveal a considerable social adherence to the use of pharmaceuticals to manage sleep problems in the older population; 46.2% were using sleeping pills. This adherence is socially differentiated. First, in terms of gender: women use significantly more medication for sleep than men (51.5% versus 31.8%). Second, in terms of age: younger members of the older population show lower levels of consumption (about 41% for individuals aged 65–74 years versus about 48% for those over 74). Finally, in terms of older people's living contexts: those integrated in institutional settings show greater consumption than those living in their own domiciles (about 50% versus about 41%). Consumption practices do not always presuppose a strong conviction on the use of pharmaceuticals for sleep problems. Among those who take them, strategies used to diminish the associated risks were common. Besides recourse to pharmaceuticals, individuals use various non-therapeutic strategies to deal with sleep problems. These practices stem from a resistance to sleep medication, fuelling a search for alternative ways to deal with a condition thought to be undesirable and that can be improved.

Discussion and conclusions: The results reveal the existence of different social rationales [2] underlying the use of sleep medication and the way those rationales are built upon different conceptions of sleep problems. Rationales favoring the use of medication are associated with a naturalization of consumption, which is regarded as a prosthesis to deal with ageing effects. They usually correspond to long trajectories of consumption. Rationales resisting the use of medication are associated with a naturalization of poor sleep, which is accorded the status of non-disease, and with the belief in the body's natural ability to adapt itself to ageing effects.

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Factors leading elderly people to abandon medication

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Introduction: Aging is commonly associated with an increased of pathologies and consumption of medications, which may result in total or partial cessation of therapy. The general purpose of this study was to "explore the factors of abandonment of medication by the elderly and the understanding how this abandonment is intertwined with the sociocultural contexts of belonging and reference of individuals." The objectives were (1) to identify and characterize reasons/motivation/determinants for the abandonment of medication; (2) characterize the diversity underlying abandonment patterns, type of medication, context, motivations; (3) recognize and understand the main factors of abandonment variability, according to gender; age; place of residence and schooling.
Materials and methods: An explanatory, descriptive and longitudinal study was carried out on a random sample of residents in Évora and some surrounding villages, constituted by citizens aged 65 years old and over, without cognitive alterations that limited their understanding and non-institutionalized persons, who attended the services of the National Health System and the local pharmacies of their neighbourhood. Two questionnaires were used, one for USF [Family Health Units] ($n = 202$) and another for pharmacies ($n = 336$), obtaining 538 valid questionnaires. The objectives of these questionnaires were to obtain data about socio-demographic characterization, conceptions about health/disease, health care, existing pathologies, prescribed medication and medication taken and abandoned.
Results: There was a 29.2% drop in total medication and 15.8% in partial abandonment, with the following allegations: side effects; health improvement; difficulties; uncertainty about the effectiveness of treatment; self-perceptions based on acquired lay knowledge, due to their experience or experience of others; restricting/avoiding medication; forgetfulness and lack of knowledge/information about the prescribed medication and its therapeutic effect. Likewise, the lack of information and clarification by health professionals about the patient's clinical situation and the reasons for the prescription and effect of the medications, especially in the asymptomatic pathologies, contribute to the abandonment. It has been observed that the most prescribed, most taken and most abandoned drugs are in tune, being the ones for the central nervous system. It was verified the existence of polymedication. The total abandonment of medication occurs more in women, in the city residents, with the first complete cycle, over 75 years old, with more economic difficulties, with greater number of diseases, with higher averages in prescribed and taken medications. On the other hand, they are more careful about their health, with more consultations and medical examinations, more care about food. They have a more negative view of their current and future health.
Discussion and conclusions: Polymedication and use of free prescription drugs led to a consequent increase in the use of medications, and was one of the aspects that stood out in this study. The prescription of several drugs with the same therapeutic effect, often recommended by the same doctor, has been found in a considerable number of patients. Failure to adhere to therapy is a multifactorial social problem that is difficult to account for precisely and depends on several factors, such as demographic, socioeconomic, disease-related and therapeutic regimen, factors dependent on patients and their relationship with health professionals. Each individual makes an unscientific and subjective self-assessment of his or her state of health, which may interfere with adherence. Also, the knowledge acquired during their experience and contacts with health professionals, as well as those transmitted through their peers, such as the existence of "prejudices" about medicines and their efficacy, are also important factors. Definitely health professionals, particularly physicians, should pay more attention to patients, simplify the treatment regimen avoiding major changes, and explain the reasons for the medication and possible side effects as well as the pathologies affecting them.

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The Judicialization of Health in Brazil

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Introduction: The aim of this communication is to present Brazilian context in relation to access the right to health of the population. The Judiciary system in Brazil receives daily requests and law actions, these requests are focused in access to medicines and health treatments, which were not available or sustained by public healthcare. This phenomenon is called the Judicialization of Health rights. These actions are brought by people who are sick and do not have safeguarded their right to attend a clinical or surgical treatment, or do not have access to medicines that are nonexistent in the public health network. The Health Rights in Brazil had a great landmark when the Federal Constitution of 1988 was enacted, also present later in the Organic Law of Health (Law 8.080/90), consequently this laws mandate that, the Brazilian State is legally responsible for maintaining health and free access to all Brazilian citizens.
Materials and methods: The present summary is characterized as a theoretical essay, and aims to present a problem of major repercussion in SUS – Brazilian Health System. The initial investigation was carried out through bibliographic research in books and articles related to the issue.
Results: The first lawsuits providing health rights to those who required through courts, began with requests for medicines for the treatment of HIV (human immunodeficiency virus) [1], which is now available to the entire population. In Brazil, these actions are currently for the supply of medicines, obtainability of exams and coverage of treatment for diseases [2], even though the costs are varied, from cheap medicines to complex and expensive exams. The number of such actions in the country is showing a great growth and, in the year 2016, R$16 billion were spent by Brazilian federal government, according to data from the Ministry of Health. The subject has been targeted in many studies and reflections.
Discussion and conclusions: When it comes to the historical trajectory of health in Brazil, it is possible to observe that this has not always been considered a right to all. It was witnessed that the innovations related to health in the post-1988 period in the country brought very significant changes in the legal provision of the health Rights. In other side, represent a challenge for health professionals, since that the practice of good Health to everyone it is still an utopic subject. It should also be pointed out that the judicialization of the right to health, the way it is taking place in Brazil, does not guarantee the effective access of for the ones who normally attend health institutions. In reality, only those who are aware of such possibility (of appealing to the Judiciary). Therefore, we perceive that it is possible to offer an health treatment, oppositely to the ones that follows the normal assets. This denounces the absurdity between the legal and the real and the failures of the current health system, indicating that, despite the changes in the last decades, there is still a long path to be covered.

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NURSING

Development of functional magnetic resonance imaging (FMRI) paradigms for Primary Progressive Aphasia: a pilot study

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Introduction: Primary progressive aphasia (PPA) is a neurodegenerative condition associated with atrophy of the frontotemporal region of the left hemisphere. A gradual and isolated impairment of language function for at least two years is the core feature of this disease [1,2]. fMRI is emerging as an outcome measure used to report treatment-induced changes of brain activity in PPA. Yet most fMRI designs consist of a single picture-naming task, while other tasks are equally important to assess other domains affected in PPA. Additionally, some paradigm designs fail to include a control condition. The aim of this study was to design and test adequate language paradigms that can be used in the context of PPA treatment in the future.
Materials and methods: Three tasks were designed for this experiment: confrontation naming; semantic association; and phonological decision-making. A list of stimuli was elaborated for each task. The confrontation naming task consisted of 20 black and white pictures from Snodgrass and Vanderwart corpus [3]. The semantic task included 160 word pairs semantically related, unrelated and partially related. The stimuli chosen for the phonological task consisted of 40 nonword pairs that followed the European-Portuguese syllable structure rules. A group of 14 healthy European-Portuguese university students volunteered to participate in this study. In order to elaborate the final set of words for the semantic task, participants were asked to classify 160 word pair relations in a scale from 1 (unrelated) to 7 (highly related). The phonological decision-making task required participants to decide whether two nonword letter strings sounded the same or not.
Results: After testing 160 word pairs with volunteers for the semantic task, words that scored 6–7 points were assigned to the related words group and words that scored 1–2 points were assigned to the unrelated words group. All participants classified 33 word pairs with 1–2 points (unrelated) and 20 word pairs with 6–7 points (related). From each group, only 15 word pairs were selected to the final set. These stimuli will be tested using a block design in six healthy adults in a near future.
Discussion and conclusions: In the confrontation naming task, we tried to avoid pictures that could retrieve more than one target word. Hence, only one correct answer was associated to each picture. In the phonological task, we chose nonwords instead of words, because words are linked to a meaning, which would recruit semantic areas in addition to phonological areas. In conclusion, testing this paradigm with healthy subjects provided useful information that will be used to design an adjusted paradigm for patients with PPA. The use of an appropriate fMRI paradigm is a valuable tool in providing evidence of therapy-induced neurofunctional reorganization in PPA patients.

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Healthcare associated infections perceptions amongst healthcare professionals: the role of the nurse manager

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Introduction: Healthcare Associated Infections (HAI) are responsible for increasing mortality, prolonging hospitalizations and increase health-related costs [1]. Management is essential in the infection control. Organizational factors such as safety culture influence adherence to hand hygiene (HH). So, the nurse manager is required a set of managerial and leadership skills that facilitate the practice of quality care [2]. The aim of this study was to analyze the healthcare professional's perception and those practices relatively to HH and its influence on HAI.
Materials and methods: It is a case study, defined as an observational cross-sectional, using a quantitative methodology and use a descriptive analysis and is retrospective. It was applied a questionnaire in a sample of 47 health professionals (nurses and auxiliaries) in a hospital. The questionnaire was based on the "Basic Questionnaire on the perception of health professionals about infections associated with health care and hand hygiene" [3]. Data processing made up of SPSS (Statiscal Package for Social Sciences), version 21.0.
Results: Of the total sample 44.7% professionals have a high perception that an HAI will have a great impact on the clinical evolution of the patient and that HH is effective in preventing HAI. It appears that professionals with more years of profession (31.9%) consider necessary a greater effort to perform hand hygiene. In general, professionals' perception

those managers give priority to HH, 55.3% considered that priority was high and 31.9% considered very high. The moments in which the hands are less hygienic are before contact and after contact with the whole skin of he client and surfaces close to the client. On the other hand, professionals often sanitize their hands when there is a risk to them. As causes of non-adherence to HH, the professionals (53.2%) pointed the overload of work and shortage of human resources.
Conclusions: Healthcare professionals hold knowledge on HAI and HH, however, according to the literature, adherence to HH is low and this leads to the conclusion that professionals devalue this practice. It is the responsibility of the nurse manager to develop strategies to increase adherence to this practice, such as training, feedback on results and frequent audits.

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Hospitalization of school age children – parents satisfaction with nursing care

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Introduction: A child hospitalization represents to parents a moment of crisis, despite their permanent presence in hospital with their child. Because of parents decisive role, the use of family-centered philosophy of care is consensually seen as the "golden standard of care" [1]. Nurses are the professionals that most influence global satisfaction [2] and the positive association between satisfaction and adherence to care is recognized [3]. This study aims to characterize the satisfaction of school-age children parents about nursing care during hospitalization.
Materials and methods: An observational, cross-sectional, exploratory-descriptive study with a non-probabilistic and accidental sample was conducted. The self-completion questionnaire "Citizen Satisfaction with Nursing Care" was applied. It includes two subscales and statistical analysis was performed with SPSS (24.0). Authorization was obtained from ethics committees in each health institutions as well as National Data Protection Commission.
Results: The sample ($n = 251$) includes mainly women (83.7%, $n = 210$) with 37.65 years (SD =6.3) as mean age. In the subscale "nursing care experiences", parents felt "at ease with nurses" (76.7%, $n = 193$), "saw nurses as friends" (53%, $n = 133$) and thought that nurses promoted a pleasant atmosphere (77.6%, $n = 195$), so most parents would return to the unit if the child needed it (76.9%, $n = 193$). In the subscale "nursing care opinion", parents considered that "a nurse was always around when needed" (73.3%, $n = 184$), frequently questioning whether the child was well (74.9%, $n = 188$) and reassuring family and friends (66.2%, $n = 174$).
Discussion and conclusions: Parents are satisfied with nursing care during hospitalization, as reported in literature [4]. Relational and interpersonal aspects are highly valued [2,5] and closely related to overall satisfaction and intention to return/recommendation of services [2]. In general, the results point to high satisfaction scores in all areas.

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REATIVA: an effective program to increment self-efficacy perception during retirement

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Introduction: Retirement is a transitional life event whose adaptive responses can originate different stats of vulnerability in their protagonists that can affect their active aging [1–3]. REATIVA Program was designed to promote a successful retirement adjustment. This conference aim to present the methodology used to evaluate the effectiveness of REATIVA Program, expressed by self-efficacy evolution during transition to retirement.
Materials and methods: REATIVA Program resulted from a quantiqualitative empirical research, conducted over two years, in newly retires, which was structured to be implemented in Primary Health Care [4]. Three distinct groups were conducted to evaluate its efficiency: a control group (CG) that, has not undergone any prescriptive intervention, and two experimental groups (GE1, composed of newly retirees and GE2, composed of newly retirees and their spouses), both subject to REATIVA Program. All groups were submitted to evaluation, by filling out of a questionnaire that was applicate in three different stages of program implementation [initial (A1), immediate (A2) and final (A3)]. Different statistical measures were used to study the effects of the REATIVA program. In what concerns self-efficacy, the Manova test was selected as well as the t test for paired samples, in order to determine his evolution. p Values less than .05 were considered significant.
Results: A total of 87 newly retirees participate in the effectiveness evaluation of REATIVA Program. 56 participated in its implementation: 15 integrated Experimental Group 1 (GE1 = 26.8%) and 12 the Experimental Group 2 (GE2 = 21.4%). Twenty-nine participated as Control Group (GC = 51.8%). Participants were aged between 48 and 72 years (M = 62.8 years, SD = 3.1 years), were mostly female (57.1%), married (85.7%), and with qualifications higher than the 9th grade of schooling (39.3%). The results shows a positive evolution over time in the experimental groups, showing the beneficial effect that the program will have on the self-efficacy of the subjects participating in it [GE1(A3–A1) = 0.11; GE2(A3–A1) = 0.35]. This evidence was reinforced by the fact that in the control group there was a slight decrease in the mean of the perceived self-efficacy [GC (A3–A1) = −0.02].
Discussion and conclusions: Self-efficacy perception increased in all newly retirees who participated in REATIVA Program, particularly at those who participated individually. This evidence demonstrates that REATIVA Program can improve health status perception during retirement transition.[d] REATIVA Program is now being analysed by the Portuguese General Direction of Health, to be a part of the health surveillance programs to be implemented in primary health care.

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The two-dimensional perspective on nurses' conflict management, in a health unit

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Introduction: Health professionals, particularly nurses, are permanently exposed to internal and external conflicts. However, in order for the conflict to become an engine of development, it is necessary for nurses to identify it at an early stage, so that it can be overcome. To manage the conflict assertively, nurses must have the sensitivity to choose the more suitable conflict management strategy for each situation, towards a peaceful resolution and in order to meet the interests of the parties involved [1]. The aim of the study was to investigate conflict management styles taken by nurses and nurse managers of general care, in a health unit, understanding which conflict management strategies they adopt for each resolution.
Materials and methods: It was decided to use the two-dimensional model [2], composed of five conflict management styles, namely, collaboration (high self-interest and high interest for others), accommodation (down self-interest and high interest for others), domination (high self-interest and low interest for others), avoidance (low self-interest and low interest for others) and, finally, commitment (moderate interest for itself and for others), according to the authors [3]. This is a descriptive correlational study, of quantitative nature. It has been used the data collection instrument ROCI-II (Rahim Organizational Conflict Inventory – II). Participants (target population): nine nurse managers and 213 general care nurses of a Hospital Centre in the metropolitan area of Lisbon, in functions for more than 6 months.
Results: Regarding the conflict resolution strategies adopted by general care nurses for nurses managers (Model A) collaboration was the strategy that achieved a higher average. Dominance has a lower average face to the other strategies, showing an average of 2.89, median 3.00 and sets of 3.00. Regarding ROCI-II, Model C questionnaire (colleagues), the results show that cooperation with the colleague was the strategy that achieved a highest average. The management strategy of conflict avoidance presents a lower average of 2.74, median of 2.66 and sets of 2.33.
Discussion and conclusions: After analyzing the obtained results, it was found that the highest averages are related to collaboration conflict management strategy, regardless the type of nurses studied (general care nurses or nurse managers). An article was published about the conflict management strategies, in the Sultanate of Oman, which is directed only to the chief nurses [4]. Through this study, the authors were able to draw the following conclusions: nurse managers have, as a strategy of choice in their daily practice, the collaboration strategy, because, in the opinion of these authors, the collaboration allows you to satisfy fully the interests of the parties involved at the time of conflict.

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Nursing professional practice environment – influence on nurse burnout

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Introduction: Nurses have faced some difficulties and challenges regarding good care delivery, to ensure the safety and quality of care provided [1], in a work environment, which is not always the most favorable for the professional performance. This study is pioneer in the Hospital Center in study, having as a research question "will the characteristics of the organizational environment of care influence nursing burnout in a hospital context?". The overall goal was: to analyze the influence of organizational characteristics on the burnout of nurses in hospital context.
Materials and methods: This is a quantitative, descriptive, transversal and correlational study, carried out with nurses of all Medicine Services. Of the 110 nurses who were invited to compose the sample, 80 nurses collaborated, responding to the questionnaires, of which 62.72% were female. Data were collected with the help of the questionnaire Measuring Organizational Traits of Hospitals: The Revised Nursing Work Index [2], and the Maslach Burnout Inventory [3], both Portuguese versions, authorized by the ESEL research line. To perform the analysis, the IBM SPSS Statistics for MAC software was used.
Results: The dimensions of the nursing professional environment studied were: Autonomy, Control over Professional Practice, Multidisciplinary Relation and Organizational Support, which presented a positive average, between 3.23 and 3.83, which means that the Environment of the professional practice was considered favorable, since the overall mean of agreement of the nurses, at the time it was performed, was higher than 3.00. *Burnout* was assessed according to the dimensions: Emotional Exhaustion, Personal Realization and Depersonalization, with mean values between 3.08 and 4.95, with a low risk of burnout.
Discussion and conclusions: There was a low relation between the dimensions of the NWI-R and the MBI. It was concluded that, for the nurses participating in the present study, at the time it was carried out, the relationship between the characteristics of the environment of the professional practice of nursing and the influence on nursing burnout is practically non-existent.

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Organizational traits of hospitals and their contribution to the quality of nursing care

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Introduction: In a scenario of constant change and, most importantly, demands, nursing care requires the attainment of individual and collective objectives. The quality of the care provided to the clients is directly linked to the quality of the practice of all nurses and is guaranteed by valuing the competences of the nursing managers and can equally be influenced by the organizational environment [1], as all the conditions, circumstances and influences that surround and affect the practice of quality care. The aim of this study is to analyze the elements that determine the organizational environment that contribute to the quality care provided by nurses.
Materials and methods: A quantitative and descriptive-correlational analysis, with a transversal focus, was carried out. The data were collected using the questionnaire "Organizational Traits of hospitals and their contribution to the quality of nursing care", based on the questionnaire by Aiken and Patrician [2], translated and adapted to the Portuguese cultural context. The sample consists of 321 nurses working in a hospital unit, part of the National Health Service, located in the regional health administration of Lisbon and Tagus Valley, in Portugal.

Results: The nurses put more emphasis on the factors that are related to the dimension that concerns the grounds of nursing for quality care (60.10%), what seems to suggest that they are pleased with the work that they are developing, even if they did not give so much importance to their area of autonomy (46.10%). They value more the improvement of the continuous quality (57.30%), followed by the quality of the practice (56.40%) and the multidisciplinary relation (54.50%).
Discussion and conclusions: Nurses consider that the organizational characteristics of service that can most contribute to a good organizational environmentare: nurse's clinical competence; effective knowledge of organization; belonging to nursing and organizational working groups; assertive, attractive and empathic work environment [3–5]. They value the improvement of the continuous quality, the quality practiced and the multidisciplinary relation, being less evident the importance given to the control on the environment, organizational support and autonomy.

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Scoping review: work environment and its relationship with Chinese nurse satisfaction in a hospital setting

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Introduction: China is a country with a large population of about 1.4 billion inhabitants [4]. Following international context of cost reduction in the healthcare sector, hospitals implemented policies that will potentially generate negative repercussions, such changes that often lead to the shortage of nurses and the decrease of the safety/quality of patient's care [1,5,6]. The aim of Scoping Review is to map out concept of work environment and nurses' job satisfaction, also, to explore the relationship between those concepts.
Methods: Twelve studies were identified through a Scoping Review of the literature. The first step was searched in CINAHL and MEDLINE databases with the following keywords: job satisfaction, Quality of Health Care, environments, nurses, hospitals, China. A second search using all identified keywords and index terms then was undertaken across all included databases. Thirdly, the reference list of all identified reports and articles was searched for additional studies. Eligibility criteria included the studies that examined factors correlating the nursing professional practice environment and job satisfaction between 2011 and 2016. Data extraction and analysis of the studies were based on The Joanna Briggs Institute criteria's.
Results: A lot of research has documented the relationship among the quality of work environment, nurse satisfaction, patient satisfaction and quality of care [1–3]. The frequent reason for dissatisfaction of nurses is: high levels of burnout, job dissatisfaction, salary and intention to leave [1–5]. Research demonstrated the positive work environment increase levels of job satisfaction and staff retention of nurses [1–3,5,6].
Discussion and conclusions: Results showed a stable nurse workforce, is associated with favorable perceptions of nurse work environment factors, adequate workload, autonomy to make decisions, and low levels of burnout. Then, nursing leaders and executives share responsibility to create an environment supportive for nurse's development that might be an effective strategy for better nursing outcomes and patient satisfaction. As the same time, they can develop strategies to contribute to the retention of nurses and to promote the better quality of care.

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The organizational environment and customer safety in the practice of nursing care in a hospital context

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Introduction: The environment of nursing practice can condition nursing care, influence quality, safety and the outcomes [1,2]. Is fundamental for the provision of health care with quality and safety that institutional structures recognize the importance of the organizational environment and that it can condition the quality and safety of the health care provided to clients, the satisfaction and safety of the professionals, besides compromising the performance of the institution itself [3]. This study aims to evaluate the relation between the organizational environment and client safety from the perspective of nurses in a private hospital context, as well as to identify the organizational factors of care environment, client safety aspects and the relation between care practice environment and client safety in a hospital context.
Materials and methods: A quantitative, observational, descriptive, cross-sectional and correlational study, having as research question "How does the care practice environment relate to client safety from the perspective of nurses in a hospital context?" was distributed by 180 nurses, with 104 validated, fully-answered questionnaires. As a resource, the Portuguese version of the Revised Nursing Work Index (NWI-R) [4] was used to evaluate the nursing practice environment. The safety of clients, from the perspective of nurses, was evaluated through some dimensions of the safety questionnaire – Hospital Survey on Patient Safety Culture (HSPSC) [5], which were related to the organizational environment and were included in the questionnaire used for this study.
Results: The environment of professional nursing was considered favourable by nurses, with NWI-R subscales obtained a positive average of 3.03. The dimension "Support Management for Client Security" showed a disagreement percentage between 57.7% and 61.5%.
Discussion and conclusions: The nursing professional environment positively influences the client safety perceived by the nurses, a result in line with studies by Aiken et al. [1] and Laschinger et al. [2].

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How to improve nursing care? Perspectives of hospitalized school aged children parents

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Introduction: Consumer satisfaction is considered the golden standard of care in nursing. Questioning directly the users is a current practice in order to identify aspects of care that can be improved, from the patient point of view [1]. This study aims to identify aspects to improve nursing care, as referred by parents of hospitalized school aged children.
Materials and methods: An observational, cross-sectional, exploratory-descriptive study with a non-probabilistic and accidental sample was conducted. As data collection technique, the questionnaire "Citizen Satisfaction with Nursing Care", adapted from the original to this population, was used and integrates the open-ended question: "which aspects of nursing care could be improved?". Responses were submitted to qualitative analysis, with content analysis technique. Authorization was obtained from ethics committees in each health institutions as well as National Data Protection Commission.
Results: The sample ($n = 251$) includes mainly women (83.7%, $n = 210$) with 37.65 (SD =6.3) as mean age. Most parents did not respond to this question (53.4%, $n = 134$) and among respondents ($n = 117$), five categories were identified: "satisfaction" ($f = 71$), "personal domain" (F = 22), "environmental domain" ($f = 18$) "professional domain" ($f = 1$) and "nursing assignment" ($f = 6$).
Discussion and conclusions: The results point to satisfaction with care [2] and among the items to be improved, personal and relational aspects are emphasized as highly valued by parents [3,4] and closely related to overall satisfaction and intention to return/recommend services [3].

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PHYSIOTHERAPY

Patients' perceptions regarding handling and using of the Wii Sports System in Parkinson disease

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Introduction: People with Parkinson disease (PwP) benefit from ongoing exercise to manage their disease. This can be obtained through home-based programs, such as the Wii Sports System (WiiSS). However, these programs raise fundamental questions regarding what barriors are percieved by PwP that will influence the adherence of its use. These may be important issues for physical therapists to target in PwP. The aim of the present study is to identify the usability to exercising with the WiiSS in PwP with special focus on the handling requirements and using it as exercise regime.

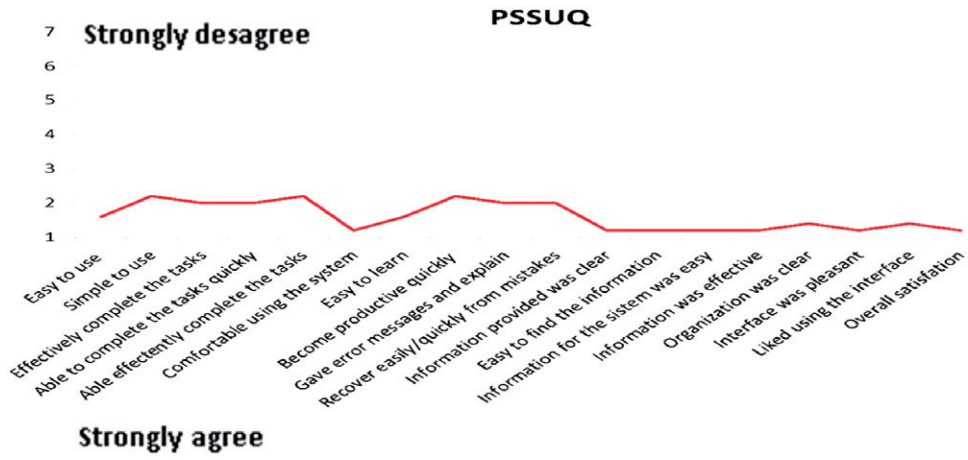

Figure 1. Satisfaction of participants based on the PSSUQ.

Materials and methods: PwP, dwelled in the community, in stages I to III on the Hoehn and Yahr scale (H&Y) participated in this study. Participants were characterized according to: disease staging (H&Y scale), clinical status (MDS-UPDRS), cognition (Mini-mental MMSE), balance (Berg Scale) and functionality (Timed Up & Go test). Study duration was planned for 3 months with nine practical sessions of 1 h. Participant satisfaction level was assessed based on replies to the Post-Study Usability Questionnaire (PSSUQ). Additionally, adherence to the sessions was assessed based on number of sessions attended or number of withdraws from the study. Anonymized patients data were analyzed after study approval by the Ethics Commissions of Cooperativa de Ensino Superior Egas Moniz.
Results: Ten PWP (7 Men) with mean age of 68 ± 5.4 years old participated in our study. All patients participated in all sessions with a 100% adherence rate. The mean value of satisfaction of the participants with the PSSUQ was 1.6 ± 0.41. Replies with best scores included "overall satisfaction", "interface was pleasant", "information was effective", "information for the system was easy", "easy to find the information", "information provided was clear" and lastly "comfortable using the system" (Figure 1).
Discussion and conclusions: Handling and use of the WiiSS was well received in our group of PwP, who were ambulatory and living in the community. Addressing knowledge requirments and perceptions relating to the handling of technology, as well as recognizing the challenges specific to this patient group in terms of need for more learning processes, may assist in increasing the use of such promising training devices as means of improving patients' physical, psychological and social well-being.

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The effect of assistive devices on gait patterns in Parkinson's disease: a pilot study

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Introduction: To assess the influence of 6 different assistive devices (ADs) on gait patterns in people with Parkinson (PwP) and the frequency and type of problems that arise from the use of each AD (i.e. freezing episodes; anxiety and/or panic attacks; stumbles and/or falls; patient perception of feeling safe; and patient level of satisfaction).
Materials and methods: Prospective, single-center pilot study involving PwP. Patients were consented in to the study and after were instructed to walk on a comfortable, self-selected, walking speed across the 2' by 16' Zeno walkway (ProtoKinetics, Havertown, PA). Spatiotemporal gait measures were collected when patients walked without and with different ADs: an aluminium straight cane (Cane); a tripod cane (TCane); a pair of Nordic walking sticks (NWS); a standard walker (StW); a two-wheeled walker (2WW) with fixed wheels and a four-wheeled walker (4WW) with front wheels casters. Gait parameters were analyzed and presented in a previous study [1]. This study was leaded through a Case Report Form (CRF) that included a self-perceived questionnaire about each AD approved by the Ethic Commission of the Cooperativa de Ensino Superior Egas Moniz.

Results: Fifteen PwP were included with a mean age of 65.2 ± 7.9 years old. Analysis of differences between gait parameters across ADs shows results [1] that were in line with previous findings in PD [2]. The information collected in CRF regarding patient perception of feeling safe and patient level of satisfaction with each AD was lined up with gait performance. Despite de fact participants did not like the idea of seeing themselves using a 4WW, because it looked like they were too dependent, 4WW (40%) and Cane (40%) were the ADs that gave more confidence when walking. Right after came the NWS (20%) described as the ones that most favors the gait, but it general opinion were not user-friendly at first contact.
Discussion and conclusions: The impact of different ADs in gait patterns should be considered when prescribing ADs to PwP. When walking with these ADs patients may encounter some difficulties but overall feel they improve their safety. In order to mitigate those difficulties and increase patients confidence a proper training most be carried with the AD to optimize the results of its use. Further research must be carried out to expand these finds.

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Exercise training in patients with chronic obstructive pulmonary disease. Effectiveness 10 years after participation

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Introduction: Patients with chronic obstructive pulmonary disease (COPD) may respond to exercise training in different ways compared to healthy subjects since the determinants of exercise limitation appear to be widely multi-factorial [1]. Such factors may include gas exchange abnormalities, dynamic lung hyperinflation, insufficient energy supply to the peripheral and respiratory muscles, morphological alterations in leg and diaphragm muscle fibers and reduced functional metabolic capacities [2]. The strength exercise can improve the respiratory muscle performance with potential positive effects on blood oxygenation, and consequently, muscular performance of patients with COPD [3]. The purpose of this study was to assess the effectiveness of attending an exercise program, combined (aerobic and strength exercise) or aerobic training alone in patients with COPD compared with subjects not submitted to exercise programs. The study was approved by the Ethics Committee of the Garcia de Orta Hospital and all participants gave their written informed consent prior to inclusion in the investigation.
Materials and methods: Thirty men with moderate COPD, were randomly assigned for two groups: 15 patients (age, 66.5 ± 6.2 years; FEV1, 55.8 ± 9.9%) to a combined exercise training program (CG), and 15 (age, 65.4 ± 3.6 years; FEV1, 59.1 ± 9.1%) to an aerobic training program (AG), for 10 weeks, three times a week. Outcome variables included cardiopulmonary function (cardiopulmonary exercise test (CPET) and 6-min walk distance (6MWD), muscular strength (1-RM); and quality of life (HRQL) with SF-36 and QRSG. Ten years after the programs, both groups were compared with ten patients who were not submitted to exercise programs, by evaluating health service recurrence (HSR) and mortality for respiratory cause.
Results: Both exercise groups increased ($p < .05$) functional capacity (CG, 24 ± 16%, AG, 26 ± 25%); O_2pulse_{peak} (CG, 24 ± 0.1%, AG, 21 ± 0.2%); CPET time and power (CG, 28.6 ± 8% AG, 23.0 ± 17%) 6MWD (CG, 12.7 ± 4%; AG, 6.8 ± 3%) and HRQL immediately after exercise, with greater benefits for the CG group ($p < .05$) in all variables. Ten years later, there were no differences between the exercise groups on mortality and HSR. Between exercise groups and subjects who did not integrate the program, there weren't differences on mortality, but there were significant differences on HSR ($p < .05$).
Discussion and conclusions: Combined exercise training was more effective than aerobic exercise alone with greater improvement in muscular strength, functional capacity and HRQL after program reducing morbidity by improving functional capacity through exercise. It is still unknown if improvements in functional capacity are maintained in the long-term

and if this leads to increased physical activity levels as measured by a free-living activity monitor [4]. Nevertheless participation in exercise programs seems to reduce HSR at long-term follow-up.

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Cardiorespiratory fitness in patients with chronic obstructive pulmonary disease

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Introduction: Cardiorespiratory fitness (CRF) reflects the integrated ability to transport oxygen from the atmosphere to the mitochondria to perform physical work. It quantifies the individual functional capacity and it is directly related to the integrated function of numerous systems as pulmonary ventilation and diffusion, cardiovascular functions and muscle cells capability to receive and use the O_2 and nutrients delivered by the blood, as well as to communicate these metabolic demands to the cardiovascular control center and, thus it is considered a reflection of total body health [1]. Chronic Obstructive Pulmonary Disease (COPD) is a chronic lung disease characterized by a progressive airflow limitation that is not fully reversible [2]. Daily life activities (DLA) in patients with COPD are limited by exertional dyspnea and reduced exercise capacity with reduction in exercise tolerance measured as peak oxygen uptake (VO_{2peak}) [3]. It has been found to be related to ventilatory limitations, pulmonary gas exchange abnormalities, peripheral muscle dysfunction or any combination of these factors [4]. The purpose of this study was to assess differences in CRF after a combined exercise program in COPD patients.
Materials and methods: Twenty men with moderate COPD (FEV1 49.8 ± 10.5%) were randomly assigned; age 65 ± 6.5 year; weight, 72.9 ± 10 kg; height, 167 ± 7.8 cm, were included to a combined exercise (strength and aerobic exercise) training program, for 6 month, three times a week. Cardiopulmonary exercise test (CPET) and 6-min walk distance test (6MWD), were performed before and after the training program. The study was approved by the Ethics Committee of the Garcia de Orta Hospital and all participants gave their informed consent.
Results: The values of the CRF measured by $\dot{V}O_{2peak}$ increased ($p<.001$) (15.38 ± 5.0 versus 25.7 ± 5.4 $mLkg^{-1}\,min^{-1}$); ventilatory efficiency improved $\dot{V}E/\dot{V}CO_2peak$ (36.7 ± 15.1 versus 29.2 ± 6.6), V_t (1.52 ± 0.35 L min^{-1} versus 1.82 ± 0.41 L min^{-1}); increased work peak (4.3 ± 1.7 min versus 9.18 ± 1.9 min) and also 6MWD (491.8 ± 74.0 m versus 620.2 ± 50.4 m); peak dyspnea decrease (4.6 ± 2.6 1.2 ± 1.2).
Discussion and conclusions: An increase in cardiorespiratory capacity reflects a better integrated response of the pulmonary, cardiovascular and neuromuscular systems in exercise and an improvement in functional fitness for DLA of these subjects, which was observed by an increase in exercise tolerance, in distance of 6MWD, and VO_2peak, accompanied by a decrease in dyspnea at the exercise peak. These improvements demonstrate a good correlation with the capability of effectively perform DLA [5]. In conclusion, combined exercise training provides an increased cardiorespiratory fitness in COPD subjects.

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Benefit of balance retraining after stroke using a force platform biofeedback – case study

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Introduction: Impaired balance is a major cause of locomotor disability after stroke [1], and is one of the main objectives of the Physiotherapy programs. According to some studies, these individuals present alterations in posture, poor alignment of the head and trunk, asymmetry in weight distribution on the lower limbs, and increased postural sway [2]. Recent advances in technology have resulted in numerous force platform systems with biofeedback, which can be used to retrain balance after a stroke [3]. This method involves constant self-correction by visual biofeedback, in which motor planning and motor control skills are continuously stimulated, contributing to neural plasticity [4]. The main goal of this case study was to analyse if a balance exercise program with visual biofeedback had any benefit in post-stroke.
Materials and methods: Woman, 54 year old with ischemic stroke on the vertebrobasilar territory of the right hemisphere with one month post-stroke. The subject completed 4 weeks balance therapy program 3 days per week. Each session consisted on 30 min of conventional therapy and additional 20 min of exercises in platform Physiosensing®. The Exercises on the Physiosensing platform included exercises in keeping symmetrical mass center in the sagittal plane, frontal plane, in both planes simultaneously, and dynamic balancing exercises, which consisted in the displacement of the center of mass in multiple directions simultaneously (movements in circle, square shape and labyrinth paths). The balance was assessmed by the Berg Balance Scale (BBS), postural control was assessed by Postural Assessment Scale for Stroke (PASS) and the weight and the execution time of the exercises were assessed with the platform. The subject was assessed at the beginning and end of sessions. The study was approved by the Ethics Committee of the Garcia de Orta Hospital and the patient gave her informed consent to participating in the investigation.
Results: The result indicated that subject improved on both scales. The subject improved on the Berg scale 20 points (27–47 points) and on the PASS scale 5 points (25–30 points). Moreover, the subject improvement in symmetry load distribution at the frontal and sagittal plane and showed a decrease in the execution time of the exercises (1.24–62 s).
Discussion and conclusions: The pilot study suggests that an exercise program of balance with visual biofeedback may be useful as a complement to physical therapy after stroke in the vertebrobasilar artery, 1 month post-stroke, with improvements of dynamic balance, postural control and symmetrical distribution of load in the lower limbs.

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Characterization of forearm muscle fatigue indicators in tennis right stroke

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Introduction: Tennis is a racket sport that comprises the execution of repetitive movements of varying intensities during a variable period of time. The same player can be involved in matches with diverse lengths, enduring movements that involve changes in direction, type of stroke, and variations in acceleration [1]. During a match, high-intensity repetitive movements may lead to muscle fatigue, which is expected to reduce the precision of the stroke in 81%. The decrease in muscle maximal strength or power may lead to a change in technical aspects and reduce the reaction time. These factors are crucial on the performance of professional players and their results [2]. The purpose of this paper is to analyze if there are any changes on the strength pattern of forearm muscles that may be caused by fatigue during the right-side stroke in tennis.
Method: Our sample was extracted from the Portuguese Tennis Federation (FPT) and the International Tennis Federation (ITF) members. Only male professional athletes, aged between 16 and 25, who trained 5 days a week, had been playing tennis for at least 8 years, and had no previous history of epicondylitis were included in our sample. Data collection was conducted on an indoor tennis court located at the Jamor Tennis Complex (Oeiras, Portugal). Initially, each athlete participated in a warm-up activity (running and active mobilization of the upper limbs). This was followed by a training period of 25 straight strokes in a row. A Jamar dynamometer was used to evaluate the hand grip strength on three different moments [3]: the first, immediately after the warm-up period; the second, after the training period; and the third, after 15 min resting period, where each participant had to perform a maximal voluntary contraction for 3 s. All participants gave their informed consent in writing prior to inclusion in the investigation.
Results: A total of 11 athletes were evaluated, which participated on average for 18.18 (±7.17) tournaments per year. For the hand grip strength, mean and standard deviation values were 52.91 ± 8.11 after the warm-up period; 46.9 ± 7.36 after the training period, and 52.3 ± 9.07 after the resting period. By *t*-tests and ANOVA statistical significant differences ($p<.001$) were found between the warm-up and training periods and between the training and resting periods, with no differences between the warm-up and resting periods.
Conclusion: It has been found that there are differences on the pattern of development of forearm muscle strength, which may indicate the onset of muscle fatigue after 25 right strokes, but these differences are attenuated after a 15-min resting period.

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Relation between functional ankle instability and physiotherapy in ballet dancers

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Introduction: The practice of ballet presents a high incidence of injuries [1], the lateral sprain of the ankle (LAS) is one of the most common [2], which can lead to Functional Ankle Instability (FAI). When LAS occurs repeatedly, it is a risk factor for new sprains, and repetitive sprains can lead to chronic ankle instability (CAI) [3]. Thus, we believe that physiotherapy plays an extremely important role in the prevention, treatment and reduction of complications of injuries resulting from ballet practice, especially in LAS [4]. The objectives of this study were to determine the prevalence of FAI in professional ballet dancers and verify the relation between Physiotherapy intervention (PTI) after LAS in the development of FAI. A secondary objective was to identify the reasons why dancers do not seek physiotherapy after LAS.
Materials and methods: Fifteen professional ballet dancers from Companhia Nacional de Bailado, who speak Portuguese or English with history of LAS were included in the study. Ten were female and 5 male, the mean age was 31.1 ± 6.9 years old, and they have been practicing dance for 23.6 ± 8.7 years, with 7.2 ± 1.1 h per day. The FAI evaluation was done through the Cumberland Ankle Instability Tool (CAIT), a self-completion questionnaire and to determine if there was a physical therapy intervention for the last LST, as well as the type of intervention and the number of sessions, was through the characterization questionnaire sample. All participants gave their informed consent in writing prior to inclusion.
Preliminary results: Seventy three percent of dancers have suffered one LAS. From those, 81.8% seek for health professional after LAS and received PTI. Of all dancers surveyed, 66.7% had FAI. Only two did not seek a health professional after LAS. All the other dancers who suffered an ankle sprain received PTI. The reasons for not seeking a health

professional after LAS were the dancer's believe that they could treat LAS on their own and the believe that the LAS was not a significant injury. There was no relation between the PTI after ankle sprain in the development of FAI, but there was a negative correlation between the LAS number and the CAIT score.
Discussion and conclusions: The prevalence of FAI in this group of dancers is high, especially when compared to other athletes. The dancer's believes of LAS as being a simple injury, might make them not seeking treatment. Although there was no correlation between the intervention of Physiotherapy after LAS and the development of FAI, those dancers who did not received Physiotherapy treatment, both developed bilateral FAI.

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Impact of a proprioceptive training for the ankle joint in basketball

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Introduction: Basketball is a sport that requires a good dynamic stability on the lower limbs, enabling athletes to respond quickly to constant accelerations, decelerations and jumps [1]. Ankle instability is complex since it involves several factors, which can be either mechanical or functional deficits [2]. Proprioceptive training aims to optimize the proprioceptive function, thus reducing some of the functional deficits which lead to ankle instability [3,4]. Therefore, the objective of this study was to understand whether or not performing proprioceptive exercises could prevent ankle injuries in a female basketball team.
Materials and methods: Fifteen female basketball players from the main team of Basket Almada Club (BAC) were submitted to a proprioceptive training program in ankle injury prevention, applied for 4 months during the second round of the regular season. The training program used was based on a training program developed by FIFA and F-MARC and adapted to basketball. The athletes performed this program as part of their warm up in practice. The Star Excursion Balance Test was used to evaluate each athlete's balance on three different moments, at the beginning of the study, at second and fourth months after. All participants gave their writing informed consent prior to inclusion in the investigation.
Results: The mean age of the athletes is 20.9 ± 2.8 years old and they have been playing basketball for 11 ± 4.4 years. The mean days of practice were 4 days per week with a total of 6 h per week. None of the athletes practices another sport and three of them are basketball coaches as a hobby. After the modification rates were calculated the data showed a mean increase of the athletes' balance, between the first and third evaluation, of 29.97 ± 11.1 cm on the Star Excursion Balance Test score. The increase in the scores was greater between the first and second evaluation (23.45 ± 8.8 cm) than it was between the second and third evaluation (8.12 ± 2.2 cm). Although two athletes got injured during this study, only one had her ankle injured which means that, compared with last season, there was a decrease in the number of ankle injuries.
Discussion and conclusion: After spending two months performing a training program which consists of proprioceptive exercises, the athletes' dynamic balance was increased, and therefore the ankle injury risk decreased. The greater increase on the first two months, was interpreted as first time adaptation, since the athletes' had never performed a proprioceptive training program before, their bodies were not used to this kind of training and, therefore, the gains were greater on the first two months.

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Ankle sprain prevention in juvenile football players

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Introduction: The most injuries in youth soccer are acute, usually affecting the ankle and knee joints and muscles of the thigh and calf. The most common injuries are sprains, strains, and contusions [1]. Balance and coordination training are common components of intervention programs for the prevention and treatment of acute lateral ankle sprains and chronic ankle instability [2]. With this study, we intend to analyze the benefits occurred after implementing an ankle sprain prevention training. This study will be applied to a local football club, having as goals the evaluation of the ankle stability on the juvenile players and the decrease of the number of ankle sprains comparing with the period before the training implementation. Other objective of this study was to raise awareness of club leaders and trainers for the necessity of structured train to prevent injuries, mainly in younger players.
Material and methods: The sample was composed of 17 Portuguese male juvenile football players with mean age 16.1 ± 0.5, who have been playing football for 6.6 ± 4.3 years, with mean training time 7.2 ± 1 h per week. All the participants filled out the validated questionnaire Cumberland Ankle Instability Tool (CAIT). The Star Excursion Balance Test (SEBT) was used to evaluate each athlete's balance at the beginning and after of the study. After collecting the data, it was implemented a prevention plan, composed by three stages: warming exercises, strength exercises and proprioception training. This prevention plan should be implemented for six weeks, three times a week, for 15–20 min a session. As the participants were minors, informed consent was given by their parents.
Results: After the CAIT questionnaire was applied three players presented instability in both ankles, two players presented instability in the right ankle one player presented instability in the left ankle. Unstable ankle was considered for a score <25. It was possible to obtain a significant difference ($p < .05$) in the displacements Left A, M, PM, P, PL and AL and Right A and AM. For the remaining displacements, differences were not observed. Nevertheless higher mean values were obtained in the re-evaluation compared to the mean values of initial evaluation.
Discussion and conclusions: Based on the results, it can be concluded that the proprioceptive training relative to the ankle was effective in this small sample, showing an increment of the stability in this joint, which may lead to lesser injuries.

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Benefits of manual therapy in temporomandibular joint dysfunction treatment

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Introduction: Temporomandibular disorders (TMD) are a heterogeneous group of musculoskeletal and neuromuscular conditions involving the temporomandibular joint complex (TMJ), and surrounding musculature and osseous components, that occurs predominantly in women [1]. TMD is classified as intra-articular or extra-articular and its common symptoms includes limited or asymmetric mandibular motion, joint noises (clicking, popping or crepitus), pain in the masticatory muscles, TMJ pain, earache, headache, tinnitus and dizziness [2]. Diagnosis is most often based on history and physical examination. Treatment of patients with TMD should, initially, be based on the use of conservative, reversible, and evidence-based therapeutic modalities like physiotherapy, therefore a multidisciplinary approach is essential [3]. The purpose of this study is to evaluate the benefits of manual therapy (MT) when compared with self-rehabilitating exercises at home in patients with TMD.
Material and methods: A randomized, controlled clinical trial was performed involving a group of 20 subjects of both genders with TMD diagnosed according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). The subjects were assigned to two groups: 10 in the experimental group (EG) and 10 in the control group (CG). A pre-teaching session for both groups was held to explain the therapeutic exercises (TE) at home and delivered a handout with the TE. This study took place in a 6 weeks period where pain scores, perception of tinnitus/deafness, headache, joint noises, and range of mandibular (vertical) motion were evaluated and recorded at stage 0 and again at the end of the clinical trial for both groups. In addition to the TE, the EG was submitted to physiotherapy sessions with manual mobilization, massage and relaxation techniques, twice a week, while the CG only performed the TE referred above. All participants gave their writing informed consent prior to inclusion in the investigation
Results: The results evidence that the treatment with MT and TE is more effective that TE applied on a standalone basis. There was a decrease in all aspects of pain in the EG pain referred by the patient: decrease of 3.5 values in pain numeric rating scale (PNRE), TMJ pain: decrease of 2.9 values in PNRE, muscle pain: decrease of 2.9 values in PNRE) In the CG, there were no significant changes in the aspects of pain. In the range of motion (ROM) there was an increment of 5.39 mm in the EG; however, the CG had a decrease of 0.01 mm in the ROM.
Discussion and conclusions: MT combined with TE showed promising results to treat TMD. The EG showed reduction of symtoms and increase of mandibular ROM. However, the CG did not show significant improvements, the subjects remained overlapping compared with the first evaluation at stage 0. Therefore, this program brings promising effects in reducing symptomatology and improving quality of life.

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Relationship between body composition, sprint performance and vertical jump tests in young elite soccer players

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Introduction: Elite soccer requires high-level competencies across many specific physical qualities [1]. Being able to ensure players' progress at the required levels in order to achieve elite status is of paramount importance to scientists and practitioners. We analyzed anthropometric and sports performance data of young elite soccer players at 2 different times over a competitive season and investigated the relationship between body composition and physical tests of vertical jumps and sprint.
Materials and methods: Body weight, height and sum of skinfolds, squat jump (SJ), countermovement jump (CMJ) and sprint tests (V5 and V20) were evaluated at two different times over a competitive season. To analyze the results we used anova function provided by the statistical computing program R where a linear mixed model to assay the effect of height, Body Mass Index (BMI) and total value of the skinfolds (SSF) on the performance of athletes in each test was performed. All procedures were approved by an ethical committee.
Results: Eighty-eight male with mean age of 15.53 ± 1.45 years were included in this study and significant relationships were observed between the results of physical tests of vertical jump and sprint speed and SSF and BMI. SSF positively

correlated in SJ test ($F = 9.96$, $p < .002$), CMJ ($F = 11.29$, $p < .001$), V5 ($F = 5.94$, $p < .016$) and V20 ($F = 33.41$, $p < 4.327e-08$). Such as SSF, BMI positively correlated in SJ test ($F = 9.68$, $p < .002$), CMJ ($F = 5.58$, $p < .019$) and V20 ($F = 22.58$, $p < 6.731e-06$). If we use a significance value $p < .10$ the BMI influences not only the running times in V20 but also in V5. We could also verify that the older athletes were the ones that presented better results in all the tests.
Discussion and conclusions: It was hypothesized that a low adiposity would be associated with a better performance, which is confirmed by the results of this investigation showing that SSF negatively correlates with sprint performance in the V20 test and jump tests. These results are in line with previous findings which revealed a positive correlation between total body fat with sprint and jump performances [2]. With the relationships observed, the importance of a body optimization for better sports income is clear. In a game as demanding as soccer, where there are constant jumps and sprints, it is essential that athletes have an amount of muscle mass within the recommended values so that their performance is not affected during the exercise [5]. An unbalanced body composition, with a high amount of fat mass, represents a greater wear in the motor actions, negatively influencing the athlete's activity [3]. To obtain an ideal body composition, the role of the Nutritionist and Coach is crucial in order to guide and provide the athletes with the necessary bases for a balanced and autonomous diet and training so that they can optimize their performance and thus achieve excellent performances. Additionally, body composition may be used for talent selection process.

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Physical exercise and functional fitness of older adults in day care center

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Introduction: The physical and cognitive decline that occurs during aging translates into an inability to carry out daily living tasks with consequent impacts on social relationships. Functional fitness (FF) is generally defined as the ability to perform daily living activities without difficulty [1] and represents a powerful and independent risk factor for premature mortality [2]. However, the decline in FF with aging does not occur in a linear rate; a significant decrease occurs with advancing age, and between 70 and 80 years old appears to be a critical period of life [3]. This is due to the progressive decline in FF, which includes muscular strength, flexibility, balance, agility, gait velocity, and cardiorespiratory fitness [4]. It has been well established that elderlywho spend more time in physical activity (PA) or less time in sedentary behaviors exhibit better FF. Assessing elderly FF performance is an essential element in designing effective exercise programs for them [5]. The purpose of this study was therefore to assess elderly FF whom are users of day care Center.
Materials and methods: Forty-eight elderly non-institutionalized, users of a day care center, with 79.7 ± 7.1 years old, and a body mass index, 27.7 ± 3.5 kg m^2, were assigned to a specific multicomponent training composed by an aerobic endurance, strength, balance/coordination exercises, 45-min session, for 8 weeks, twice a week. The physical parameters assed were strength, aerobic endurance, flexibility and agility/balance. For upper body strength maximal amount of weighted arm curls was measured; for lower body strength the maximal amount of chair-ups were registered. Aerobic endurance was measured as the number of steps performed in 2 min (2-min step test). Upper body flexibility was assessed by back-scratch, and lower body flexibility was assessed by sit-and-reach test. For testing agility/balance, an 8-foot up-and-go test was used. All subjects gave their written informed consent prior to inclusion.
Results: The FF values before and after the intervention test were different ($p < .05$); 30-s chair stand 10.2 ± 1.4 versus 16.1 ± 2.6 times; arm curl 10.9 ± 3.2 versus 18.9 ± 3.5 time; 2-min step (70.5 ± 16 versus 98.3 ± 12.4 step), 8-foot up-and-go (9.8 ± 4.7 versus 7.1 ± 2.4 s), chair sit-and-reach (12.9 ± 11.2 versus -8.1 ± 9.9 cm) and for the back scratch test (−14.1 ± 9.8, −10.2 ± 6.1 cm) respectively.
Discussion and conclusions: This combined exercise was effective in improving all FF components related to daily living activities. The results demonstrate the importance of elderly participation in physical exercise leading to a lower FF decline. Despite exercise is often portrayed as inaccessible without expensive gym facilities and a grueling experience for those who take part, it seems to be an inexpensive, easily accessible approach to improve health and overall well-being.

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