

## Effect of albumin, urea, lysozyme and mucin on the triboactivity of Ti6Al4V/zirconia pair used in dental implants

H. Teixeira<sup>a</sup>, A.C. Branco<sup>a,b,c</sup>, I. Rodrigues<sup>a,d</sup>, D. Silva<sup>b</sup>, S. Cardoso<sup>e</sup>, R. Colaço<sup>f</sup>, A.P. Serro<sup>b,c,\*</sup>, C.G. Figueiredo-Pina<sup>a,c,d</sup>

<sup>a</sup> Centro de Desenvolvimento de Produto e Transferência de Tecnologia (CDP2T), Department of Mechanical Engineering, Escola Superior de Tecnologia de Setúbal, Instituto Politécnico de Setúbal, Setúbal, Portugal

<sup>b</sup> Centro de Química Estrutural (CQE), Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

<sup>c</sup> Centro de investigação interdisciplinar Egas Moniz (CiEM), Instituto Universitário Egas Moniz, Monte de Caparica, Portugal

<sup>d</sup> Centro de Física e Engenharia de Materiais Avançados (CeFEMA), Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal

<sup>e</sup> Instituto de Engenharia de Sistemas e Computadores-Microsistemas e Nanotecnologias (INESC-MN), Lisboa, Portugal

<sup>f</sup> Instituto de Engenharia Mecânica (IDMEC), Departamento de Engenharia Mecânica, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

### ARTICLE INFO

#### Keywords:

Saliva biomolecules  
Adsorption  
Zirconia/Ti6Al4V pair  
Wear  
Tribocorrosion

### ABSTRACT

The titanium implant/zirconia abutment interface can suffer failure upon mechanical and biological issues, ultimately leading to the loss of the artificial tooth. The study of the effect of the organic compounds present in saliva on the tribological behavior of these systems is of utmost importance to understand the failure mechanisms and better mimic the in vivo conditions. The aim of the present work is to evaluate the effect of the addition of albumin, urea, lysozyme and mucin to artificial saliva, on the triboactivity of Ti6Al4V/zirconia pair commonly used in dental implants and then, compare the results with those obtained with human saliva. The solutions' viscosity was measured and the adsorption of the different biomolecules to both Ti6Al4V and zirconia was accessed. Tribological tests were performed using Ti6Al4V balls sliding on zirconia plates inside of a corrosion cell. Friction and wear coefficients were determined, and the open circuit potential (OCP) was monitored during the tests. Also, the wear mechanisms were identified. The presence of mucin in the artificial lubricant led to the lowest wear coefficients. The main wear mechanism was abrasion, independently of the used lubricant. Adhesive wear was observed for the systems without mucin. Tribocorrosion activity and wear coefficient were lower in the presence of mucin. None of the studied artificial lubricants mimicked the effect of human saliva (HS) on the tribological behavior of the studied pair since this lubricant led to the lowest friction coefficient and highest corrosion activity.

### 1. Introduction

Teeth loss has strong implications in phonetics, aesthetics and mastication processes. This problem is usually overcome by the implantation of an artificial tooth constituted by three single pieces that are connected together: a dental crown that is normally screwed on the top of an abutment previously fixed onto the implant which mimics the root of the tooth, after its osseointegration. Titanium alloys and zirconia are the most used materials for implants and abutments, respectively (Adatia et al., 2009; Kerstein and Radke, 2008; Özcan and Hämmerle, 2012; Taylor et al., 2014; W. Nicholson, 2020).

The failure of the implant/abutment interface, due to biological and

mechanical issues, can lead to the loss of the artificial tooth. The accumulation of bacteria in this interface and their microleakage to the surrounding tissues may lead to peri-implantitis, which may result in decreased osteointegration. The mechanical concerns are associated to micromovements that can occur in this interface and induce wear. Besides, bending moments may enhance wear and the consequent fracture of the abutment. The continuous displacement at this interface in an aqueous environment induces tribocorrosion, i.e. the degradation of the material due to the combined effect of corrosion and wear (Noumbissi et al., 2019; Revathi et al., 2017).

The saliva composition and the microbiome of each individual is determinant for the tribological response of this interface. Saliva

\* Corresponding author. Centro de Química Estrutural (CQE), Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

E-mail address: [anapaula.serro@tecnico.ulisboa.pt](mailto:anapaula.serro@tecnico.ulisboa.pt) (A.P. Serro).

consists in roughly 98% water and 2% inorganic and organic substances, including electrolytes (e.g. sodium, potassium, calcium, magnesium, bicarbonate, and phosphates), immunoglobulins, proteins (mucin, albumin, lysozyme) and nitrogenous products, such as urea and ammonia (Aguirre et al., 1987; Humphrey and Williamson, 2001), with a pH ranging between 5.3 and 7.8 for healthy individuals (Khemiss et al., 2017). Urea, mucin, albumin and lysozyme are the molecules chosen for the lubricants in the present study. Urea (or carbamide) is a small organic molecule ( $\text{CO}(\text{NH}_2)_2$ ) with molecular weight 60 Da that has a dual effect in saliva: apart from inhibiting the metabolism and multiplication of bacteria, it also regulates saliva acidity acting as buffer, which contributes to reduce the appearance of dental caries (Zabokova Bilbilova et al., 2012). Mucin is a high-molecular-weight (0.5–20 MDa) glycosylated protein with an high amount of negatively charged oligosaccharide side chains (Sarkar et al., 2019). Although human saliva has only about ~0.3 wt% mucin, it has been reported that this protein plays an important role in the rheological properties of saliva (viscosity, elasticity, stickiness/adhesiveness), and in oral lubrication (Schenkels et al., 1995; Tabak, 1990). Mucin also offers an antibacterial protection by minimizing/preventing the adhesion of microorganisms to oral tissue surfaces, thus contributing to the control of bacterial and fungal colonization (Humphrey and Williamson, 2001). Albumin has a molecular weight of ~67 kDa and has an overall negative charge in the physiological conditions. Although this protein is found in high quantities in plasma, as it diffuses into mucosal secretions in the mouth, it has been detected in low concentrations in the saliva of healthy individuals (Meurman et al., 2002). However, when oral diseases are present (e.g. gingivitis or periodontitis), the amount of this protein increases (Schenkels et al., 1995). Finally, lysozyme, which is much smaller (~14 kDa) and is positively charged, has anti-bacterial properties due to its catalytic activity, being able to destroy the protective layer of bacteria, mainly of *Streptococcus* (Zalewska et al., 2011). The contents of these biomolecules in human saliva have been reported to be in the ranges: 0.14–0.75 g/L for urea (Ratner et al., 1996), 0.44–0.83 for mucin (Edgar et al., 2012), 0.008–1.92 g/L for albumin (Henskens et al., 1996; Hershkovich and Nagler, 2004), and 0.0035–0.092 g/L for lysozyme (Hershkovich and Nagler, 2004).

Saliva acts as a lubricant and therefore plays a protective role of the teeth, by reducing the effect of shear stresses, minimizing the friction between opposing surfaces and preventing excessive wear (Prinz et al., 2007; Ranc et al., 2006a, 2006b; Sajewicz, 2009). The influence of saliva on the tribological response of titanium alloys against ceramic materials was already investigated by some authors. Branco et al. (2019) studied the microtriboactivity of the zirconia-Ti6Al4V pair in dry and lubricated conditions (water, saliva electrolyte solution and human saliva) and observed that Ti6Al4V wear rate was neglectable when tests were performed with human saliva, which confirms the protective role of this lubricant. Mathew et al. (2012a) studied the tribocorrosion behavior of commercially pure (cp) titanium against alumina in the presence of artificial saliva at different pH values (3, 6 and 9) and observed the tribological tests performed at pH = 6 led to the highest titanium wear and also induced high tribocorrosion damage with appearance of cracks. Licusi et al. (2013) did a similar study, but with Ti6Al4V instead of cp titanium. Contrarily to Mathew et al., they observed that the three pH values led to the same tribocorrosion mechanisms: plastic deformation with passive dissolution.

In most of the studies, artificial saliva used as lubricant does not contain the organic compounds usually present in human saliva. Biomacromolecules, like proteins, tend to adsorb onto the surfaces of biomaterials when these contact the biological fluids. This phenomenon is almost instantaneous and constitutes the first step of a complex series of biochemical and biophysical processes that determine the biocompatibility of the material and its behavior in vivo, namely their tribological performance (Serro et al., 2012). As far as the authors know, there are no studies in the literature concerning the effect of saliva organic compounds on the tribological performance of titanium alloys against

ceramic materials. Thus, the aim of this study is to evaluate the effect of the addition of urea, mucin, albumin and lysozyme to artificial saliva, on the tribological performance of the pair Ti6Al4V-zirconia commonly used in dental implants, using a ball (Ti6Al4V) on plate (zirconia) test configuration, and compare the results with those obtained with human saliva. The following null hypothesis were considered: H01: The tribological behaviour of the studied system in the presence of the different biomolecules is the same; H02: The tribological behaviour of the studied system in the presence of saliva is mimicked by at least one of the studied biomolecules solutions. The viscosity of the solutions was measured, as well as the hardness and roughness of the counterfaces. The biomolecules adsorption onto the surfaces was investigated and the tribological behaviour was extensively studied, namely in what concerns to the wear and friction coefficients, wear mechanisms and open circuit potential (OCP) evolution.

## 2. Materials and methods

### 2.1. Materials preparation

#### 2.1.1. Human saliva collection

The collection of human saliva was performed by gravity, without stimulation of the salivary glands, from 3 healthy donors without any smoking habits. One week before collection, the donors did not ingest medicines and did not have any deviant behavior. Three hours before harvesting, the donors did not ingest food. All donors signed informed consents for saliva collection. The saliva samples were mixed and stored at 4 °C.

#### 2.1.2. Lubricants preparation

Artificial saliva solution was prepared by dissolving the following salts in distilled and deionized (DD) water: NaCl = 0.4 g/L, KCl = 0.4 g/L,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  = 0.906 g/L,  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  = 0.390 g/L,  $\text{Na}_2\text{HPO}_4$  = 0.142 g/L,  $\text{Na}_2\text{S}_9\text{H}_2\text{O}$  = 0.005 g/L (Fusayama et al., 1963). The artificial saliva pH was set at 6.4 with 1M NaOH. The prepared solution was sterilized through ultraviolet radiation (UV,  $\lambda = 254$  nm) for 15 min.

Solutions with different organic molecules (albumin, urea, lysozyme, and mucin) were prepared by dissolution of the compounds in the sterilized artificial saliva, to mimic the concentrations found in natural human saliva: lysozyme (Merck) 0.042 g/L, mucin from bovine submaxillary glands (Sigma) 0.83 g/L, albumin (Merck) 0.1 g/L and urea (Panreac) 1 g/L. The prepared solutions are referred in Table 1.

#### 2.1.3. Zirconia and titanium samples preparation

Zirconia ( $\text{ZrO}_2$ ) plates  $1 \times 1 \text{ cm}^2$  (Zirkonzahn GmbH, Gais, Italy), containing 5% yttria were sintered at 1500 °C (Zirkonofen 600/V2, Zirkonzahn GmbH, Gais, Italy) for 2 h. The polishing of the zirconia surfaces was performed using 320, 600, 800, 1000 and 2500 grit size sandpapers and 9  $\mu\text{m}$  polishing cloth. Balls of Ti6Al4V titanium alloy with 6 mm diameter were purchased from Luis Aparicio, S.L.

**Table 1**  
Lubricant solutions used for the tribological tests.

Solution	Description
HS	Human saliva
AS	Artificial saliva
AS + A	Artificial saliva with albumin
AS + U	Artificial saliva with urea
AS + L	Artificial saliva with lysozyme
AS + M	Artificial saliva with mucin
AS + A + U + L	Artificial saliva with albumin, urea and lysozyme
AS + A + U + L + M	Artificial saliva with albumin, urea, lysozyme and mucin

## 2.2. Materials characterization

### 2.2.1. Solutions viscosity

Viscosity measurements of the different solutions were carried out in a rheometer (MCR 92, Anton Paar). Tests were performed at 37 °C, at a shear rate range of 25–1000 s<sup>-1</sup>, using 1.5 mL of solution for each measurement. Three measurements of  $\approx$  10 min were done per system.

### 2.2.2. Surface hardness

Vickers hardness was measured using a hardness tester (INNOVATEST Falcon 450) with an applied load of 294.21 N during 15 s for zirconia plates and Ti6Al4V balls. The balls were previously mounted in resin, cut and metallographically prepared. Five indentations were carried out per sample.

### 2.2.3. Surface roughness

Topographical images of Ti6Al4V balls and zirconia plates (20 × 20 μm<sup>2</sup>) before the tribological experiments were obtained using an Atomic Force Microscope (AFM) Nanosurf Easyscan 2. After the wear tests, the worn areas of the Ti6Al4V balls were also observed for all systems. Two images per ball, in a total of 6 images per system were obtained randomly in contact mode, at room temperature, using a silicon probe with an applied force of 25 nN, at a scan rate of 1 Hz. The average surface roughness, S<sub>a</sub>, and maximum peak to valley height, S<sub>z</sub>, was determined based on the AFM images using the WSXM 4.0 software.

### 2.2.4. Biomolecules adsorption

The interaction between the biomolecules presents in the different solutions and Ti6Al4V and ZrO<sub>2</sub> surfaces was monitored with a quartz crystal microbalance with dissipation (QCM-D, E4 from Q-Sense). Quartz crystals (5 MHz) with gold electrodes were pre-coated with a thin layer of Ti6Al4V (80 nm) and ZrO<sub>2</sub> (60 nm). The deposition of the films was done in a Nordiko 3000 ion beam tool (Cardoso et al., 2000), using a 10 cm diameter ion source. Beam parameters were 1.8 sccm Xenon flow and 110 W rf power, with 0.04 mTorr. The 24 mA Xe<sup>+</sup> beam was accelerated towards the metallic targets (Zr or Ti6Al4V) with +1022 V acceleration voltage and -300 V extraction voltage. For oxidation of the Zr layer, the 25 cm diameter assist gun was used simultaneously with the deposition gun (Zhang et al., 2001) with an argon-oxygen plasma formed inside the assist gun, accelerated with +50 V and extracted with -200 V, at 0.24 mTorr. The deposition was carried out at room temperature.

Before each measurement the crystals were cleaned in ultrasounds bath with Hellmanex solution (2% v/v, Hellma) for 15 min, and with DD water (2 × 15 min) and dried with nitrogen. The baseline was obtained in the presence of artificial saliva (AS). Variations of frequency (Δf/n) and dissipation (ΔD) for the 3rd, 5th, 7th, 9th and 11th harmonics upon addition of the solution under study were registered. Δf/n is

proportional to the adsorbed mass, while ΔD depends on the rigid/viscoelastic character of the adsorbed film. Rinsing with AS was carried out after stabilization of the signals ( $\approx$ 1 h). Four independent measurements at 37 °C were done for each system.

### 2.2.5. Wear tests

Reciprocating pin-on-plate tribocorrosion tests were performed using Ti6Al4V balls and polished zirconia as plates (Fig. 1). The tests were carried out inside a corrosion cell (Fig. 1) containing the different solutions (lubricants). The temperature was maintained at 37±1 °C, through a water jacket that was connected to a thermal bath. The balls were connected through a wire to a potentiostat (Gamry 600) and a saturated calomel electrode was used as reference electrode. During the first two hours, the ball was placed in the solution without contacting the plate for open-circuit potential (OCP) stabilization. The tests were performed with an applied vertical load of 1 N, sliding distance 2.4 mm/cycle and frequency 2 Hz during 2 h. Using the Hertzian elastic contact equation for ball on plate and values of 114 GPa and 205 GPa for the Young's modulus and 0.342 and 0.310 for the Poisson's ratio of Ti6Al4V and zirconia (Boyer et al., 1994; Branco et al., 2019; Green, 1998; Loginov et al., 2017), respectively, the initial average contact stress was estimated to be 350 MPa. At the end of the wear test, the ball was raised from the plate and stayed immersed in the lubricant for one more hour, for repassivation. The OCP was recorded during the stabilization, wear and repassivation periods. The friction coefficient was also monitored during wear testing. Three tests were performed per condition.

At the end of the test, the ball's wear scars were observed by Light Optical Microscope (LOM, Olympia BH), and the scars radius were measured using the software Dino Capture. The cup wear volume loss was obtained by integration taking into consideration the worn area radius and the ball radius. The specific wear coefficient (K) was calculated through the equation:

$$K = \frac{V}{P \cdot S} \quad (1)$$

where V is the titanium ball volume loss, P is the applied normal load and S is the sliding distance.

In order to evaluate the wear mechanisms, the worn surfaces of the titanium balls and zirconia plates were observed by Scanning Electron Microscope (SEM, Hitachi S2400), at 25 kV. For that, zirconia plates were coated with a thin layer of Au-Pd. Qualitative chemical analyses were performed through Energy Dispersive Spectroscopy (EDS, Bruker).

## 2.3. Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics software. Shapiro-Wilk test was carried out to verify the normality of the results. ANOVA (one-way analysis of variance) was performed to

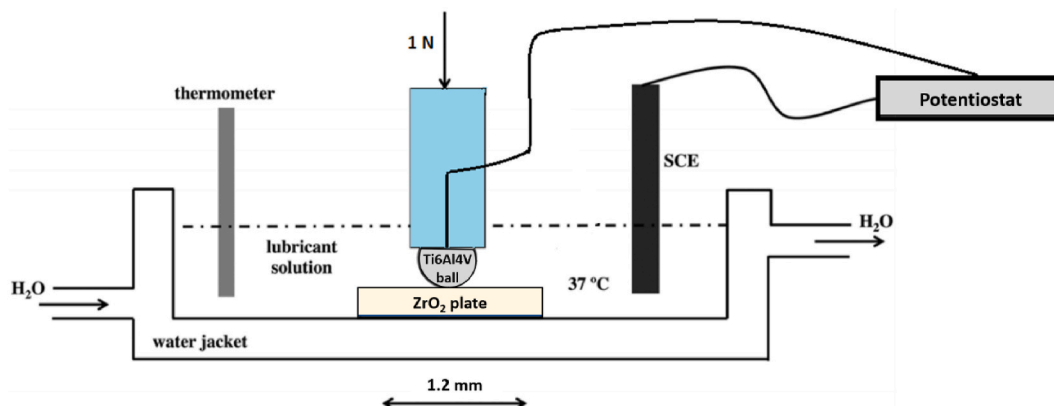


Fig. 1. Schematic of the tribocorrosion cell (adapted from (Figueiredo-Pina et al., 2019)).

compare three or more independent groups, while t-Student test was used for two groups. The level of significance chosen was 0.05.

### 3. Results

#### 3.1. Materials characterization

##### 3.1.1. Lubricants viscosity

Fig. 2 shows the viscosity of the different lubricants as a function of the shear rate. For all the shear rate range studied, the viscosity values of HS were always higher than those observed with the other lubricants, which were similar among them. Contrarily to HS, whose viscosity decreases with shear rate, all the other solutions present a constant viscosity, independent of the shear rate.

##### 3.1.2. Surface hardness and roughness

The hardness values obtained for Ti6Al4V and zirconia were  $364 \pm 11$  HV and  $1200 \pm 16$  HV, while the average surface roughness ( $S_a$ ) was  $39 \pm 5$  nm and  $14 \pm 2$  nm, respectively. AFM images depicted in Fig. 3 show that the initial contact surfaces of Ti6Al4V and zirconia exhibit nanometric scratches.

##### 3.1.3. Biomolecules adsorption

Changes in frequency ( $\Delta f/n$ ) and dissipation ( $\Delta D$ ) of quartz crystals coated with Ti6Al4V and ZrO<sub>2</sub>, upon addition of the different lubricant solutions were measured in a QCM-D. Frequency changes for the 3rd harmonic are compiled in Fig. 4A. The highest frequency decrease was registered for HS, followed by the solutions containing mucin and albumin. For each lubricant, the decrease in frequency was similar for Ti6Al4V and zirconia, except for AS + U, AS + M and AS + A + U + L + M, where it was higher for Ti6Al4V ( $p = 0.018$ ,  $p < 0.001$  and  $p = 0.006$ , respectively). Concerning dissipation (Fig. 4B), considerable changes were only observed for the lubricants containing mucin and HS. The observed increase was more evident for HS. Artificial saliva solutions with mucin led to a higher increase of dissipation for Ti6Al4V ( $p < 0.001$ ), while for HS the highest variation occurred for zirconia ( $p < 0.001$ ).

The values of frequency and dissipation for the remaining harmonics (5th, 7th, 9th and 11th) were similar to those obtained for the 3rd, except for HS where a visible shift was observed for the different harmonics both on Ti6Al4V and zirconia. Although not so evident, smaller shifts were also observed for the lubricants containing mucin. Typical curves for AS + L, AS + A + U + L + M and HS are shown in Fig. S1 (Supplementary material).

##### 3.1.4. Wear and friction coefficient

The friction coefficients for all studied systems are depicted in Fig. 5A. The average values range between 0.43 and 0.86. No

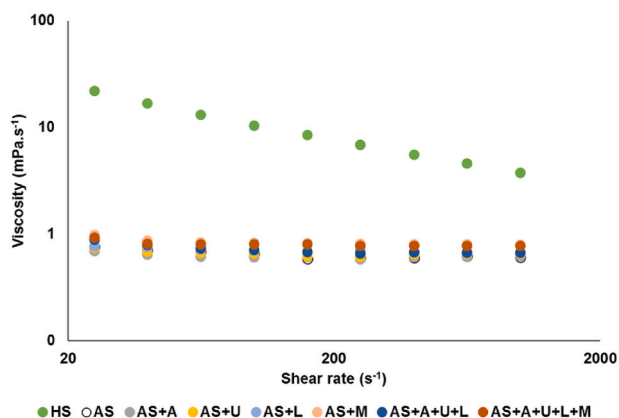


Fig. 2. Viscosity of the different lubricants as a function of the shear rate.

meaningful tendency was observed.

Concerning the wear of Ti6Al4V/zirconia pair, only Ti6Al4V suffered measurable wear. Fig. 5B depicts the Ti6Al4V wear coefficient for the different studied tribological systems. No statistically significant differences were observed among the different systems, except from AS + A + U + L + M which led to a much lower wear coefficient ( $p = 0.001$ ). Although the average value obtained for AS + M was similar to the latter, the difference to the other systems was not statistically significant in this case due to the large standard deviation of the results.

##### 3.1.5. Ti6Al4V worn surface characterization

Regarding the wear mechanisms observed on the titanium balls after the wear tests, HS, AS + M and AS + A + U + L + M originated the appearance scratches in the interior of the scars (Fig. 6). The system AS + M and AS + L + A + U + M present the lower wear scar width. The artificial lubricants without mucin (AS, AS + A, AS + U, AS + L and AS + A + U + L) have very similar wear scars among them, showing debris accumulation on their periphery, as well as particles' adhesion and marked scratches inside of the wear scars. EDS was done to perform the chemical composition analysis of the worn surfaces. For all systems, there was no evidence of adhered zirconia particles. Only elements from the salts existent in the artificial saliva and small amounts of sulfur, when proteins were present, were identified. In the systems where debris accumulation on the scar's periphery occurred, a higher amount of those elements was observed in that zone (see for example Fig. S2, Supplementary material).

Fig. 7 depicts AFM images of selected representative areas of the worn surfaces of Ti6Al4V, as well as the surface roughness,  $S_a$ , and maximum peak to valley height,  $S_z$ , for all the tested systems. It was observed that systems containing mucin present similar roughness to HS ( $p = 0.912$ , for AS + M and  $p = 0.184$  for AS + A + U + L + M) and lower roughness than AS ( $p < 0.001$  for both systems). AS + A system presented the highest roughness among all systems ( $p < 0.001$ ). Maximum peak to valley height results show a similar tendency to surface roughness. In all cases, AFM images show scratches, whose depth is influenced by the lubricant used: HS, AS + M, AS + A + U + L + M, AS + U and AS + L systems ( $\sim 300$ – $400$  nm), AS ( $\sim 500$  nm), AS + A + U + L ( $\sim 600$  nm) and AS + A ( $\sim 800$  nm).

Regarding zirconia plates, no measurable wear was observed for all the tested systems. Besides, it was found that only HS, AS, AS + U, AS + L and AS + A + U + L systems revealed the presence of adhered titanium. A typical SEM image of the zirconia surface and an EDS spectrum obtained in a zone with adhered particles is shown in Fig. 8.

##### 3.1.6. Electrochemical measurements

The OCP variation on the different tribological systems is shown in Fig. 9. The curves obtained with HS and AS were shown in each case for comparison. A significant potential drop occurred when the wear test began. The lowest OCP values were registered for HS, while de artificial saliva solutions containing mucin (AS + M and AS + A + U + L + M) showed the highest values during sliding. Once the wear tests finished, the OCP values increased progressively during time, but these values never reached the initial potential.

## 4. Discussion

In the evaluation of the tribological performance of dental materials, it is usually used artificial saliva, containing several saline compounds, as lubricant medium. However, the human saliva contains a variety of organic compounds. These molecules can adsorb on the contacting surfaces, changing the characteristics of the interface, which may lead to a significant modification of the tribological behavior. More, the rheological characteristics of the lubricant medium, may also be different and affect such behavior. Thus, the objective of this work was 1) to study the influence of some saliva biomolecules (albumin, urea, lysozyme and mucin) on the tribological response of the Ti6Al4V-zirconia pair and 2)

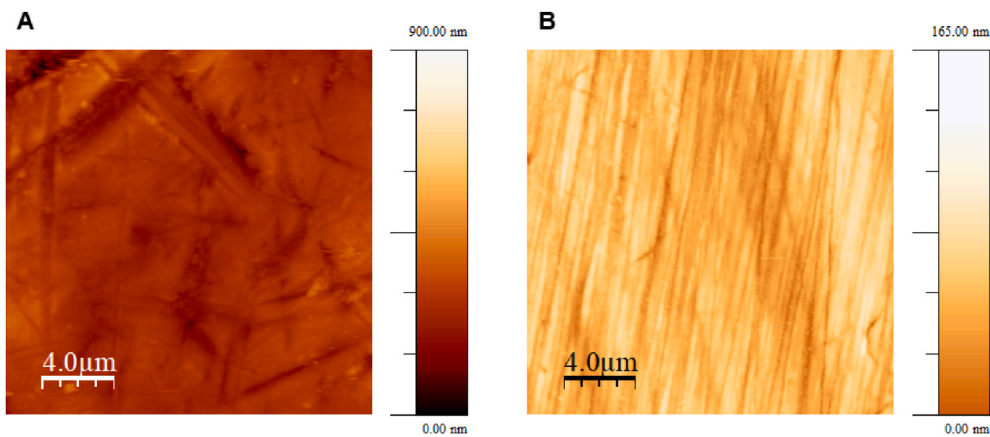


Fig. 3. AFM images of the initial surfaces of (A) Ti6Al4V balls and (B) zirconia plates.

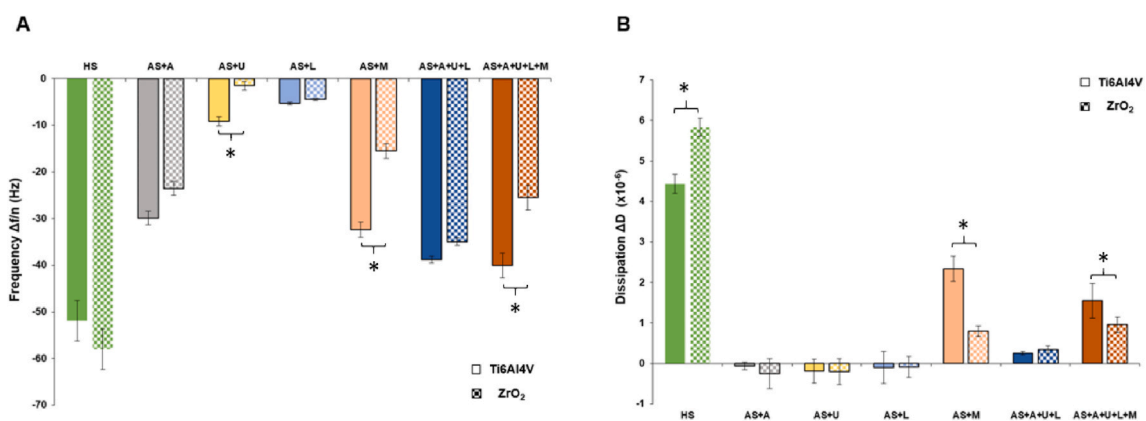


Fig. 4. Frequency ( $\Delta f/n$ ) (A) and dissipation ( $\Delta D$ ) (B) changes for the 3rd harmonic, due to the addition of lubricant solutions to Ti6Al4V and zirconia coated crystals, obtained by QCM-D. Values were obtained by difference between the final value after rinsing and the initial value correspondent to the baseline. The symbol \* identify statistically significant differences.

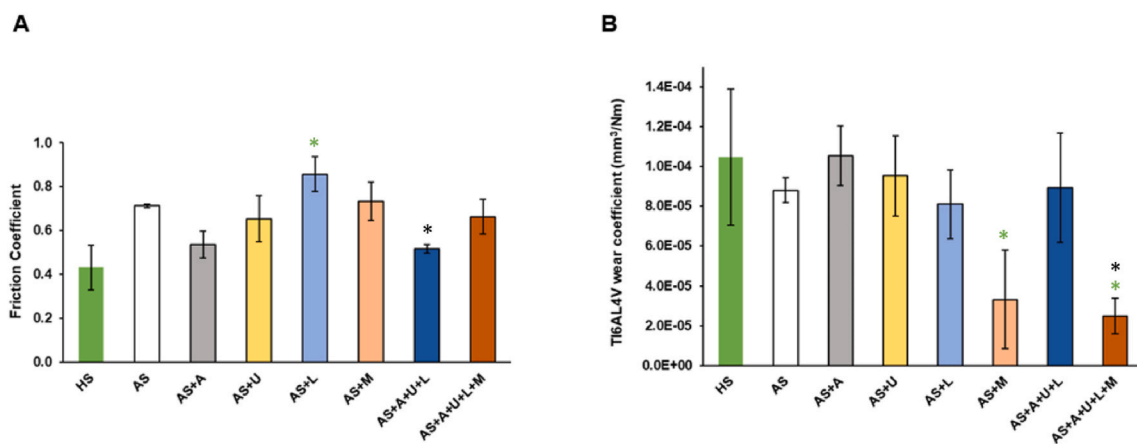


Fig. 5. (A) Friction coefficient and (B) Ti6Al4V wear coefficient of the different tribological systems. The symbols \* in green and black identify statistically significant differences relative to HS and AS, respectively.

to compare the obtained results with those observed for human saliva. This pair was chosen since it corresponds to the materials that are usually in contact in an implant-abutment junction. Zirconia is much harder than the metallic alloy. The measured values fall in both cases in the ranges found in the literature for these materials (Branco et al., 2020; Candido et al., 2014; Maj et al., 2018). The contact configuration used was Ti6Al4V ball sliding against a zirconia plate. This configuration was

already studied in another work of our group (Branco et al., 2019), where it was found that it is the most suitable one to simulate this contact in reciprocating sliding tests, because the worn metallic surface never loses contact with the ceramic surface, impairing its repassivation, as happens in the implant-abutment connection area.

Regarding the concentration of the biomolecules added to artificial saliva, these quantities were chosen based on previous works where

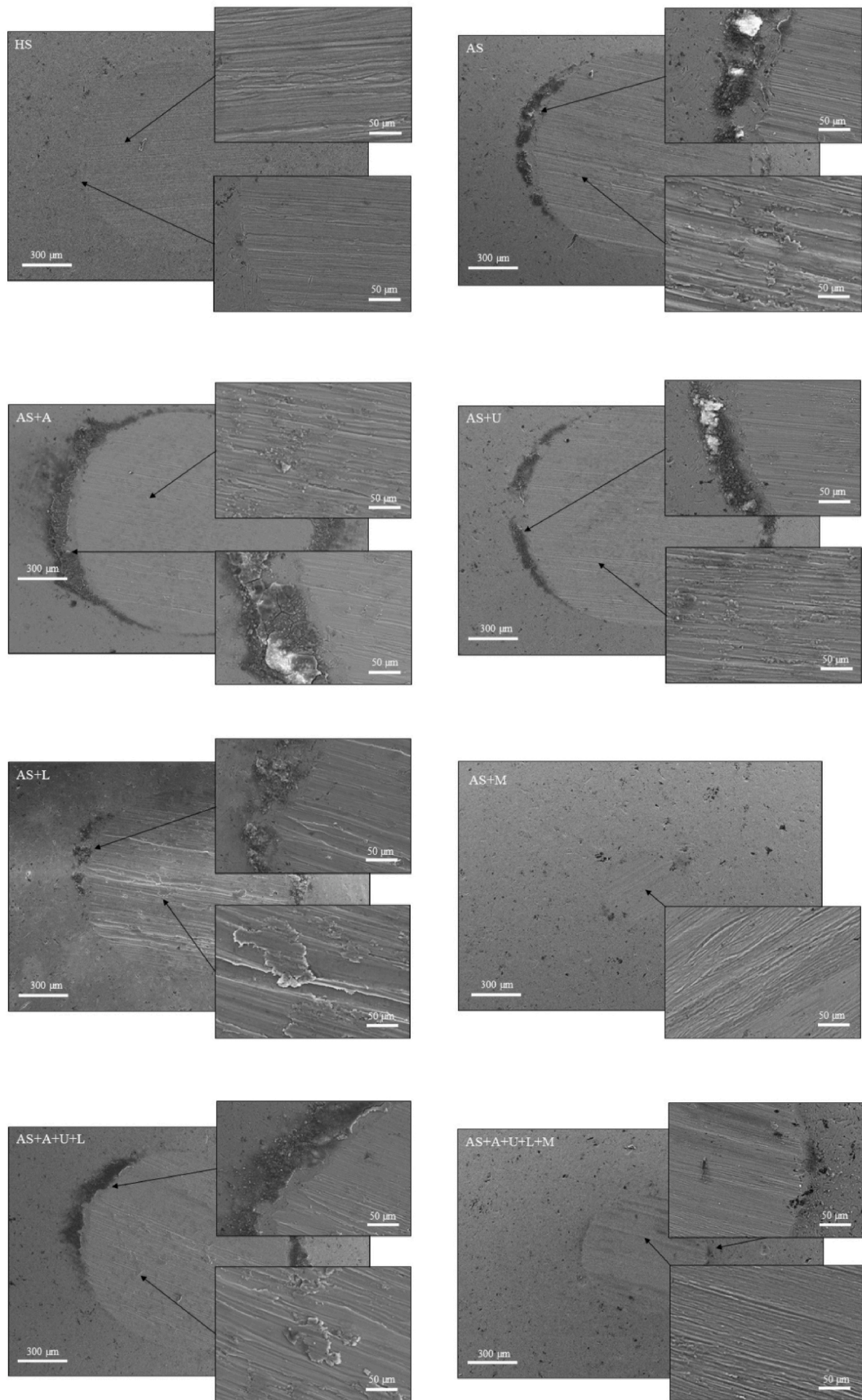


Fig. 6. SEM analysis of titanium balls wear scars for the different tribological systems.

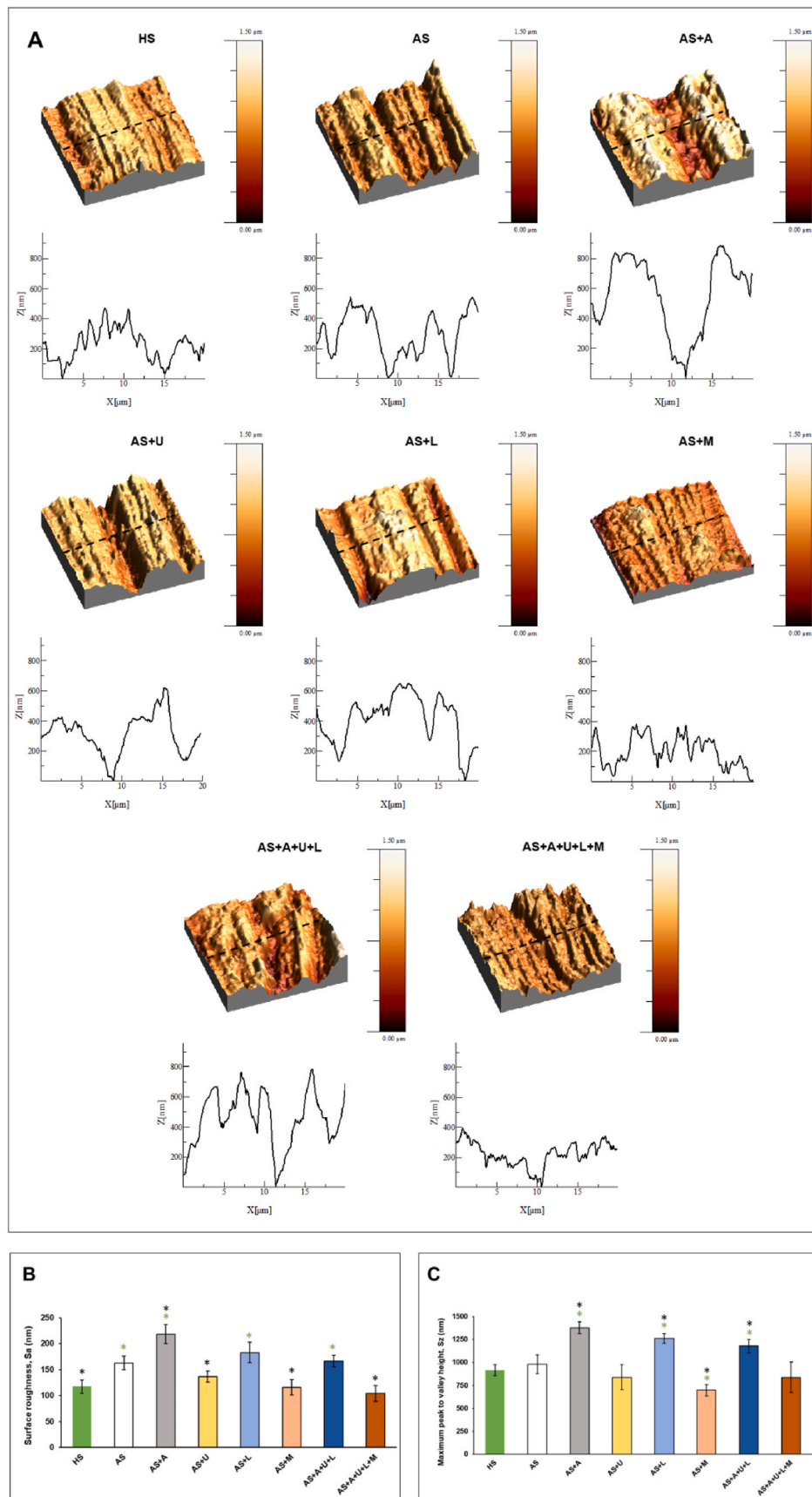


Fig. 7. (A) AFM images of the Ti6Al4V worn surface for all the tested systems and corresponding (B) surface roughness ( $S_a$ ) and (C) maximum peak to valley height ( $S_z$ ). The symbols \* in green and black identify statistically significant differences relative to HS and AS, respectively.

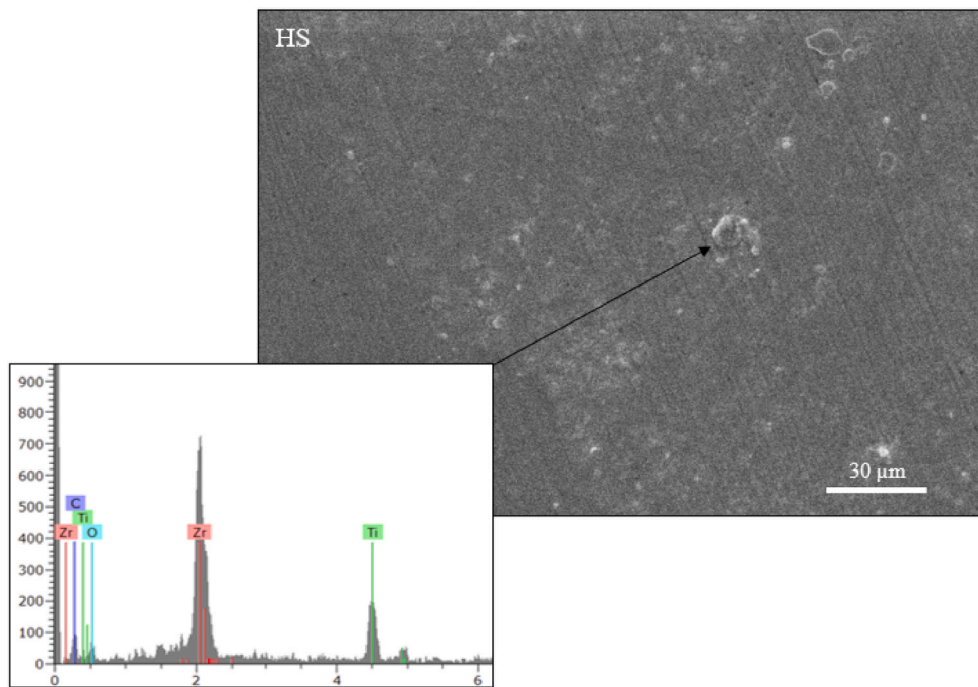


Fig. 8. SEM image and respective EDS analysis of zirconia tested with HS.

human saliva composition was studied and the contents of albumin, urea, lysozyme and mucin were determined (Edgar et al., 2012; Henskens et al., 1996; Hershkovich and Nagler, 2004; Ratner et al., 1996). The viscosity of the artificial saliva containing or not the different biomolecules was measured. It was found that all these fluids, independently of their composition, present a Newtonian behavior in the shear rate range studied. The viscosity values (in the range 0.6–0.9 mPa s<sup>-1</sup>) were analogous to those found by other authors for similar solutions (Gispert et al., 2006; Gonçalves et al., 2016). Human saliva viscosity was also determined. It presents higher viscosity values and a pseudoplastic behavior, in agreement with what was found in literature (Łysik et al., 2019; Mystkowska et al., 2016).

The interaction of the studied biomolecules with the surfaces of Ti6Al4V and zirconia was investigated by QCM-D. The decrease observed in frequency ( $\Delta f/n$ ) is due to the adsorption of the biomolecules. For rigid films, the variation of frequency is proportional to the mass of the adsorbed layer ( $\Delta m$ ), which can be calculated through the Sauerbrey equation (Serro et al., 2010):

$$\Delta m = -\frac{C \cdot \Delta f}{n} \quad (2)$$

where  $C$  is the mass sensitivity constant characteristic of the crystal (17.7 ng.Hz<sup>-1</sup>. cm<sup>-2</sup> for 5 MHz crystals) and  $n$  is the harmonic number. However, for viscoelastic layers this equation leads to an underestimation of the adsorbed mass. In this case, a more complex approach is needed, and Sauerbrey equation must be modified to take into account the viscoelastic properties of the adsorbed layer. Since upon adsorption of the different biomolecules, the variations of dissipation ( $\Delta D$ ) are low and the shifts in both frequency and dissipation signals are neglectable for the different harmonics (except for the artificial saliva solutions containing mucin, see Fig. 4 and Fig. S1), one can assumed valid the Sauerbrey equation and calculate the adsorbed mass. The thickness of the formed layer can be then estimated knowing the film's density ( $d$ ):

$$t = \frac{\Delta m}{d} \quad (3)$$

The characteristics of the adsorbed protein films depends on the proteins, adsorbing surfaces and of the medium (Serro et al., 2012).

Values between 1.05 and 1.36 g cm<sup>-3</sup> were found in literature for the density of layers of mucin, albumin and lysozyme adsorbed on different surfaces (Gispert et al., 2008; Jin et al., 2015; Rondelli et al., 2019). Considering these densities, the thickness of the albumin, lysozyme and mucin adsorbed layers onto Ti6Al4V were estimated to be 4.6 nm, 0.7 nm and 5.5 nm and onto zirconia 3.6 nm, 0.6 nm and 2.6 nm, respectively. It shall be stressed that for mucin, the values obtained shall be underestimated, since the viscoelastic behaviour of the adsorbed film is not neglectable and the application of Sauerbrey equation is not straight. For urea and the biomolecules mixtures, the layers' thicknesses were not estimated due to the absence of data in the literature. The presented values give an order of magnitude of the average thickness of the adsorbed layers, but with the data available it is not possible to infer about the surface's coverage degree, i.e., if the films are continuous or not.

#### 4.1. Influence of saliva biomolecules on the tribological behavior of the Ti6Al4V-zirconia pair

The slight differences found between the average friction coefficient obtained with the different lubricants are hard to explain. In the systems where the lubricant contains albumin (AS + A and AS + A + U + L), lower values were observed relatively to AS. Contrarily, for the system only with lysozyme (AS + L), the friction coefficient is maximum. For mucin containing lubricants (AS + M and AS + A + U + L + M) the values remain similar to AS. A drop of pH shall occur in the sliding area due to anodic character of the contacting metal. On the other hand, temperature shall rise as result of friction. Both factors can change the characteristics of the adsorbing surfaces and the biomolecules structure or even lead to their denaturation, affecting their adsorption behavior. A with range of factors may act in opposite directions, conditioning the obtained results. For example, negative surface charge of zirconia increases with temperature, but decreases with pH decrease (Muhammad et al., 2012). The net charge of albumin, which is negative at the initial pH of the solution (before the test pH  $\approx$  6.4), becomes more positive as the pH decreases (Klok et al., 2020), which impairs its adsorption to the anodic surface of Ti6Al4V. However, since this is a soft protein, with low conformational stability, it can adsorb even in electrostatic unfavorable

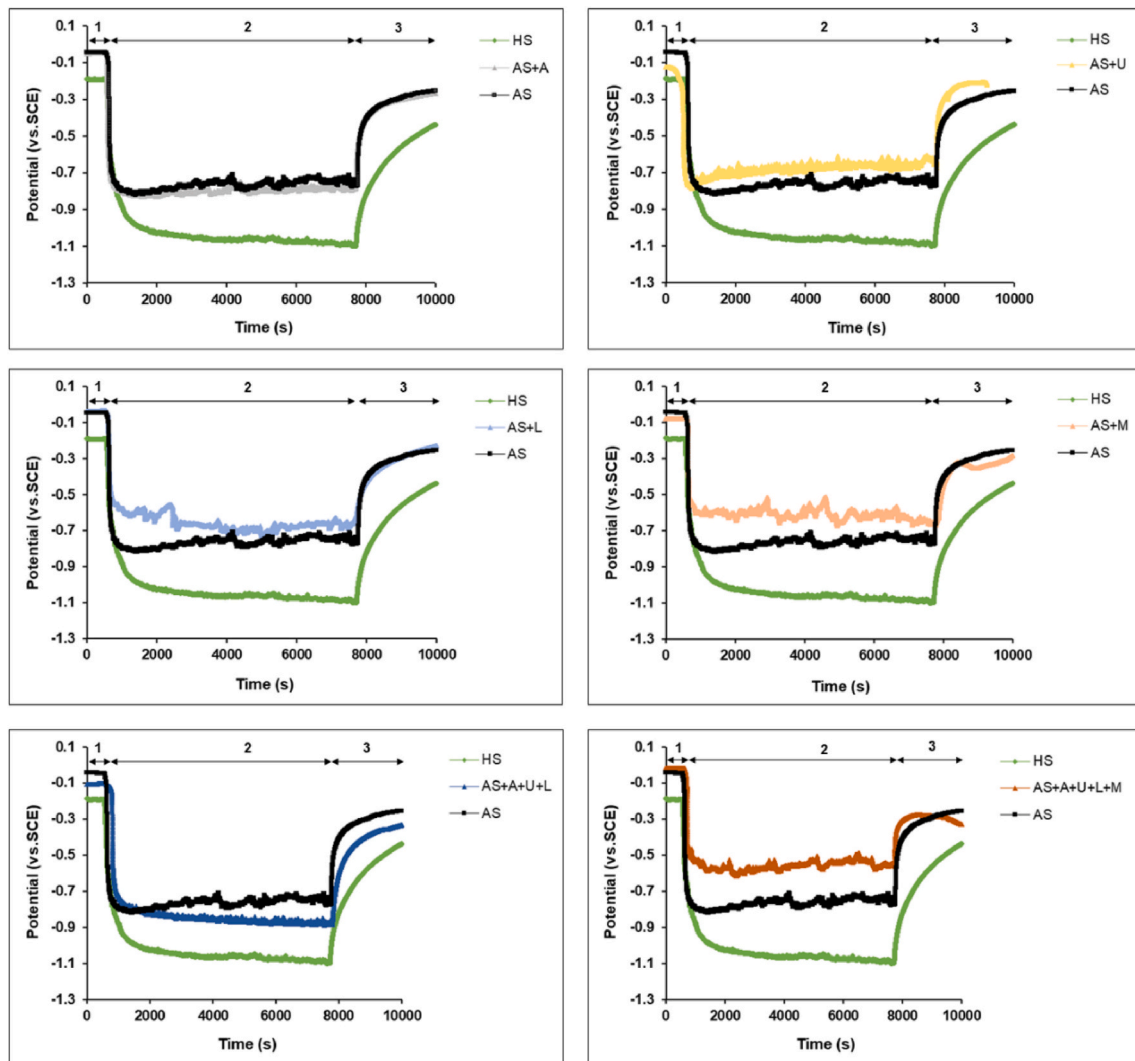


Fig. 9. OCP evolution for the different tribological systems (A) AS + A, (B) AS + U, (C) AS + L, (D) AS + M, (E) AS + A + U + L and (F) AS + A + U + L + M, being (1) the stabilization, (2) the sliding and (3) repassivation. In all cases, the OCP curves are compared to artificial saliva (AS) and human saliva (HS).

conditions. In the case of mucin, aggregates are known to form when pH decreases, leading to an increase of viscosity (Argenis Caicedo and Perilla, 2015), which may contribute to increase friction coefficient, but other authors also report that the friction behavior is not affected by the presence of this protein in acidic medium (Smart et al., 2020), although the reason is unknown. Therefore, further studies are needed to clarify these results.

No measurable wear was found on zirconia plates, independently of the tested lubricant. Zirconia can undergo ageing due to mechanical (e.g. wear) and environmental action (e.g. temperature), with intergranular fracture due to grains expansion as a result of the tetragonal to monoclinic phase transformation, followed by zirconia grains pull out (Lawson, 1995; Lughii and Sergio, 2011). However, SEM images did not reveal such features (e.g. Fig. 8), which agrees with previous observations from other studies that used counterfaces of human teeth (Santos et al., 2018) and titanium (Branco et al., 2019). Material transference onto zirconia surface was only observed for AS + L, AS + U, AS + A + U + L, corresponding to agglomerates of Ti6Al4V particles, showing that in these systems, the biomolecules did not prevent adhesion. Regarding Ti6Al4V wear coefficient, the systems containing mucin (AS + M and AS + A + U + L + M) stand out due to the lower observed values compared to those obtained with the remaining lubricants. A similar behavior was found by other authors in experiments carried out with composite resins, titanium and even enamel samples (Rocha et al., 2013; Smart et al.,

2020; Turssi et al., 2006). The protective role of mucin depends on different factors, such as the pH, the presence of salts, and the characteristics of the contacting surfaces which determine the way of interaction between the protein and the surfaces and the specificities of the protection mechanism. Scratches resultant from the abrasive action of the zirconia hard asperities that cut/plough the Ti6Al4V surface were observed in the ball worn surface in all cases (Fig. 6). However, such scratches are less evident when mucin is present. In fact, AFM topographical analysis (Fig. 7) shows that their depth is much lower in this case, suggesting a lower asperity contact between the sliding surfaces. More, no adherent particles were found on the wear scars, revealing that mucin, besides protecting the surface from abrasion, also prevents adhesion. The lower wear coefficient found for the systems where mucin is present can be attributed to the nature of the adsorbed protein layer on the sliding surfaces. In fact, proteins are known to adsorb in a wide range of surfaces (Gispert et al., 2006; Ivarsson et al., 1985; Mystkowska et al., 2019; Steinberg et al., 1995). In most of the cases these layers reduce the direct contact between the asperities of the sliding surfaces, leading to a boundary lubricating regime and decreasing wear (Fang et al., 2009). QCM-D measurements showed that adsorption of albumin and mucin onto Ti6Al4V and zirconia occurs in higher amount than urea and lysozyme. However, the wear coefficient obtained for albumin was superior to that found for mucin. This may be related with the viscoelastic characteristics of the film, evidenced by the higher dissipation of the

adsorbed mucin layers (specially onto Ti6Al4V, Fig. 4B). Similar findings were reported by Feiler et al. (2007), who observed that albumin and mucin adsorbed differently onto polystyrene surfaces: while albumin formed a flat rigid layer, mucin adsorbed in a diffuse, highly viscoelastic layer.

For all the tested lubricants, a dramatic OCP drop during the time span of the wear tests was observed, indicating that Ti6Al4V suffered tribocorrosion. This is in agreement with the results described in other works [17,57–60], and is due to the total or partial disruption of the nanometric TiO<sub>2</sub> passive film formed on the surface (Rodrigues et al., 2013). The passive film has semiconductive properties and is responsible for the protection of the metallic surface against corrosion (Pejaković et al., 2018). A higher disruption shall occur in the areas where the asperities of zirconia and titanium surfaces contact, which makes the Ti6Al4V surface more reactive and triggering the formation of metallic wear debris due to the adhesion between surfaces. The potential drop may also result from the damage induced by zirconia by the those wear debris (oxidized titanium particles) that may be part of the interfacial medium, from the deformation of the titanium sliding surfaces, the increase of temperature and the galvanic polarization coupling (Wood, 2007). The smallest potential drop was observed for the systems containing mucin (AS + M and AS + A + U + L + M) (Fig. 9 d and f), indicating that this protein induces a lower corrosion activity during sliding. The nature of the tribolayer formed when mucin is present, shall reduce the contact between the asperities of the two surfaces, resulting in a lower passive film disruption (Rocha et al., 2013) and in a lower increase of surface roughness after the wear tests (S<sub>a</sub> and S<sub>z</sub>, Fig. 7B and C).

Besides, due to the contact configuration, the worn Ti6Al4V surface is anodic, while the adjacent region outside the contact is cathodic. Thus, the formed galvanic polarization coupling may be influenced by the thickness and density of the adsorbed protein layer outside of the contact area, which can control the diffusivity of oxygen to the Ti6Al4V surface. It shall be noted that oxygen controls the cathodic reaction and the formation of the passive film.

#### 4.2. Comparison of the results obtained with artificial lubricants and human saliva

As referred above, HS is the lubricant that presents the highest viscosity among the studied lubricants. Concerning the characteristics of the adsorbed layer, the contact of both Ti6Al4V and zirconia surfaces with HS led to the highest adsorbed mass and to the highest values of dissipation. These differences may be related not only with the more complex composition of HS (it contains other proteins, enzymes and growing factors that were not considered in this work), but also with the presence of human and bacterial cells (about 8 million and 500 million per mL, respectively) (Boron, 2003).

Regarding the tribological behavior, although AS + A mimics the friction and wear coefficients, it does not lead to the same wear mechanisms. Only the lubricants containing mucin (AS + M and AS + A + U + L + M) induced wear mechanisms similar to those found with HS. However, the wear and friction coefficients were significantly different. None of the lubricants studied recreate the corrosion activity of HS during sliding. In fact, HS led to the highest OCP drop, indicating a higher corrosion relatively to the remaining lubricants. Similar results were found by Mystkowska et al. (2019) when compared the OCP during fretting corrosion of 316L stainless steel in AS + M and HS. They observed that the OCP for HS was lower. Again, these results may be explained by the complex composition of HS, that contains a variety of compounds and cells that were not included in the artificial lubricant. For instance, according to Mathew et al. (2012b), lipopolysaccharides, (constituents of gram-negative bacteria) that are highly bioactive molecules present in the gingival crevicular fluid, reduce the tribocorrosion resistance of Ti6Al4V. In contrast, the presence of bacteria on the interface reduces corrosion-wear and friction (Souza et al., 2010, 2013).

In a clinical perspective, the main conclusion of this work is that any of the studied lubricants is able to fully recreate the tribological behaviour of human saliva. This raises concerns when extrapolating the in vitro results obtained with these artificial fluids. Thus, more work needs to be conducted in order to find the composition of artificial saliva that better mimics the natural one.

## 5. Conclusions

In order to study the effect of albumin, urea, lysozyme and mucin on the tribological response of the Ti6Al4V/zirconia interface, pin-on-plate wear tests were carried out in artificial saliva solutions, with and without these organic molecules, and compared with natural human saliva. Prior to measurements, the viscosity of the solutions was measured. Besides, the adsorption of the biomolecules onto Ti6Al4V and zirconia surfaces was studied. The attained results showed that:

- The artificial lubricants present a Newtonian behavior and similar viscosities, contrasting with HS which has a pseudoplastic behavior and significantly higher viscosity.
- The adsorption studies of biomolecules to Ti6Al4V and zirconia shows that the artificial lubricants containing albumin and mucin led to higher adsorbed amounts of protein. The adsorbed films are rigid, except in the case of mucin which presents some viscoelastic character. HS induced the highest adsorption as well as the highest dissipation, indicating the formation of an adsorbed layer with distinct properties.
- The addition of mucin to artificial saliva resulted in a dramatic reduction of wear coefficient.
- In all systems, abrasion was the predominant wear mechanism. Some adhesive wear was also observed, except for mucin containing lubricants and HS.
- Tribocorrosion was present in all the studied systems, being lower when mucin was added and higher for HS.

Overall, both null hypothesis (H01: The tribological behaviour of the studied system in the presence of the different biomolecules is the same; H02: The tribological behaviour of the studied system in the presence of saliva is mimicked by at least one of the studied biomolecules solutions) were rejected. It was concluded that mucin plays a relevant role in the protection of the studied prosthetic materials against wear. Additionally, none of the artificial lubricants mimics the effect of HS on the tribological behaviour of the Ti6Al4V/zirconia pair in terms of friction, wear and corrosion.

## Authors contributions

Conceptualization: R. Colaço, A.P. Serro, C.G. Figueiredo-Pina  
 Methodology: H. Teixeira, D. Silva, A.P. Serro, C.G. Figueiredo-Pina.  
 Software: N.A.  
 Validation: N.A.  
 Formal Analysis: N.A.  
 Investigation: H. Teixeira, A.C. Branco, I. Rodrigues, D. Silva.  
 Resources: S. Cardoso.  
 Data Curation: H. Teixeira, A.C. Branco.  
 Writing – Original Draft Preparation: A.C. Branco.  
 Writing – Review & Editing: A.C. Branco, A.P. Serro, C.G. Figueiredo-Pina.  
 Visualization: N.A.  
 Supervision: R. Colaço, A.P. Serro, C.G. Figueiredo-Pina.  
 Project Administration: R. Colaço, A.P. Serro, C.G. Figueiredo-Pina.  
 Funding Acquisition: R. Colaço, A.P. Serro, C.G. Figueiredo-Pina.

## Declaration of competing interest

The authors declare that they do not have any competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

To Fundação para a Ciência e a Tecnologia (FCT, Portugal) for funding through the unit projects UIDB/00100/2020, UIDB/04585/2020, UID/CTM/04540/2020 and UIDB/50022/2020 (LAETA) from CQE, CiiEM, CeFEMA and IDMEC respectively, and for the PhD grant of A.C. Branco (SFRH/BD/145423/2019). In addition, to Gonçalo Torres for the help in the laboratory.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmbm.2021.104451>.

## References

- Adatia, N.D., Bayne, S.C., Cooper, L.F., Thompson, J.Y., 2009. Fracture resistance of yttria-stabilized zirconia dental implant abutments. *J. Prosthodont.* 18, 17–22. <https://doi.org/10.1111/j.1532-849X.2008.00378.x>.
- Aguirre, A., Tabak, L.A., Dysfunction, S.G., 1987. Artificial salivas: present and future. *J. Dent. Res.* 66, 693–698.
- Argenis Caicedo, J., Perilla, J.E., 2015. Effect of pH on the rheological response of reconstituted gastric mucin. *Ing. Invest.* 35, 43–48. <https://doi.org/10.15446/ing.investig.v35n2.50019>.
- Boron, W.F., 2003. *Medical Physiology: A Cellular and Molecular Approach*. Boyer, R., Collings, E.W., Welsch, G. (Eds.), 1994. *Materials Properties Handbook: Titanium Alloys*. ASM International, Materials Park, OH.
- Branco, A.C., Moreira, V., Reis, J.A., Colaço, R., Figueiredo-pina, C.G., Serro, A.P., 2019. Influence of contact configuration and lubricating conditions on the microtriboactivity of the Zirconia-Ti6Al4V pair used in dental applications. *J. Mech. Behav. Biomed. Mater.* 91, 164–173. <https://doi.org/10.1016/j.jmbm.2018.12.009>.
- Branco, A.C., Silva, R., Santos, T., Jorge, H., Rodrigues, A.R., Fernandes, R., Bandarra, S., Barahona, I., Matos, A.P.A., Lorenz, K., Polido, M., Colaço, R., Serro, A.P., Figueiredo-Pina, C.G., 2020. Suitability of 3D printed pieces of nanocrystalline zirconia for dental applications. *Dent. Mater.* 6, 442–455. <https://doi.org/10.1016/j.dental.2020.01.006>.
- Candido, L.M., Fais, L.M.G., Reis, J.M., dos, S.N., Pinelli, L.A.P., 2014. Surface roughness and hardness of yttria stabilized zirconia (Y-TZP) after 10 years of simulated brushing. *Rev. Odontol. UNESP* 43, 379–383. <https://doi.org/10.1590/1807-2577.1049>.
- Cardoso, S., Li, H., Freitas, P.P., Wei, P., Ramos, A.R., Soares, J.C., 2000. Characterization of nano-oxide layers fabricated by ion beam oxidation: application in specular spin-valves and tunnel junctions. *IEEE Int. Dig. Tech. Pap. Magn. Conf.* 35, 4367.
- Edgar, M., Dawes, C., O'Mullane, D., 2012. Saliva and Oral Health. <https://doi.org/10.1038/sj.bdj.2013.421>.
- Fang, H.W., Hsieh, M.C., Huang, H.T., Tsai, C.Y., Chang, M.H., 2009. Conformational and adsorptive characteristics of albumin affect interfacial protein boundary lubrication: from experimental to molecular dynamics simulation approaches. *Colloids Surf. B Biointerfaces* 68, 171–177. <https://doi.org/10.1016/j.colsurfb.2008.09.029>.
- Feiler, A.A., Sahlholm, A., Sandberg, T., Caldwell, K.D., 2007. Adsorption and viscoelastic properties of fractionated mucin (BSM) and bovine serum albumin (BSA) studied with quartz crystal microbalance (QCM-D). *J. Colloid Interface Sci.* 315, 475–481. <https://doi.org/10.1016/j.jcis.2007.07.029>.
- Figueiredo-Pina, C.G., Guedes, M., Sequeira, J., Pinto, D., Bernardo, N., Carneiro, C., 2019. On the influence of *Streptococcus salivarius* on the wear response of dental implants: an in vitro study. *J. Biomed. Mater. Res. B Appl. Biomater.* 107, 1393–1399. <https://doi.org/10.1002/jbm.b.34231>.
- Fusayama, T., Katayori, T., Nomoto, S., 1963. Corrosion of gold and amalgam placed in contact with each other. *J. Dent. Res.* 42, 1183–1197. <https://doi.org/10.1177/00220345630420051301>.
- Gispert, M.P., Serro, A.P., Colaço, R., Saramago, B., 2006. Friction and wear mechanisms in hip prosthesis: comparison of joint materials behaviour in several lubricants. *Wear* 260, 149–158. <https://doi.org/10.1016/j.wear.2004.12.040>.
- Gispert, M.P., Saramago, B., Serro, A.P., Colaço, R., 2008. Bovine serum albumin adsorption onto 316L stainless steel and alumina: a comparative study using depletion protein radiolabeling quartz crystal microbalance and atomic force microscopy. *Surf. Interface Anal.* 40, 1529–1537. <https://doi.org/10.1002/sia.2929>.
- Gonçalves, A.D., Alexander, C., Roberts, C.J., Spain, S.G., Uddin, S., Allen, S., 2016. The effect of protein concentration on the viscosity of a recombinant albumin solution formulation. *RSC Adv.* 6, 15143–15154. <https://doi.org/10.1039/c5ra21068b>.
- Green, D.J., 1998. *An Introduction to the Mechanical Properties of Ceramics*.
- Henskens, Y., van den Keijbus, P., Veerman, E., Van der Weijden, G., Timmerman, M., Snoek, C., Van der Velden, U., Amerongen, A.N., 1996. Protein composition of whole and parotid saliva in healthy and periodontitis subjects. *J. Periodontol. Res.* 31, 57–65.
- Hershkovich, O., Nagler, R.M., 2004. Biochemical analysis of saliva and taste acuity evaluation in patients with burning mouth syndrome, xerostomia and/or gustatory disturbances. *Arch. Oral Biol.* 49, 515–522. <https://doi.org/10.1016/j.archoralbio.2004.01.012>.
- Humphrey, S.P., Williamson, R.T., 2001. A review of saliva: normal composition, flow, and function. *J. Prosthet. Dent* 85.
- Ivarsson, B.A., Hegg, P.O., Lundström, K.I., Jönsson, U., 1985. Adsorption of proteins on metal surfaces studied by ellipsometric and capacitance measurements. *Colloid. Surface.* 13, 169–192. [https://doi.org/10.1016/0166-6622\(85\)80015-9](https://doi.org/10.1016/0166-6622(85)80015-9).
- Jin, J., Han, Y., Zhang, C., Liu, J., Jiang, W., Yin, J., Liang, H., 2015. Effect of Grafted PEG Chain Conformation on Albumin and Lysozyme Adsorption: A Combined Study Using QCM-D and DPI, Colloids and Surfaces B: Biointerfaces. Elsevier B.V. <https://doi.org/10.1016/j.colsurfb.2015.10.025>.
- Kerstein, R.B., Radke, J., 2008. A comparison of fabrication precision and mechanical reliability of 2 zirconia implant abutments. *Int. J. Oral Maxillofac. Implants* 23, 1029–1036. [https://doi.org/10.1016/S0022-3913\(09\)60086-X](https://doi.org/10.1016/S0022-3913(09)60086-X).
- Khemiss, M., Khelifa, M., Ben, Saad, Ben, H., 2017. Preliminary findings on the correlation of saliva pH, buffering capacity, flow rate and consistency in relation to waterpipe tobacco smoking. *Libyan J. Med.* 12. <https://doi.org/10.1080/19932820.2017.1289651>.
- Klok, O., Munoz, A.I., Mischler, S., 2020. An overview of serum albumin interactions with biomedical alloys. *Materials (Basel)* 13, 1–26. <https://doi.org/10.3390/ma13214858>.
- Lawson, S., 1995. Environmental degradation of zirconia ceramics. *J. Eur. Ceram. Soc.* 15, 485–502. [https://doi.org/10.1016/0955-2219\(95\)00035-S](https://doi.org/10.1016/0955-2219(95)00035-S).
- Licausi, M.P., Igual Muñoz, A., Borrás, V.A., 2013. Tribocorrosion mechanisms of Ti6Al4V biomedical alloys in artificial saliva with different pHs. *J. Phys. D Appl. Phys.* 46. <https://doi.org/10.1088/0022-3727/46/40/404003>.
- Loginov, Y.N., Golodnov, A.I., Stepanov, S.I., Kovalev, E.Y., 2017. Determining the Young's Modulus of a Cellular Titanium Implant by FEM Simulation. <https://doi.org/10.1063/1.5017330>.
- Lughi, V., Sergo, V., 2011. Low temperature degradation –aging– of zirconia: a critical review of the relevant aspects in dentistry. *J. Prosthet. Dent* 105, 90. [https://doi.org/10.1016/s0022-3913\(11\)60005-x](https://doi.org/10.1016/s0022-3913(11)60005-x).
- Maj, L., Morgiel, J., Mars, K., Grzegorek, J., Faryna, M., Godlewska, E., 2018. Microstructure and hardness of Ti6Al4V/NiAl/Ti6Al4V joints obtained through resistive heating. *J. Mater. Process. Technol.* 255, 689–695. <https://doi.org/10.1016/j.jmatprotec.2018.01.023>.
- Mathew, M.T., Abbey, S., Hallab, N.J., Hall, D.J., Sukotjo, C., Wimmer, M.A., 2012a. Influence of pH on the tribocorrosion behavior of CpTi in the oral environment: synergistic interactions of wear and corrosion. *J. Biomed. Mater. Res. B Appl. Biomater.* 100 B, 1662–1671. <https://doi.org/10.1002/jbm.b.32735>.
- Mathew, M.T., Barão, V.A., Yuan, J.C.C., Assunção, W.G., Sukotjo, C., Wimmer, M.A., 2012b. What is the role of lipopolysaccharide on the tribocorrosive behavior of titanium? *J. Mech. Behav. Biomed. Mater.* 8, 71–85. <https://doi.org/10.1016/j.jmbm.2011.11.004>.
- Meurman, J.H., Rantonen, P., Pajukoski, H., Sulkava, R., 2002. Salivary albumin and other constituents and their relation to oral and general health in the elderly. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 94, 432–438. <https://doi.org/10.1067/moe.2002.122345>.
- Muhammad, S., Hussain, S.T., Waseem, M., Naem, A., Hussain, J., Tariq Jan, M., 2012. Surface charge properties of zirconium dioxide. *Iran. J. Sci. Technol. Trans. A Sci.* 36, 481–486. <https://doi.org/10.22099/ijts.2012.2110>.
- Mystkowska, J., Karalus, W., Sidorenko, J., Dąbrowski, J.R., Kalska-Szostko, B., 2016. Biotribological properties of dentures lubricated with artificial saliva. *J. Frict. Wear* 37, 544–551. <https://doi.org/10.3103/S1068366616060106>.
- Mystkowska, J., Łysik, D., Klekotka, M., 2019. Effect of saliva and mucin-based saliva substitutes on fretting processes of 316 austenitic stainless steel. *Metals (Basel)* 9, 178. <https://doi.org/10.3390/met9020178>.
- Nicholson, W., 2020. Titanium alloys for dental implants: a review. *Prosthesis* 2, 100–116. <https://doi.org/10.3390/prosthesis2020011>.
- Noumbissi, S., Scarano, A., Gupta, S., 2019. A literature review study on atomic ions dissolution of titanium and its alloys in implant dentistry. *Materials (Basel)* 12, 1–15. <https://doi.org/10.3390/ma12030368>.
- Özcan, M., Hämmerle, C., 2012. Titanium as a reconstruction and implant material in dentistry: advantages and pitfalls. *Materials (Basel)* 5, 1528–1545. <https://doi.org/10.3390/ma5091528>.
- Pejaković, V., Totolin, V., Rodríguez Ripoll, M., 2018. Tribocorrosion behaviour of Ti6Al4V in artificial seawater at low contact pressures. *Tribol. Int.* 119, 55–65. <https://doi.org/10.1016/j.triboint.2017.10.025>.
- Prinz, J.F., Wijk, R.A. De, Huntjens, L., 2007. Load dependency of the coefficient of friction of oral mucosa. *Food Hydrocolloids* 21, 402–408. <https://doi.org/10.1016/j.foodhyd.2006.05.005>.
- Ranc, H., Elkhyat, A., Servais, C., Mac-mary, S., Launay, B., Humbert, P., 2006a. Friction coefficient and wettability of oral mucosal tissue: changes induced by a salivary layer. *Colloid. Surface.* 276, 155–161. <https://doi.org/10.1016/j.colsurfa.2005.10.033>.
- Ranc, H., Servais, C., Chauvy, P., Debaud, S., Mischler, S., 2006b. Effect of surface structure on frictional behaviour of a tongue/palate tribological system. *Tribol. Int.* 39, 1518–1526. <https://doi.org/10.1016/j.triboint.2006.01.017>.
- Ratner, B., Schoen, F.J., Lemons, J.E., 1996. *Biomaterials Science: An Introduction to Materials in Medicine*.
- Revathi, A., Borrás, A.D., Muñoz, A.I., Richard, C., Manivasagam, G., 2017. Degradation mechanisms and future challenges of titanium and its alloys for dental implant applications in oral environment. *Mater. Sci. Eng. C* 76, 1354–1368. <https://doi.org/10.1016/j.msec.2017.02.159>.

- Rocha, L.A., Oliveira, F., Cruz, H.V., Sukotjo, C., Mathew, M.T., 2013. 10 - bio-tribocorrosion in dental applications. In: *Bio-Tribocorrosion in Biomaterials and Medical Implants*, pp. 223–249.
- Rodrigues, D.C., Valderrama, P., Wilson, T.G., Palmer, K., Thomas, A., Sridhar, S., Adapalli, A., Burbano, M., Wadhvani, C., 2013. Titanium corrosion mechanisms in the oral environment: a retrieval study. *Materials (Basel)* 6, 5258–5274. <https://doi.org/10.3390/ma6115258>.
- Rondelli, V., Di Cola, E., Koutsioubas, A., Alongi, J., Ferruti, P., Ranucci, E., Brocca, P., 2019. Mucin thin layers: a model for mucus-covered tissues. *Int. J. Mol. Sci.* 20, 1–15. <https://doi.org/10.3390/ijms20153712>.
- Sajewicz, E., 2009. Effect of saliva viscosity on tribological behaviour of tooth enamel. *Tribol. Int.* 42, 327–332. <https://doi.org/10.1016/j.triboint.2008.07.001>.
- Santos, F., Branco, A., Polido, M., Serro, A.P., 2018. Comparative study of the wear of the pair human teeth/Vita Enamic® vs commonly used dental ceramics through chewing simulation. *J. Mech. Behav. Biomed. Mater.* 88, 251–260. <https://doi.org/10.1016/j.jmbbm.2018.08.029>.
- Sarkar, A., Xu, F., Lee, S., 2019. Human saliva and model saliva at bulk to adsorbed phases – similarities and differences. *Adv. Colloid Interface Sci.* 273, 102034. <https://doi.org/10.1016/j.cis.2019.102034>.
- Schenkels, L.C.M., Veerman, E.C.I., Amerongen, A.V.N., 1995. Biochemical composition of human saliva in relation to other mucosal fluids. *Crit. Rev. Oral Biol. Med.* 6, 161–175.
- Serro, A.P., Degiampietro, K., Colaço, R., Saramago, B., 2010. Adsorption of albumin and sodium hyaluronate on UHMWPE: a QCM-D and AFM study. *Colloids Surf. B Biointerfaces* 78, 1–7. <https://doi.org/10.1016/j.colsurfb.2010.01.022>.
- Serro, A.P., Colaço, R., Saramago, B., 2012. Effect of albumin adsorption on biotribological properties of artificial joint materials. In: *Proteins at Interfaces III – State of the Art. ACS Symposium Series*, pp. 497–523. <https://doi.org/10.1021/bk-2012-1120.ch023>.
- Smart, P., Neville, A., Bryant, M., 2020. Tribocorrosion of dental tissues: the role of mucin. *Tribol. Int.* 148, 106337. <https://doi.org/10.1016/j.triboint.2020.106337>.
- Souza, J., Henriques, M., Oliveira, R., Teughels, W., Celis, J., Rocha, L., 2010. Do oral biofilms influence the wear and corrosion behavior of titanium? *Biofouling J. Bioadhesion Biofilm Res.* 26, 471–478.
- Souza, J.C.M., Ponthiaux, P., Henriques, M., Oliveira, R., Teughels, W., Celis, J.P., Rocha, L.A., 2013. Corrosion behaviour of titanium in the presence of *Streptococcus mutans*. *J. Dent.* 41, 528–534. <https://doi.org/10.1016/j.jdent.2013.03.008>.
- Steinberg, D., Klinger, A., Kohavi, D., Sela, M.N., 1995. Adsorption of human salivary proteins to titanium powder. I. Adsorption of human salivary albumin. *Biomaterials* 16, 1339–1343. [https://doi.org/10.1016/0142-9612\(95\)91050-9](https://doi.org/10.1016/0142-9612(95)91050-9).
- Tabak, L.A., 1990. Structure and function of human salivary mucins. *Crit. Rev. Oral Biol. Med.* 1, 229–234. <https://doi.org/10.1177/10454411900010040201>.
- Taylor, T., Klotz, M., Lawton, R., 2014. Titanium tattooing associated with zirconia implant abutments: a clinical report of two cases. *Int. J. Oral Maxillofac. Implants* 29, 958–960. <https://doi.org/10.11607/jomi.3700>.
- Turssi, C.P., Faraoni, J.J., De Menezes, M., Serra, M.C., 2006. Analysis of potential lubricants for in vitro wear testing. *Dent. Mater.* 22, 77–83. <https://doi.org/10.1016/j.dental.2005.02.010>.
- Wood, R.J.K., 2007. Tribo-corrosion of coatings: a review. *J. Phys. D Appl. Phys.* 40, 5502–5521. <https://doi.org/10.1088/0022-3727/40/18/S10>.
- Zabokova Bilbilova, E., Sotirovska Ivkowska, A., Ambarkova, V., 2012. Correlation between salivary urea level and dental caries. *Prilozi* 33, 289–302.
- Zalewska, A., Waszkiewicz, N., Szajda, S.D., Waszkiel, D., 2011. Impact of salivary flow and lysozyme content and output on the oral health of rheumatoid arthritis patients. *Postepy Hig. Med. Dosw.* 65, 40–45. <https://doi.org/10.5604/17322693.932232>.
- Zhang, Z., Cardoso, S., Freitas, P.P., Wei, P., Barradas, N., Soares, J.C., 2001. Annealing effect of magnetic tunnel junctions with one FeOx layer inserted at the Al<sub>2</sub>O<sub>3</sub>/CoFe interface. *Appl. Phys. Lett.* 78, 2911–2913. <https://doi.org/10.1063/1.1371538>.
- Lysik, D., Niemirowicz-Laskowska, K., Bucki, R., Tokajuk, G., Mystkowska, J., 2019. Artificial saliva: challenges and future perspectives for the treatment of xerostomia. *Int. J. Mol. Sci.* 20 <https://doi.org/10.3390/ijms20133199>.