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Low friction hydrogel with diclofenac eluting ability for dry eye therapeutic contact lenses

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ABSTRACT

When placed in the eye, contact lenses (CLs) disturb the tear fluid and affect the natural tribological behaviour of the eye. The disruption in the contact mechanics between the ocular tissues can increase frictional shear stress and ocular dryness, causing discomfort. Ultimately, continuous CLs wear can trigger inflammation which is particularly critical for people suffering from dry eye. In this work, a double strategy was followed to obtain therapeutic daily disposable CLs for dry eye: a hydroxyethyl methacrylate (HEMA) based hydrogel was coated with two natural polysaccharides, chitosan (CHI) and hyaluronic acid (HA) and posteriorly loaded with an anti-inflammatory drug (diclofenac, DCF). Material sterilisation was carried out by high hydrostatic pressure (HHP) combined with moderate temperature. The friction coefficient (μ) was determined in the presence of different tear biomolecules (cholesterol, lysozyme and albumin) using a nanotribometer. Drug release experiments were performed in static and in hydrodynamic conditions. The material was extensively characterised, regarding surface morphology/topography, optical properties, water content and swelling behaviour, wettability, ionic and oxygen permeability and mechanical properties. It was found that the coating did not impair the physico-chemical properties relevant for the material's application in CLs. Besides, it also ensured a sustained release of DCF for 24 h in tests performed in hydrodynamic conditions that simulate those found in the eye, increasing significantly the amount of drug released. It reduced friction, improving the lubrication ability of the hydrogel, and presented antibacterial properties against *S. aureus*, *P. aeruginosa* and *B. Cereus*. The coated samples did not reveal any signs of cytotoxicity or potential eye irritation. Overall, the coating of the hydrogel may be useful to produce daily CLs able to alleviate dry eye symptoms and the discomfort of CLs wearers.

1. Introduction

Contact lenses (CLs), widely used for the correction of vision refractive errors, affect the natural stability of the ocular surface, by dividing the tear fluid film in post-lens tear film (POLTF) and in pre-lens tear film (PLTF) [1,2]. Their use also impacts the tribological behaviour of the eye, as the contact mechanics between the tissues changes and the blinking mechanism and frequency is also altered [3]. The disruption of the tear film can increase the evaporation and enhance frictional shear stress, causing ocular dryness and discomfort, which often leads to ocular inflammation. These factors can be further aggravated by several disorders or diseases, as dry eye disease (DED), chronic non-infectious inflammation, or seasonal allergic conjunctivitis, which affect millions of individuals worldwide, significantly impairing their quality of life. For example, it is estimated that DED affects 5–50 % of the global population, depending on the geographic region [4], with prevalence

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increasing with age, particularly among women. The severity of DED ranges from mild discomfort to severe pain, blurred vision, and ocular surface damage.

The most common treatments include artificial tears, topical medication (immunosuppressant agents like cyclosporine or anti-inflammatories), punctual plugs to retain tears, and lifestyle changes to reduce environmental triggers. Regarding anti-inflammatories, non-steroidal drugs (NSAIDs) are currently preferred to steroids, due to their less-severe side effects and because steroids locally suppress the immune response in patients with an already compromised ocular surface [5]. Short-term use of NSAIDs can help to reduce the ocular discomfort caused by DED, without major risks for the corneal epithelium [6]. Diclofenac (DCF), bromfenac and pranoprofen are among the most used NSAIDs to treat eye pain, redness, and swelling [7,8]. Studies performed with animal models showed that, contrarily to bromfenac, DCF diminished the corneal surface damage without affecting the volume of tear fluid and was able to avoid the apoptosis of ocular epithelial cells induced by hyperosmolarity (a key pathological factor of DED) and cell growth arrest [9]. Regarding pranoprofen, its application at a dose of 0.1 % was found to be advantageous for patients with mild to moderate DED [10]. More, Akyol-Salman *et al.* [11], found that pranoprofen was as effective as DCF in the same dose to reduce ocular inflammation and pain. DCF mechanism of action involves the inhibition of the stage of conversion of arachidonic acid to prostaglandin H2, which is mediated by the cyclooxygenase isoenzymes COX-1 (constitutive) and COX-2 (activated only during the inflammatory process) [12]. DCF decreases corneal sensitivity and has an analgesic and anaesthetic effect, reducing ocular discomfort. Some reports also state that it impairs bacterial colonisation of CLs and prevents bacterial adhesion to human corneal epithelial cells [13,14].

Although the most common form of ocular drug administration is through eye drops, a large number of studies have focused on the possibility of using CLs as drug delivery systems [15]. These allow increased drug bioavailability compared to conventional eye drops (it is estimated to increase from 1-5 % to ~ 50 % [16]), lower drug losses and less side effects due to the lower doses involved. The sustained delivery of drugs from CLs can be a patient-friendly way of prolonging the drug pre-corneal retention time, improving the ocular tolerance and enhancing its therapeutic efficacy [5]. However, their use also faces challenges e.g., related with patient's comfort and their compliance. A high degree of ocular irritation and/or dryness may discourage prolonged wear. On the other hand, users may forget or improperly use the CLs, affecting consistent drug dosing. Yet, this drug vehiculation method may be quite useful for non-independent people (as bedridden and very old patients), whose medical care is provided by caregivers.

Several authors have studied the release of DCF from hydrogels for CLs and/or commercial CLs, using different strategies to ensure a controlled drug release. For example, Morgan *et al.* [17] loaded silicon-based CLs with DCF by a 'breathing in' technique (soaking of dry lenses in the drug solution) and achieved a sustained drug release over 6 h. Santos *et al.* [18] developed acrylic hydrogels with grafted β-cyclodextrins that improved DCF loading by 1300 %, enhanced drug affinity 15-fold and were able to sustain drug delivery in the lacrimal fluid for two weeks. In turn, Torres-Luna *et al.* [19], evaluated the *in vitro* release of DCF from CLs based on hydroxyethyl methacrylate (HEMA) hydrogels, containing embedded microemulsions of the non-ionic surfactant Brij 97 with low amounts of the cationic surfactant cetalkonium chloride (CKC). The CLs exhibited an extended DCF release, excellent optical transparency and water content. Finally, Silva *et al.* [16,20–22] performed several studies where layer-by-layer (LBL) coatings, prepared with natural based polyelectrolytes (e.g. alginate (ALG), chitosan (CHI), poly-L-lysine (PLL) and hyaluronate (HA)), were used to control the DCF release from silicon-based hydrogels for CLs and commercial CLs. The barrier effect of the LbL coatings for DCF was attributed to electrostatic interactions and hydrogen bonds between the polyelectrolytes and the drug.

The modification of the CLs materials have been attempted, not only to improve drug release kinetics [23–26], but also the material's wettability [27,28], water content [29], mechanical properties [27] and to reduce the coefficient of friction [30]. Friction assumes a particularly relevant role for the comfort provided by the CLs, largely determining their acceptance by patients [31]. To reduce CLs friction, several strategies may be employed, e.g. plasma treatments, incorporation of lubricant agents, or deposition of hydrophilic coatings. Although a significant number of studies have focused on the CLs coating to modify their properties or control their performance, only a small part (~ 1 %) perform friction measurements [32].

Another important factor affecting the tribological behaviour of the CLs when placed in the eye is their interaction with the biomolecules of the tear fluid. Proteins and lipids can adsorb/be absorbed by the CLs materials and significantly affect not only their properties (e.g., water content, oxygen permeability, optical properties) [33–35], but also the biological response, since they may impair or enhance bacterial adhesion [36]. Although this is an important issue, information about the influence of such biomolecules on the tribological behaviour of the CLs materials is still scarce. In a previous work, Silva *et al.* [37] observed distinct behaviours regarding the adsorption of albumin and cholesterol (two of the major components of the tear fluid) on HEMA- and silicone-based hydrogels for CLs. Differences in the structural changes induced by the molecules on the hydrogels, and on the amount and nature of the adsorbed films were advanced to explain their distinct performance.

In this work, a HEMA-based hydrogel was coated with a CHI/HA layer and loaded by soaking in a DCF solution. The main goal was to design a material with a lower μ that simultaneously provided a controlled release of DCF, that could be used in therapeutic CLs to manage dry eye symptoms. Several authors also attempted to modify HEMA-based hydrogels, to obtain CLs capable of alleviating dryness associated with CLs wearing. For example, Maulvi *et al.* [38] investigated the release of HA incorporated in a HEMA hydrogel by soaking or direct entrapment, while Korogiannaki *et al.* [39] grafted the hydrogel surfaces with a hydrophilic layer of HA. Mun *et al.* [40], developed a cholesterol-HA micelle-embedded CL for extended controlled release of cyclosporine, a hydrophobic drug commonly used in the treatment of dry eye. In the present study, the hydrogel was coated by sequential deposition of CHI and HA. CHI is a cationic polysaccharide with antimicrobial and permeation enhancing properties, which make it quite attractive to produce coatings on drug delivery ocular devices [41]. HA is a glycosaminoglycan that presents negative charge at the physiological pH [42]. It is highly hydrophilic and retains large amounts of water, which favours biocompatibility and avoids biofilm formation [43]. This, together with its unique viscoelastic characteristics, confer it excellent lubricating properties. Besides, depending on its molecular weight, it may have antioxidant and anti-inflammatory action [41]. The combination of CHI and HA has been proven to enhance antimicrobial capacity [44].

Since sterilisation is a mandatory step to ensure the biological safety of the CLs, a suitable sterilisation method was pursued to process the hydrogels produced in this work. To avoid the degradation of the coating by conventional sterilisation agents (heat or radiation), an alternative method based on the application of high hydrostatic pressure (HHP) combined with moderate temperature was attempted. The use of this method to sterilise other hydrogels for ophthalmic lenses (CLs and intraocular lenses) was previously explored by the authors successfully [16,45]. In fact, HHP preserved the materials' properties, and contrarily to other methods (e.g. ozone [46]) did not affect the therapeutic activity of the loaded drugs, nor significantly impair the drug release profiles (in some cases, even improved them). More, it has the advantage of being fast, simple, economic and may be considered a green process since it does not produce any toxic residues and only consumes water and electric energy.

The sterilised drug loaded and coated hydrogels were then characterised regarding their frictional behaviour in the presence of different

biomolecules present in the tear fluid (albumin, lysozyme, cholesterol). The DCF release profiles were obtained in static and in hydrodynamic conditions, using in this case a microfluidic cell that approaches the eye functioning (lacrimal fluid volume and renovation rate). Several relevant properties for CLs application were accessed (e.g. morphology, water content, wettability, optical properties, ionic and oxygen permeability) and the stability of the produced layer was also investigated. The antibacterial properties of the samples against *S. aureus*, *P. aeruginosa, S. pneumoniae* and *B. cereus* were evaluated. The first three are among the leading isolates in ocular infections [47], while the last one, although much more rare, is one of the most feared ocular pathogens, since it may cause uniquely rapid ocular infections responsible by quite serious complications [48]. Finally, the sample's biocompatibility was accessed through the choriollantoic membrane test (HET-CAM) and by cytotoxicity assays using human corneal epithelial cells.

2. Experimental

2.1. Materials

2-hydroxyethyl methacrylate (purity ≥ 99 %, HEMA), ethylene glycol dimethacrylate (purity ≥ 98 %, EGDMA), 2,2′-azobis(2-methylpropionitrile) (purity ≥ 98 %, AIBN), acetic acid (purity ≥ 99.7 %), diclofenac sodium salt (purity ≥ 98.5 %, DCF), cholesterol (purity > 99 %), toluene, phosphate buffered saline (PBS) tablets, sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl) and calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) were provided by Sigma-Aldrich (Germany). Poly vinylpyrrolidone (PVP) Kollidon 30 was provided by BASF (USA). Sulphuric acid (purity ≥ 98 %, H_2SO_4), hydrogen peroxide (30 % (w/v), H_2O_2) and polystyrene were purchased from PanReac (Spain). Sodium hydroxide (purity ≥ 99 %, NaOH), octylphenoxypolyethoxyethanol (IGEPAL®) and lysozyme chicken egg white (MW 14300 Da, pH 6.5) were obtained from Merck (USA). Albumin bovine Fraction V standard grade (MW 66500 Da, pH 7.0) was supplied by Serva (Germany). Sodium hyaluronate (weight-average molecular weight 1,000,000–2,000,000 g/mol, HA) was purchased from Biosynth (UK). Hellmanex®II was obtained from Hellma GmbH (Germany). Chitosan (acetylation degree > 90 %, MW 750,000–1,000,000 g/mol, CHI) was obtained from Bioceramed (Portugal). Corneal Epithelial Cell Basal (ATCC PCS-700-030) supplemented with Corneal Epithelial Cell Growth kit components (ATCC PCS-700-040) and 1 % antibiotics (penicillin–streptomycin solution) and human corneal epithelial cells (ATCC 700–010) were purchased to LGC Standards, S.L.U. (Spain). Mueller-Hinton broth (MH) was obtained from Scharlau (Spain), while Mueller-Hinton agar (MHA) was from VWR (Portugal). Ultrapure water (resistivity > 18 MΩ.cm) was obtained from a Millipore system and used to prepare all solutions.

2.2. Hydrogel preparation

The HEMA based hydrogel was synthesised through the dissolution of appropriate amounts of the EGDMA (81 mM), PVP (1.3 mM) and AIBN (12 mM) in a HEMA and ultrapure water 1:1 vol ratio solution. The compounds were added sequentially to the aqueous solution of HEMA, under magnetic stirring. Each one only was added after the complete dissolution of the previous. The solution was then degassed by bubbling a gentle stream of nitrogen during 2 min, followed by a 5 min ultrasonic bath. The obtained solution was poured using a syringe, into a mould constituted by two silanised glass plates separated by a polyurethane thread prepared according to a procedure described elsewhere [21]. Polymerisation was achieved through exposure to UV radiation (UV – Exposure box 2, 350 nm, 230 W/4 × 15 W, Gie-Tec GmbH, Eiterfeld, Germany) for 100 min, at a distance of 15 cm. The polymerised hydrogels were detached from the moulds and washed with ultrapure water for 5 days (changed three times a day) to remove unreacted monomers and other impurities. The hydrogel sheets, with a thickness of 1 mm when fully hydrated, were cut with a scalpel in squares with 2x2 cm^2 for wettability and friction measurements. For all the other analysis, discs with 10 mm diameter (except when specified other dimension) were cut using appropriate punches. Finally, all samples were dried for 72 h in an oven at 36 °C, and stored.

2.3. Hydrogel coating

Dried hydrogel samples were treated with argon plasma prior to coating deposition, using a compact Harrick PDC-32G Plasma Cleaner/Steriliser (115 V, Harrick Plasma, Ithaca, NY, USA). The equipment was connected to a vacuum pump (LVO 100, Leybold, Cologne, Germany) and to an argon gas bottle (20 MPa, Alphagaz™, Air Liquid, Lisbon, Portugal). The samples were placed inside the glass chamber of the equipment and exposed to ionised argon for 10 min (power 18 W). Then, they were immersed for 3 min in 1 mg/mL CHI aqueous solution (1 % v/v of acetic acid) with pH = 5 to avoid CHI precipitation (pH was adjusted by adding NaOH 0.1 M). The samples were rinsed with ultrapure water and dipped for 30 min in a HA + DCF solution (containing 3 mg/mL of HA and 1 mg/mL of DCF). Uncoated samples were immersed for 30 min in 1 mg/mL of DCF to allow a better understanding on the effect of the coating. Samples were dried as outlined in the previous section. A scheme that resumes the procedure is provided in Fig. 1.

2.4. Drug loading and drug release tests

Both uncoated and coated dry samples were individually placed in falcon tubes with 3 mL of DCF solution (1 mg/mL in PBS) and incubated at room temperature for 24 h, 15 days and 30 days to study the influence of the loading time. To evaluate the amount of drug that was loaded into the samples, the drug was extracted by boiling the samples in water during 1 h.

The drug loaded samples were then rinsed with ultrapure water, gently blotted with lab paper, and used immediately in drug release tests. These were done in sink conditions, in vials containing 3 mL of simulated lacrimal fluid (SLF), during 24 h at 36 °C and 180 rpm. SLF (pH = 7.4) was prepared by dissolving NaCl (6.8 g/L), $NaHCO_3$ (1.1 g/L), KCl (1.4 g/L) and $CaCl_2{\cdot}2H_2O$ (0.04 g/L) in ultrapure water [49].

At pre-determined times, 200 μL aliquots were taken and replaced by the same volume of fresh medium. DCF concentration was determined measuring the absorbance of the collected supernatant solutions by UV–VIS at a wavelength of 276 nm, using a microplate reader (Platos R 496). A calibration curve was done using DCF solutions with concentrations between 0 and 50 μg/mL. Measurements were carried out in quadruplicate.

The drug released from the uncoated and coated samples after 30 days of loading was also studied under hydrodynamic conditions, to simulate the *in vivo* eye conditions. The experiments were carried out before and after sterilisation (see section 2.5). A lab-made microfluidic cell with a chamber of volume 45 μL was used to approach the tear fluid real volume in the eye [21]. SLF was fed using a syringe pump, at a flow rate of 3 μL/min, to simulate the tear turnover. Measurements were done in quintuplicate, during 24 h and at 36 °C. The exit solution was collected at pre-determined times and analysed as described above to determine DCF concentration.

2.5. Hydrogel sterilisation

Uncoated and coated HEMA hydrogels were placed inside bags of polyamide/polyethylene (90 μm thickness, Penta Iberica, Portugal) with 3 mL of SLF or DCF solution and sealed. The samples were submitted to high hydrostatic pressure (HHP), at 600 MPa, using a high-pressure equipment (Hiperbaric 55, Burgos, Spain). Briefly, the samples were pre-heated in a water bath until reaching 70 °C, and then placed for 10 min in a polypropylene insulated basket equipped with a piston for pressure transmission [45].

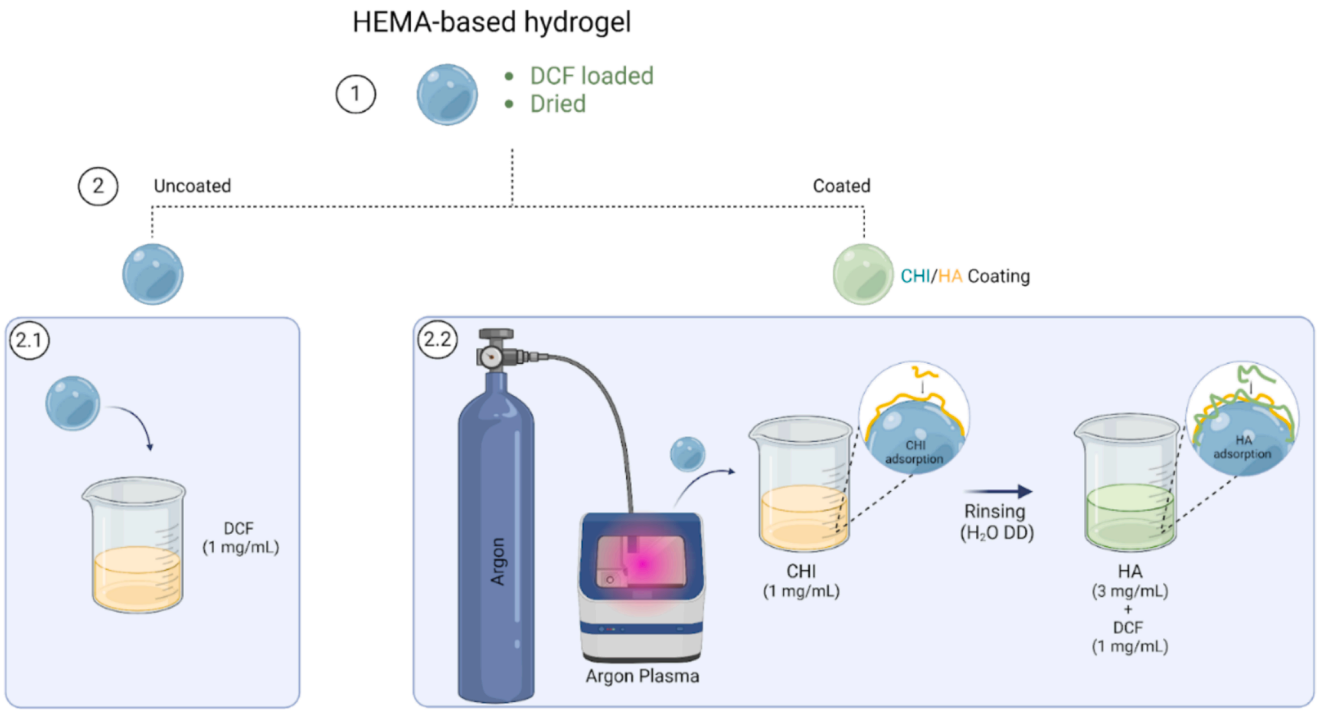

Fig. 1. Schematic representation of the coating process. (Created with BioRender.com).

2.6. Friction coefficient

The friction coefficient (μ) of the sterile uncoated and coated samples was determined using a linear nanotribometer (CSM Instruments, Needham, Massachusetts, USA) [37]. A glass tube with 1.65 mm diameter and 6.25 mm of length was glued to the cantilever of the nanotribometer and used as counterbody, sliding over the samples in reciprocating movement (800 cycles, 0.8 mm/cycle, sliding speed 7 mm/s, and normal force 20 mN, which according to Hertz theory corresponds to a load of 10 kPa). The applied load is of the same order of magnitude of that induced by the eyelid on the ocular surface during the blinking movement (1–5 kPa in the absence of CLs, 12–18 kPa when wearing CLs [29,50]). Regarding the average sliding speed, it is estimated that during blinking, the eyelid accelerates to a maximum of ~ 100 mm/s [51], a value that could not be reached in the performed experiments due to equipment limitations. The run distance was found to be enough to reach stationary conditions during the experiment (constant μ). The friction tests were conducted using ultrapure water, and solutions of different biomolecules present in the lacrimal fluid as lubricants, in concentrations that fall within the range of the values reported in literature for the lacrimal fluid: lysozyme (1.9 mg/mL) [52], albumin (0.05 mg/mL) [53,54], and cholesterol (1.8 mg/L) [37]. Solubility issues impaired the use of higher concentrations of cholesterol. Measurements were carried out in triplicate for each system.

To evaluate the stability of the coating, the coated samples underwent additional testing in ultrapure water, 1 h and 24 h after the first assay. The samples were kept in ultrapure water between experiments.

2.7. Chemical structure

The chemical structure of the non-loaded HEMA hydrogels, with and without coating, was studied using Fourier transform infrared spectroscopy (FTIR, model Spectrum Two from PerkinElmer, Waltham, MA, USA), with attenuated total reflectance (ATR), equipped with a lithium tantalate ($LiTaO_3$) mid-infrared (MIR) detector (signal/noise ratio 9300:1). Samples were analysed in the dry state. An efficient contact between the sample and the crystal (diamond crystal ATR accessory, model UATR Two) was ensured by manual control. Infrared spectra were obtained with a 4 cm^{-1} resolution, and with 8 scans of data accumulation. Normalisation of the spectra was carried out using the GraphPad Prism 9 software.

2.8. Adsorption studies

The deposition of the coating, and its interaction with lysozyme, albumin and cholesterol were monitored with a quartz crystal microbalance with dissipation (QCM-D, E4 from Q-Sense, Biolin Scientific, Gothenburg, Sweden). Gold-coated quartz crystal sensors (5 MHz) were first coated with the hydrogel following the protocol described in [37]. In short, a thin layer of polystyrene was deposited by spin coating (2000 rpm, 30 s) of a polystyrene solution (2 % wt in toluene), followed by two layers of the monomer mixture used in the preparation of the HEMA-based hydrogel (5000 rpm, 30 s). The crystal was then exposed to UV radiation for 20 min to polymerise the hydrogel. The baseline was obtained by pre-hydrating the HEMA-coated crystals in ultrapure water for 5 min. Variations of frequency (Δf/n) and dissipation (ΔD) for the 3rd, 5th, 7th, 9th and 11th harmonics upon addition of CHI and HA solutions, were monitored. Rinsing with ultrapure water was carried out between each addition. The interaction of the formed coating with biomolecules of the lacrimal fluid (lysozyme, albumin and cholesterol) was studied through the injection of aqueous solutions of those molecules in the concentrations referred in section 2.6. Six independent measurements were done in each case. To recover the sensors, they were immersed in piranha solution (H_2SO_4/H_2O_2, 7/3 v:v) and thereafter washed in a ultrasonic bath in a 2 % v/v Hellmanex solution (15 min) and in ultrapure water (2 × 15 min). Finally, they were dried with a nitrogen flux and stored.

2.9. Morphology and topography

The morphology of the samples was analysed by scanning electron microscopy (SEM, Hitachi S2400, Chiyoda, Tokyo, Japan). Hydrated samples were frozen with liquid nitrogen (−195.8 °C) and immediately placed in a freeze-dryer (Alpha 1–2 LDPlus, Martin Christ,

Gefriertrocknungsanlagen, Germany) for 24 h, at −60 °C and 0.026 Pa. The surfaces were coated with a Au/Pd film (100 nm) in a sputter coater and evaporator (Polaron Quorum Technologies, Laughton, East Sussex, United Kingdom).

Topographic images (10x10 μm^2) of the surfaces were obtained by atomic force microscopy (Nanosurf EasyScan 2, Liestal, Switzerland) in non-contact mode, using silicon probes (resonance frequency: 204–497 kHz) at a scanning rate of 0.7 Hz. The average roughness of the surfaces (R_a) was estimated from the images total area using the WSxM 5.0 develop 9.1 software. Three images were acquired for each type of hydrogel.

2.10. Physical characterisation of the hydrogels

Both uncoated and coated samples were HHP sterilised and subsequently characterised regarding a set of physical properties relevant for CLs application.

2.10.1. Water content and swelling capacity

The water content (WC%) and the swelling capacity (SC%) of the samples was determined by weighting them in the dry state (dry weight, *Wd*) and after hydration in 3 mL of ultrapure water (wet weight, W_w, obtained after carefully blotting the samples with lab paper), using Equation 1 or 2, respectively:

$$WC\% = \frac{W_w - W_d}{W_w} \times 100 \quad (1)$$

$$SC\% = \frac{W_w - W_d}{W_d} \times 100 \quad (2)$$

Experiments were also conducted in SLF and in DCF solution.

2.10.2. Water contact angle

The captive bubble method was used to evaluate the hydrophilicity of the HEMA hydrogels, with and without coating. Air bubbles were formed underneath the hydrated hydrogels immersed in ultrapure water using a micrometric syringe. Images were acquired at pre-defined times using a video camera (jAi CV-A50, Spain) mounted on a microscope Wild M3Z (Leica Microsystems, Germany), and analysed using the ADSA software (Axisymmetric Drop Shape Analysis, Applied Surface Thermodynamics Research Associates, Toronto, Canada). At least 10 bubbles were done for each type of hydrogels.

2.10.3. Light transmittance and refractive index

The transmittance of the uncoated and coated hydrogels (discs with 8 mm diameter) was analysed in the wavelength range 400–700 nm range with a scanning interval of 1 nm, using a UV–Vis spectrophotometer (Multiscan GO, Thermo Scientific, Waltham, USA).

The refractive index of both samples was measured at room temperature at a wavelength of 599.6 nm by using ABBE Refractometer AR4 (KRÜSS, Hamburg, Germany).

Measurements were done in triplicate.

2.10.4. Ionic permeability

Hydrogel samples (14 mm diameter) hydrated in ultrapure water were placed between the donor chamber (filled with 24 mL of NaCl 0.9 %) and the acceptor chamber (filled with 32 mL of ultrapure water) of a lab-made cell. The conductivity (μS/cm) of the acceptor solution was measured during 4 h (1 point/min), using a conductivity meter (HI2030-02 edge^{EC}® from HANNA instruments, Padova, Italy) and converted into NaCl concentrations using a calibration curve previously obtained. The ionic permeability (D_{ion}) was calculated using Equation (3):

$$\frac{F.V}{A} = D_{ion}\frac{dC}{dx} \quad (3)$$

where *F* is the ion transport rate (slope of the linear regression concentration vs time), *V* is the volume of the acceptor solution, *A* is the sample cross-sectional area and *dC/dx* is the initial NaCl concentration gradient across the sample.

2.10.5. Oxygen permeability

The oxygen permeability (Dk) of the uncoated and coated samples was determined using the following equation [55–57]:

$$Dk = 1.67e^{0.0397WC\%} \quad (4)$$

Additionally, the oxygen transmissibility (Dk/t) was obtained by:

$$Dk/t = \frac{Dk}{t} \quad (5)$$

where *t* is the samples thickness.

2.11. Irritability

The Hen's Egg test – Choriollantoic Membrane (HET-CAM) was performed to predict the ocular irritability potential of the produced samples, following the procedure described in [21]. Basically, fertilised hen's eggs (Sociedade Agrícola da Quinta da Freiria, SA, Portugal) were placed in an egg incubator (56S, China) at 37.2 ± 0.7 °C and with 59 ± 2 % RH, for 9 days. On the 9th day, the eggs shell was cut with a rotary saw (Dremmel 3000, Breda, Netherlands) to expose the inner membrane, and 500 μL of saline solution (NaCl 0.9 %) were poured over the membrane and left for 30 min in the incubator to hydrate it. The membrane was then carefully removed, exposing the CAM. The hydrogel samples were then placed on the top of the CAM and after 5 min, possible irritability related signs were evaluated, i.e., lysis, haemorrhage and coagulation. The irritation score (IS) was calculated as previously described [58]. A positive and a negative control (NaOH 1 M and NaCl 0.9 %, respectively) control were done for comparative purposes. The experiments were carried out in triplicate for each system.

2.12. Cell viability

The viability of primary human corneal epithelial cells (ATCC 700–010) after exposure to DCF released from the coated HEMA hydrogels was assessed, following ISO standard 10993-5 [39]. Cells were routinely cultured in corneal epithelial cell growth medium (Oculife, CellSystems) supplemented with Gentamicin (30 mg/ml) and Amphotericin B (15 μg/ml) at 37∘C in a humidified 5 % CO_2 atmosphere. Cells were seeded at a density of 1.5×10^4 cells/well on a 96 well-plate and incubated for 24 h. After incubation, the medium was replaced with culture medium containing DCF 0.1 mg/mL (the concentration obtained at the 4th hour of release of the tests performed in hydrodynamic conditions with 30-day loaded sterile coated samples) and diluted 1:2 and 1:5 with medium. The DCF used to prepare these solutions was obtained from drug release tests performed in the microfluidic cell (see Section 2.4 Drug loading and drug release), using water (to avoid hyper salinity), after lyophilisation of the collected supernatant solutions. Two controls were performed, one positive (culture medium with 10 % v/v DMSO) and one negative (culture medium only). Cells were incubated in the solutions for 12 h and 24 h, under the conditions referred above. The MTT assay was used to assess cell viability. The medium was removed, the cells were washed with PBS and incubated with 0.5 mg/mL MTT (in culture medium) for 3 h. Formazan crystals were dissolved with DMSO. Absorbance was read at 595 nm in a microplate reader (Infinite® 200 Pro plate reader, TECAN, Männedorf, Switzerland). Eight experiments were carried for each system.

2.13. Antibacterial activity

The antibacterial activity of the produced samples against *S. aureus*

(ATCC 25923), *P. aeruginosa* (ATCC 15442), *S. pneumoniae* (ATCC 10015) *and B. cereus* (ATCC 1178) was evaluated by turbidimetry in aseptic conditions, using a flow chamber. The strains were grown in MHA overnight (16 h at 37 °C). Afterwards, inoculums of the bacteria were prepared by adjusting the turbidity to 0.5 McFarland (1.5×10^8 bacteria/mL) for *P. aeruginosa*, *S. pneumonia* and *B. cereus,* and 1 McFarland (3×10^8 bacteria/mL) for *S. aureus*, respectively. The uncoated and coated samples, loaded in PBS or in DCF solutions, were individually placed in wells of a 24-well plate, together with 500 μL of MH and 10 μL of each bacterial suspension. Negative (MH) and positive (MH + bacteria suspension) controls were also done. All plates were incubated at 37 °C for 24 h. Then, homogenised 200 μL aliquots were taken, placed in a 96-well plate and their optical density was measured at 630 nm using a spectrophotometer (Infinite® 200 Pro plate reader, TECAN, Männedorf, Switzerland). The samples were tested in quadruplicate for all bacteria.

2.14. Statistical analysis

Statistical analysis was carried using the software R Project v. 4.2.1. The normality of the data was verified through the application of the Shapiro-Wilk test. The parametric test one-way Student's *t*-test was used to compare two sets of data when normality was observed. Otherwise, Willcoxon test was applied. A level of significance of 0.05 was considered. Statistical differences were signalled with * when $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.005$ and **** for $p < 0.001$.

3. Results and discussion

3.1. Drug loading and release

The use of CLs as platforms for the direct administration of drugs to the anterior segment of the eye has been gaining interest, as it can be more efficient than conventional eyedrops [2,59,60]. Although the potential of such systems can only be fully known through *in vivo* tests (first with animal models and later in clinical trials), these shall be restricted to the most promising systems due to ethical reasons. In an early-stage evaluation, *in vitro* experiments are critical to compare the performance of the drug release systems and understand the effect of different variables. Hence, in this work, the DCF release behaviour of uncoated and coated HEMA-based hydrogels loaded by soaking in the drug solution for different periods was first evaluated *in vitro* under static sink conditions. The cumulative drug release profiles are presented in Fig. 2A.

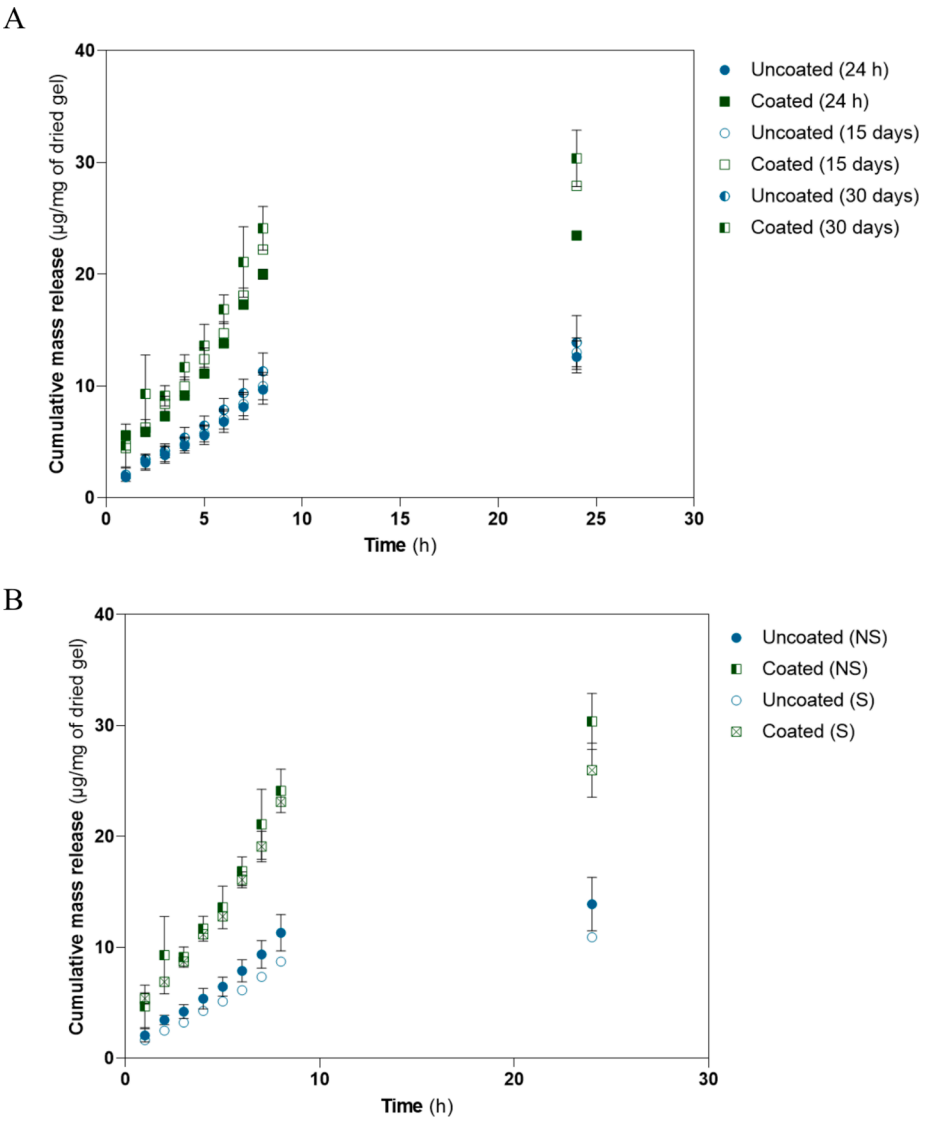

Fig. 2. Cumulative DCF release profiles obtained for uncoated and coated HEMA based hydrogels in static sink conditions (A) after 24 h, 15 days and 30 days of loading (B) after 30 days of loading, before (NS) and after (S) HHP sterilisation. The error bars are the ± standard deviations ($n = 4$).

The presence of the coating significantly increased the amount of drug released (about 2.5 times, both for sterile and unsterile samples). This should be due to the higher amount of DCF loaded in the coated samples (2134 ± 456 μg/sample) compared to the uncoated samples (469 ± 103 μg/sample), resultant from its entrapment in the CHI + HA film. CHI and HA present opposite charges at physiological pH. The establishment of electrostatic interactions between the amine groups (NH^{3+}) of CHI and the carboxylate groups (COO^{-}) of HA, leads to the formation of complexes that have demonstrated self-healing ability [61,62]. At neutral pH, DCF is negatively charged [42] and therefore can interact with the positive charges of CHI. Some authors have also suggested that in addition to electrostatic interactions, DCF may be physically entrapped within the CHI chains [63]. Regarding HA, the establishment of hydrogen bonds between the DCF molecule, and the OH groups of HA and the possible interaction of DCF with hydrophobic domains of HA, may have contributed to the higher amount of drug loaded [22]. HA is widely used as drug carrier and is known to enhance the delivery of DCF to sites of inflammation through a mechanism that is still not fully elucidated [42,64]. The drug release profiles obtained for the coated samples (Fig. 2A) show a sustained release for 24 h (although at a higher rate during the first 8 h). This suggests that the interactions between the drug and the components of the coating and/or of the CLs matrix have a reversible character.

It can also be observed that the increase in the time of loading leads to an increase in the amount of drug released, especially in the coated samples. The evaluation of the effect of soaking/storage time in the loading solutions is often put aside in research, due to lack of time available for the analysis, as the soaking/storage period can take months to achieve equilibrium. At the end of the release tests, the amount of drug released increased by ~ 19 % when the loading time increased from 24 h to 15 days and an additional ~ 10 % for the samples loaded for 30 days. This suggests that the time needed to achieve equilibrium may exceed 30 days. Therefore, 30 days was selected as the loading time for further experiments, which means that the drug release capacity of the CLs will be slightly underestimated.

In order to be commercialised, CLs must be sterilised in a final packaging containing a storage buffered solution. Several terminal sterilisation methods can be used for these devices, namely steam and

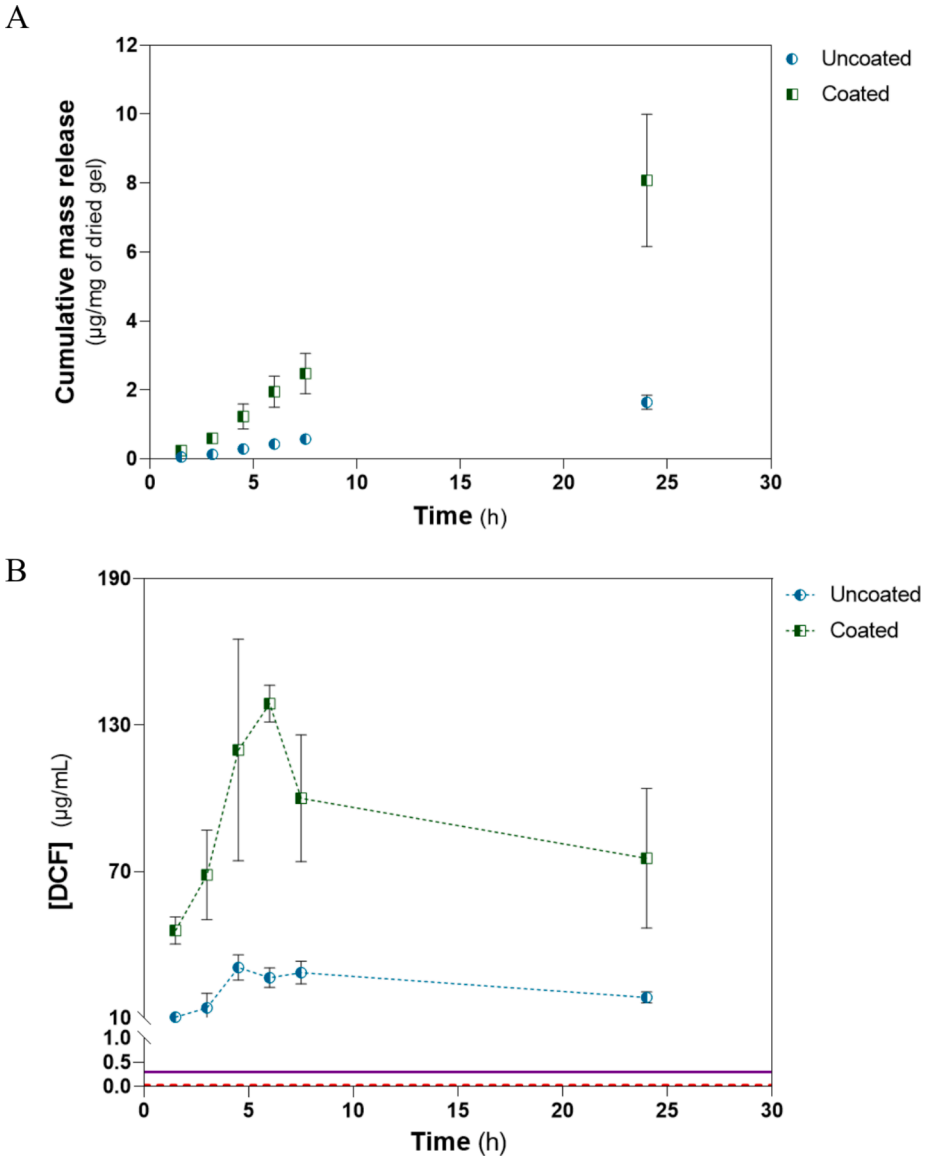

Fig. 3. DCF release profiles from uncoated and coated HEMA based hydrogels after 30-days of DCF loading and HHP sterilisation, obtained in hydrodynamic conditions. (A) Cumulative drug release profile (B) Non-cumulative drug release profile. IC_{50} for cyclooxygenase-1 (COX-1; 0.30 μg/mL, purple continuous line) and cyclooxygenase-2 (COX-2: 0.03 μg/mL, red dotted line) are shown in the last figure. The error bars are the ± standard deviations ($n = 4$).

pressure, γ-irradiation, and gas sterilisation (ethylene oxide) [65]. Sterilisation poses a big challenge for drug-loaded CLs, as it is crucial to maintain the material's properties while ensuring that the sterilisation procedure does not negatively affect the drug release profiles or the drug bioactivity. Previous studies showed that natural based polyelectrolytes, e.g., alginate and chitosan are degraded by conventional sterilisation methods [16]. As such, HHP combined with moderate temperature has been investigated to ensure sterilisation while maintaining the overall drug delivery system efficacy [45]. As seen in Fig. 2B, the sterilisation resulted in a decrease of the amount of drug released from both types of samples. This could be related to additional crosslinking of the coating as referred by Silva *et al.* in a previous work [16], which will impair the drug release. In fact, submitting the samples to extremely high pressures may bring the polymer chains closer to each other and favour the interactions among them.

Lastly, a microfluidic cell was used to perform drug release tests in hydrodynamic conditions, to simulate the lacrimal fluid turnover and approximate its volume [66]. This experiment provides a significant insight into the potential *in vivo* behaviour and efficacy of the studied systems. The cumulative drug release profiles obtained in such conditions for uncoated and coated samples, loaded with DCF by soaking in the drug solution for 30 days, are presented in Fig. 3A.

The initial burst of drug released greatly decreased in hydrodynamic conditions and a slower release was achieved, meaning that lower amounts of drug are released compared to static conditions. Similar conclusions were reached by the authors in previous works [66], being the differences attributed to the distinct driving forces for drug release in both cases. In fact, in static conditions, the driving force resultant from the gradient of concentrations between the drug eluting samples and the supernatant medium is much higher than in hydrodynamic conditions, since the volume of supernatant in contact with the sample is ~ 67 times higher (3 mL in static conditions *vs* 45 μL in hydrodynamic conditions). It is also clear that, as observed in static conditions, coated samples release a much higher amount of drug than uncoated samples (in this case ~ 3.8 times higher). Fig. 3B shows the concentration of DCF at the exit point of the microfluidic cells. For comparison purposes, the maximum values of the half-maximum inhibition concentrations (IC_{50}) found in literature for cyclooxygenase-1 (COX-1: 0.04 – 0.30 μg/mL) and cyclooxygenase-2 (COX-2: 0.01 – 0.03 μg/mL) [67] regarding DCF, are also marked in Fig. 3B. The measured DCF concentrations are remarkably higher than these values throughout the 24 h experiment, indicating that the hydrogels, and in particular the coated ones, could be suitable to be used in daily disposable CLs effective for treating inflammation.

3.2. Friction coefficient

In a healthy eye, a complex lubrication system enables the rapid movement of the palpebral conjunctiva while minimising strain-induced wear on the epithelium.

As for a non-healthy eye, several factors, like the tear film composition, tear film viscosity and/or a reduced number of blinking cycles, can alter the lubrication process, and ultimately lead to stress signs and discomfort symptoms in the patient. In these cases, the presence of a CL can aggravate the situation. CLs wearers present a higher rate of evaporation of the tear, changes in tear reposition rate, and alterations in the tear film stability [68,69]. The biomolecules present in the tear fluid can adsorb onto the CLs surfaces or even be absorbed, affecting the material's properties and their interaction with the sliding counterfaces, and in particular the μ. Fig. 4 shows the μ values for uncoated and coated samples, using ultrapure water and solutions containing some of those molecules (albumin, lysozyme, and cholesterol) as lubricants. Albumin and lysozyme are proteins present in the tear fluid, the latter being the most abundant (it can reach 40 % of the tear total protein content) [70,71]. Although present in lower concentrations, albumin plays an important role in the ocular surface's physiology: it serves as a transport

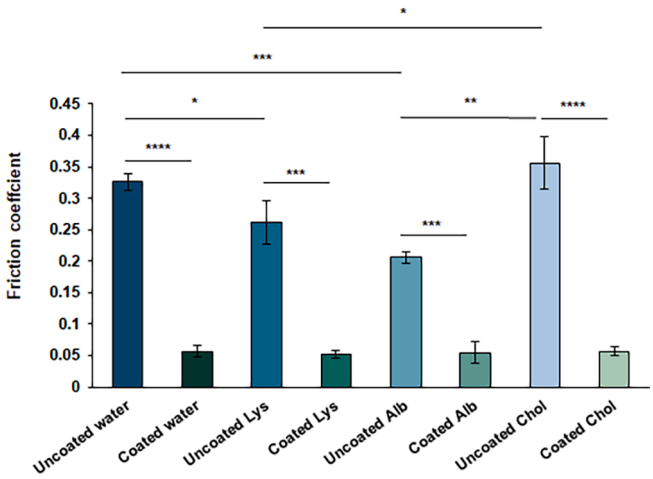

Fig. 4. Friction coefficient (μ) for the uncoated and coated samples in pure water, and in solutions of albumin, lysozyme and cholesterol. The error bars are the ± standard deviations ($n = 4$).

protein, binding and carrying various molecules such as lipids, hormones, and drugs, which can influence the bioavailability of therapeutic agents like DCF [72]. Cholesterol is one of the major non-polar lipids segregated by the meibomian glands [73] and plays, together with wax esters and cholesterol esters, a key role in retarding the tear film evaporation and keeping the ocular surface homeostasis and visual acuity [74,75]. Thus, understanding how it interacts with CLs surface is also useful to provide insights into optimising CLs for individuals prone to evaporative dry eye.

Uncoated samples presented a lower μ when the lubricant contained lysozyme or albumin ($p < 0.001$ in both cases), which may be attributed to the adsorption of the proteins onto the hydrogel's surface. Chang *et al.* [76], studied the effect of lysozyme adsorption on HEMA-based contact lenses (made of Polymacon) over their tribological behaviour against quartz glass and found that it was widely influenced by the structure of the adsorbed protein. Higher lysozyme concentrations led to higher surface coverage of the hydrogel surface, and to stronger protein–protein interactions, which preserved the native structure of the adsorbed molecules. This gave rise to a high shear strength layer that increased μ. Contrarily, at lower protein concentrations, a lower surface coverage was achieved. This allowed lysozyme molecules to unfold, undergoing significant secondary structural changes. The exposure of hydrophilic moieties to the aqueous environment shall result in the entrapment of water molecules, creating a lubricious, low-shear-strength, fluid film, that decreases μ. Su *et al.* [77] found the same tendency in the frictional behaviour when used albumin solutions as lubricants in studies with HEMA-based contact lenses (made of Polymacon and Etafilcon-A) rubbing against glass: a decrease in μ with a concentration of the protein of 0.2 mg/mL, but an increase for 50 mg/mL. However, with silicone-based contact lenses, the presence of albumin always improved lubrication, independently of the protein concentration. This could be related to the higher hydrophobicity of these hydrogels, which favours albumin denaturation [78]. Other factors, such as the existence of monomers/polymers with different characteristics in the hydrogel matrix or as coatings, the porosity of the material, its water content and surface roughness, may also play an important role. In another work, Silva *et al.* [37] also concluded that the effect of albumin adsorption depended on the characteristics of the formed film. The frictional behaviour of HEMA-based and silicone-based hydrogels against polymethylmethacrylate (PMMA) was studied, using an albumin solution at 4 mg/mL as lubricant. Distinct behaviours were found: while with the HEMA-based hydrogel μ remained almost equal to that observed in water, with silicone μ increased significantly. This was explained by the viscoelastic nature of the albumin film formed on the first hydrogel. In contrast, in

the silicone hydrogel, a lower amount of protein was adsorbed and the resultant film was essentially rigid. The results reported by Su *et al.* [77] and Silva *et al.* [37] clearly show that comparing conclusions of different studies found is not straightforward. The observed behaviours and the μ values depend on the counterbody, system geometry, applied load, sliding velocity and protein concentration, among other factors.

As for the results obtained in cholesterol solution, a slight increase of μ occurred for the uncoated samples, when compared to the tests performed in water ($p < 0.001$). As explained by Silva *et al.* [37], the lipid led to the collapse of the hydrogel structure, and to a dehydration process. In fact, these authors found a lower amount of free and loosely bound water in the samples hydrated in cholesterol solution compared to those equilibrated in pure water. These resulted in the reduction of the amount of water being expelled to the interface during the friction tests, thereby reducing the lubricant effect of the fluid, as the separation between the counterbody and the sample decreased. The release of water increased the surface rigidity of the hydrogel, contributing to an increase in μ.

The coating of the hydrogels led to a significant decrease of μ values relatively to the uncoated ones. This drop ranged from 4-fold in the case of albumin solution ($p < 0.001$) up to 6-fold for the cholesterol solution ($p < 0.001$). Similarly, when ultrapure water was used as lubricant, there was also a 6-fold decrease in μ ($p < 0.001$). It shall be stressed that the value of μ did not change significantly after incubating the coated samples for 1 h or 24 h in ultrapure water, demonstrating the stability of the coating.

The decrease in μ may be attributed to the high hydrophilicity, ability to retain water and viscoelastic properties of HA present in the coating [79]. The use of HA in the field of CLs to enhance wearer comfort and treat dry eye has raised significant interest in the last years [41]. Several authors have investigated the effect of HA when administrated through eye drops [80,81], incorporated into lenses for elution [81–83] or adsorbed/immobilised onto their surfaces [84,85]. It is widely recognised that HA in its free from (non-surface-bound) can provide enhanced lubrication and hydration.

Hynnekleiv *et al.* [86] demonstrated its ability to significantly increase the tear film thickness and diminish evaporation, thereby stabilising it. However, HA's impact on reducing boundary friction of CLs in the eye has been shown to depend on HA concentration, molecular weight and on the characteristics of the CLs. Although diluted solutions of HA have demonstrated good lubricating properties and are recommended by many clinicians for CLs wearers, solutions with HA content superior to 0.3 % led to μ values significantly higher than those obtained with simple saline solutions [86]. It was also found that HA of medium molecular weight gave rise to lower μ values than high molecular weight HA [87]. Finally, in a study performed by Nečas *et al.* [80] with different types of CLs (Acuvue Oasys and Biofinity) to evaluate the effect of the material on the friction and lubrication of the eye/lens/lid interface in the presence of HA solutions, it was found that the lubricant film formation and μ differ depending on the CL's material.

It is known that in aqueous environment, HA poorly anchors both to hydrophobic and hydrophilic surfaces. CLs surfaces are generally negatively charged, as HA, resulting in electrostatic repulsion and expulsion of HA from the contact [88,89]. Sterner *et al.* [89] verified that hydrophilic surfaces were even less lubricated in the presence of HA, concluding that the surface-anchoring of HA was determinant for μ reduction. Both they and Singh *et al.* [90] found that surface-bound HA was an excellent boundary lubricant. Several authors attempted to follow this approach to improve CLs performance by employing different strategies to immobilise HA on the material's surfaces. For example, Deng *et al.* [84] used a click chemistry process to functionalise HEMA-based CLs with HA and found that it turned the lenses significantly more wettable, more able to retain moisture, and less protein binding, without compromising the optical and mechanical properties of the lenses. Korogiannaki *et al.* [85] also resorted to a click reaction to graft HA onto the surface of silicon hydrogel CLs, observing improved wettability and dehydration profiles, as well as antifouling properties. In turn, Yin *et al.* [91] covalently bound HA to the surface of albumin/silver porous films to fabricate CLs that revealed successful to treat alkali-burned corneal wound. Besides their healing effect, the lenses presented antibacterial surfaces and suitable key properties (e.g. transparency, water content, non-cytotoxicity). Finally, Singh *et al.* [92] produced CLs modified with a HA-binding peptide that was able to non-covalently bind HA present in eye drops and form a thin coating. This coating allowed the devices to remain hydrated for longer periods than the uncoated ones, providing both biological and physical advantages to the eye surface. It is interesting to note that none of these authors investigated the frictional behaviour of the HA-coated CLs, despite recognising the importance of studying it.

3.3. Chemical structure

Fig. 5 shows the FTIR spectra of the uncoated and coated sterilised samples, as well as of the compounds used in their preparation. Both samples gave rise to similar spectra, with many common features to that of the HEMA monomer, which was expected since it is in the higher quantity in the hydrogel formulation. The broad band in the region of 3600 – 3100 cm^{-1} may be attributed to the stretching vibrations of O–H, and in the case of the coated samples also to N–H bonds in the lower frequency range, derived from the presence of CHI and HA. In turn, the peaks from 2980 to 2800 cm^{-1} are assigned to C–H stretching frequencies [93]. The region of 1724 – 1668 cm^{-1} exhibits peaks that are due to C=O stretching, ester and carboxylic acid groups. However, all of them were less intense for the coated samples, possibly due to the action of plasma, suggesting possible breakage of the chemical bonds, as discussed by Bodas *et al* [94]. At lower wavenumbers, the band at 1070 cm^{-1}, attributed to C–O–C asymmetric stretching, is found to increase in intensity for both samples, i.e. after polymerisation. Also, the appearance of two bands in these samples, namely at 1270 and 1244 cm^{-1} might be related to C–H stretching skeletal vibrations, associated to the emergence of a band at 748 cm^{-1} due to methylene rocking vibration, indicative of a long chain linear aliphatic structure. The peaks at 1636 cm^{-1}, 941 cm^{-1} and 889 cm^{-1}, assigned to –C=C– are not present in the samples, and can be attributed to the polymerisation via double bond opening [95]. It should be noted that the peaks characteristic of both HA and CHI at 1043 cm^{-1} and of HA at 1604 cm^{-1} are not visible in the coated sample's spectra, which suggests that the CHI/HA coating may be quite thin. In fact, it is known that the depth of analysis of ATR-FTIR spectroscopy is typically of the order of a few micrometres [96], thus signals of species present in small quantity within the thin coatings may be negligible.

3.4. Morphology and topography

SEM images (Fig. 6A) demonstrate that the uncoated HEMA-based hydrogels have a smooth and homogeneous surface without any significant features, similar to those described in [37]. The coated samples present agglomerates dispersed over the surface, suggesting that CHI/HA deposition occurred in preferential zones, forming a non-uniform layer. These results were confirmed by AFM analysis (Fig. 6B) that showed the existence of agglomerates in the coated surfaces, whose height can surpass 100 nm. The samples surface's roughness calculated based on the AFM images increased more than 5 times compared to the uncoated samples. R_a values for the sterilised uncoated and coated samples are shown in Table 1.

3.5. Adsorption studies

The formation of the CHI/HA coating was monitored using a QCM-D microbalance (Fig. 7A). The changes in Δf/n and ΔD were registered for the various harmonics when CHI and HA solutions where sequentially added. After signal stabilisation of each of these solutions, a rinsing with

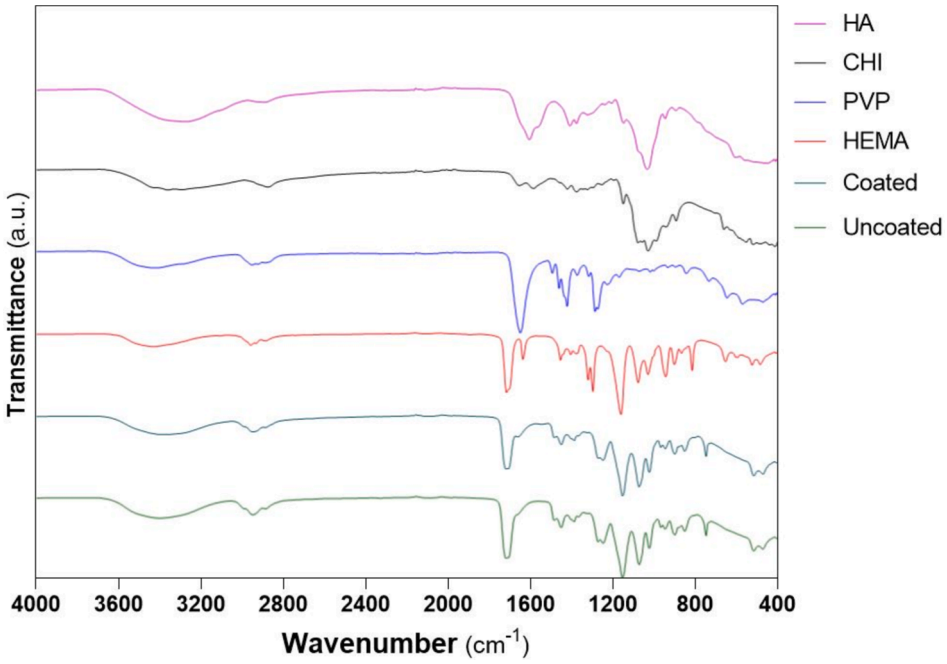

Fig. 5. FTIR-ATR spectra of the uncoated and coated HEMA based hydrogels, in the region of 4000–400 cm^{-1}.

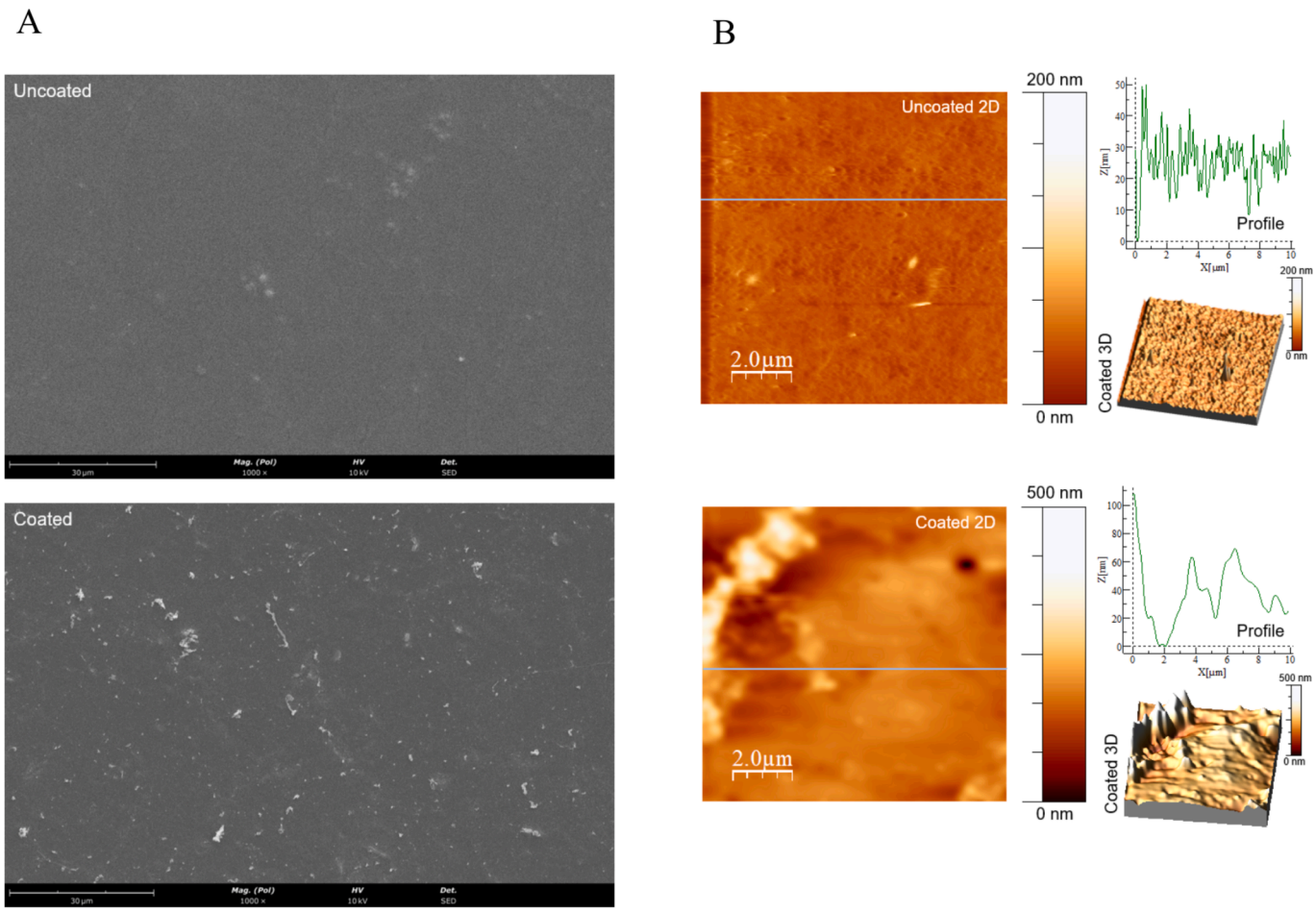

Fig. 6. (A) SEM micrographs (1000 × magnification, scale bar 30 µm) and (B) AFM 2D and 3D topographical images (10 × 10 µm) and exemplificative 2D profiles of the uncoated and coated HEMA-based hydrogels.

Table 1
Properties of the sterilised uncoated and coated HEMA-based hydrogels: Roughness (R_a), water content, swelling capacity in pure water, SLF and DCF solution, water contact angle, refractive index, transmittance, ionic permeability oxygen permeability (Dk) and transmissibility (Dk/t), and Young modulus. The errors are the ± standard deviations ($n = 3$, except for Young's modulus ($n = 5$) and water contact angle ($n = 10$)).

		Uncoated	Coated
R_a (nm)		5.1 ± 0.4	28.2 ± 0.7
Water content (%)		38.6 ± 0.3	38.3 ± 0.1
Swelling capacity (%)	Pure water	62.9 ± 0.8	62.1 ± 0.1
	SLF	59.4 ± 1.2	61.6 ± 2.0
	DCF	67.9 ± 0.4	69.3 ± 0.8
Water contact angle (°)		42.5 ± 6.1	36.0 ± 2.2
Refractive index		1.437 ± 0.001	1.432 ± 0.002
Transmittance (%) (400 – 700 nm)		98.1 ± 0.4	94.5 ± 0.6
Ionic permeability (cm^2/s)		$4.6 \times 10^{-7} \pm 2.5 \times 10^{-8}$	$4.9 \times 10^{-7} \pm 9.2 \times 10^{-8}$
Dk ((cm^2/s).(mL O_2/mL × mmHg))		7.73 ± 0.09	7.65 ± 0.02
Dk/t ((cm/s).(mL O_2/mL × mmHg))		25.8 ± 0.3	25.5 ± 0.1
Young's modulus (kPa)		412.6 ± 17.7	447.2 ± 16.4

ultrapure water was done to remove loosely bound biomolecules (steps b1 and b2 in Fig. 7A). The decrease relative to the baseline of Δf/n after each rinsing demonstrates that a significant amount of both biomolecules remained adsorbed to the surface. The rigid/viscoelastic nature of the coating can be inferred analysing the overlapping of Δf/n for the different overtones and the magnitude of the dissipative energy loss. The considerable overlapping of the frequency signal after the rinsing b2 and the correspondent small values of ΔD show that the adsorbed layer have a high stiffness [97]. For such films, the adsorbed mass (Γ) can be determined through the Sauerbrey equation:

$$\Gamma = -\frac{\Delta f}{n} C \tag{6}$$

where C corresponds to the mass sensitivity constant, which is 17.7 ng cm^{-2} Hz^{-1} for 5 MHz AT-cut quartz crystals. The obtained value, estimated based on the 3rd overtone, is 571 ± 27 ng/cm^2 (being about 24 % of this value attributed to CHI and the remaining percentage to HA).

The interaction of the substrates with the lacrimal biomolecules (cholesterol, lysozyme and albumin), was further studied (Figures S1A, B and C in Supplementary Information for the uncoated samples and Fig. 7B, C and D, for the coated ones).

In the case of cholesterol, an increase of Δf/n and a small decrease of ΔD was observed for the uncoated samples. These can be explained by the collapse of the hydrogel structure and water release induced by the lipid [37]. Interestingly, for the coated samples, the observed behaviour was quite different. The significant decrease of Δf/n and the increase of ΔD (Fig. 7B, step b3) demonstrate that the cholesterol molecules adsorbed to the surface in a considerable extent. The high spreading of the overtones points out to the formation of a layer with a viscoelastic character superior to the CHI/HA coating. For these type of films, it is known that the Sauerbrey equation underestimates the adsorbed mass [98]. A modified version of this equation should be used, but it would be needed to know the density of the adsorbed film, a value that was not found in literature for cholesterol films.

Regarding the proteins, rigid films of lysozyme and albumin were formed on the uncoated substrates, as evidenced by the low ΔD values and high overlapping of Δf/n for the different harmonics (Fig. 7 D and C, respectively, step b3). The respective adsorbed masses, obtained from the Sauerbrey equation were 364 ± 59 and 78 ± 12 ng/cm^2.

In its native state, lysozyme has an ellipsoidal shape with an approximate length of 9 nm and diameter of 1.8 nm [99], while albumin approaches to a equilateral triangular prism of with a thickness of 3 nm and triangle side of 8 nm [100]. Taking into account the molecular weight of lysozyme and the area occupied by each molecule in a side-on or end-on position, it can be estimated the adsorbed mass when a compact monolayer forms: 147 ng/cm^2 or 733 ng/cm^2. A similar rational for albumin leads to 399 ng/cm^2 and 361 ng/cm^2, respectively. Lysozyme is known to have a much greater structural stability than albumin [101], and therefore it is plausible that upon adsorption it keeps dimensions closer to the native form. The value estimated through the Sauerbrey equation for this protein (364 ± 59 ng/cm^2) is in between those calculated for side-on and end-on adsorption. It is possible that the protein molecules adsorb in both positions, but the fact that the adsorbed mass determined by QCM-D also includes a significant contribution of water entrapped in the protein layer (Komorek *et al.* [102] report values between 50–70 % for a lysozyme monolayer) suggests that most of the molecules must be adsorbed in a side-on position.

Regarding albumin, the estimated value for the mass adsorbed (78 ± 12 ng/cm^2) is significantly lower than those predicted for compact monolayers. This may result from the unfolding and spreading of the molecules over the solid surface during adsorption, since, as referred above, this protein has a low conformational stability.

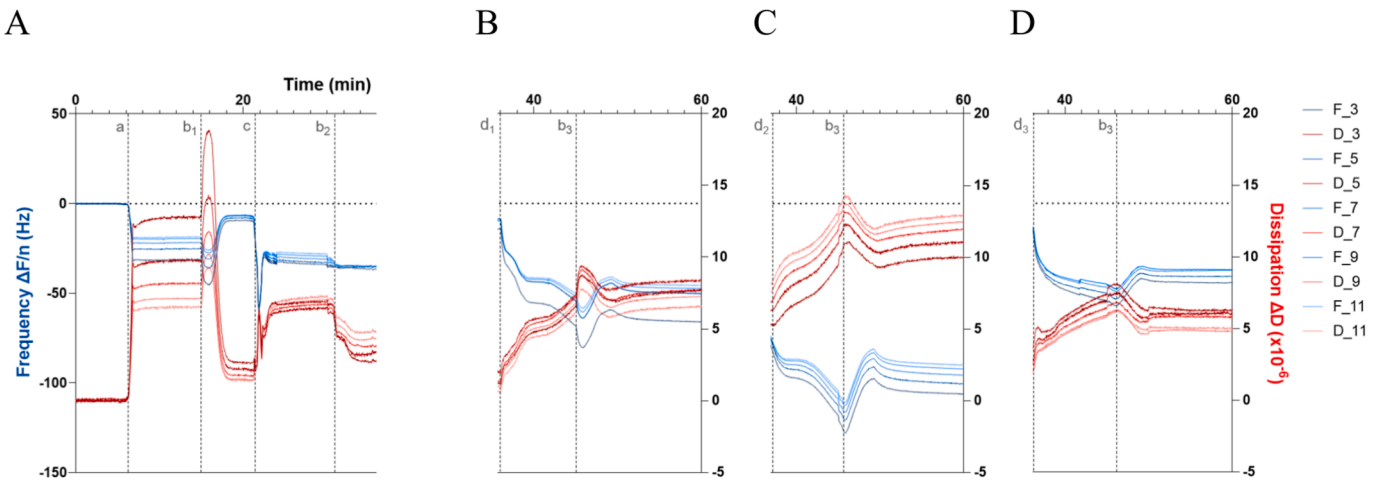

Fig. 7. Normalised shift in the frequency, Δf/n, (blue line, left y-axis) and shift in the dissipation, ΔD, (red line, right y-axis) for the 3rd, 5th, 7th, 9th and 11th harmonics of the resonant frequency of the HEMA coated quartz crystal sensor as a function of the time. The successive addition of solutions of CHI (a) and HA (c) gave rise to the formation of the CHI/HA coating (A). The final steps (d1, d2 and d3, respectively) correspond to the addition of cholesterol (B), lysozyme (C) and albumin (D). A rinsing with pure water (b1, b2 and b3) was done to separate each successive addition.

As for the coated samples, both proteins led to the formation of viscoelastic films. Assuming a density of 1.38 g/cm^3 [103] for the lysozyme film and 1.15 g/cm^3 for albumin [100], one can calculate the respective adsorbed protein amounts using the software of Q-sense (DFind, Biolin Scientific, Gothenburg, Sweden). A value of 1265 ± 46 ng/cm^2 was obtained for lysozyme and of 386 ± 102 ng/cm^2 for albumin. It shall be stressed that, although the deposition of CHI and HA is sequential, their assembling may vary and the molecules may be intermingled, which could favour the proteins adsorption in certain zones. Protein adsorption is a complex process that depends on many factors, including the substrate characteristics, and therefore, irregular films may be formed. The much higher concentration of lysozyme may have significantly contributed to the higher adsorption of this protein. Lysozyme adsorption on CLs can be beneficial. In fact, lysozyme is an enzyme with antimicrobial properties that can help reduce bacterial contamination on CLs, potentially decreasing the risk of eye infections. Additionally, the presence of lysozyme can contribute to maintaining the natural antimicrobial defense mechanisms of the eye.

3.6. Characterisation of the hydrogels

Both uncoated and coated samples were sterilised by HHP and extensively characterised regarding several properties crucial for their use in CLs. The results are summarised in Table 1.

As referred to in Section 3.4, the coating significantly increased the surface roughness of the hydrogels ($p < 0.01$). No significant changes were found before and after sterilisation.

The effect of the coating in the water content was negligible ($p = 0.340$). The same for the swelling capacity in SLF ($p = 0.342$) and in DCF solution ($p = 0.481$). It is interesting to note that both uncoated and coated samples showed a higher value of swelling in DCF solution than in SLF ($p = 0.023$ and $p = 0.04$, respectively). The fact that HEMA hydrogel has a slight negative charge, as proved by Hogt *et al.* [104] through zeta potential measurements, and that DCF sodium in solution dissociates giving rise to negatively charged DCF ions, may explain this behaviour. The entrance of negative charges in the anionic hydrogel may cause interchain repulsion and expansion of the network promoting its swelling.

Regarding wettability, both uncoated and coated samples are hydrophylic. However, a lower water contact angle was observed for the coated samples ($p < 0.001$).

As for the optical properties, the coating deposition slightly decreased the refractive index ($p = 0.03$). In addition, the transmittance showed a lower mean value in the 400–700 nm wavelength range due to the presence of the coating. Regardless, both properties are still suitable for CLs application [56].

The coated samples showed a higher value for the ionic permeability, but it was not statistically significant ($p = 0.859$). Both values were above the minimum required (1.067×10^{-9} cm^2/s) for proper contact lens movement [105].

Moving to the oxygen permeability, both uncoated and coated hydrogels exhibited low values, as expected for HEMA-based hydrogels, with no statistically significant differences ($p = 0.234$). Contrary to silicone hydrogels, in acrylic hydrogels, as the one here studied, the oxygen permeability is only related to the water content of the material [106]. Still, for the intended purpose (e.g., daily disposable lenses for dry eye management) the values are within those reported for daily CLs (7–30 Dk) [55,107,108]. It should be stressed that these values may vary according to the physiological and pathological conditions of the wearer. Variations in pH, osmolality or lacrimal fluid composition may affect significantly the lenses performance. Lee *et al* [109], for example, demonstrated that CLs oxygen permeability decreases upon deposition of proteins present in the lacrimal fluid on the lenses. This issue requires further study to fully understand the lenses behaviour *in vivo*.

Finally, the mechanical properties of the samples were evaluated, as they influence the applicability, handling and comfort provided by the CLs. Tensile tests were conducted and the Young's modulus was determined. Although an increase was observed in its value after the coating, the difference relatively to the uncoated samples is not significant ($p = 0.091$). More, the stiffness of the samples was within the range of values reported for HEMA based CLs [110].

3.7. Irritability

In order to infer about the potential of irritability of the produced CLs in the eye, hen eggs were incubated for 9 days, after which their CAMs were exposed directly to coated sterilised samples, loaded with DCF. For comparison purposes, the experiment included unloaded samples and with uncoated hydrogels, with and without DCF. The results are summarised in Fig. 8.

In all tested conditions, the samples induced a similar response to that of the negative control (NaCl 0.9 %) (Fig. 8E). In other words, none of the three signs of irritability were observed (i.e., lysis, haemorrhage or coagulation). The hydrogels were classified as non-irritant with an IS of zero. By contrast, the positive control showed severe irritation (IS = 21).

3.8. Cell viability

To ensure the biological safety of the proposed therapeutic CLs, it is necessary to evaluate their cytotoxicity and verify that is above the 70 % threshold established by the ISO standard 10993-5 (2009) [111]. Since

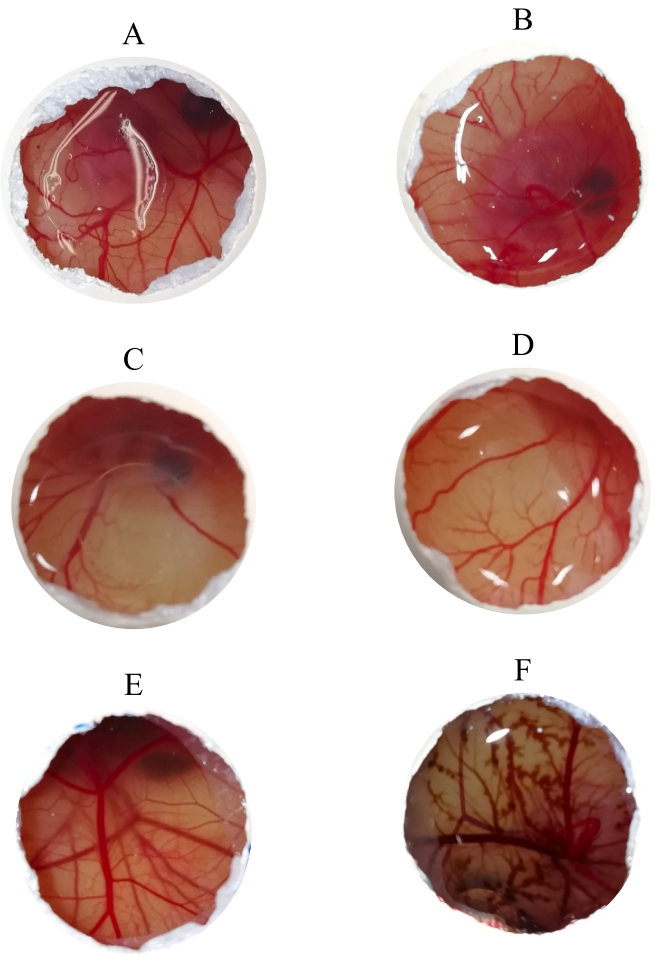

Fig. 8. Photographs of the CAM of hen eggs after 5 min of direct contact with uncoated samples with (A) and without DCF (B) and of CHI/HA coated samples loaded (C) and unloaded with DCF (D). The negative (NaCl 0.9 %) (E) and the positive controls (NaOH 1 M) (F) are also shown.

NSAIDs can have multiple adverse effects (e.g., nephrotoxicity, or gastrointestinal and cardiovascular side effects) they are usually prescribed for the shortest possible duration and at the lowest required concentration [112]. Therefore, their use at the local level is considered beneficial. Lee *et al.* [113] studied the *in vitro* toxicity of different NSAID eye drops on human corneal epithelial cells, and reported for DCF the lowest cell viability of all drugs studied. The reported higher toxicity of DCF may be due to the high sensitivity of the *in vitro* cell culture, coupled with the use of preservatives in the eye drops [114,115]. It is known that when CLs are used as a drug carrier, the bioavailability of the drugs can be increase to 50 % when compared to conventional topical application [116], which may favourably reduce the required drug concentrations. Besides, this form of drug delivery eliminates the need for preservatives [117]. However, the prolonged residence time and the direct contact of the CLs with the cornea could induce a toxic response from the highly sensitive corneal epithelial cells. Therefore, cytotoxicity tests were conducted with human corneal epithelial cells, using DCF solution 0.1 mg/mL (the concentration obtained at the 4th hour of release of the tests performed in hydrodynamic conditions with 30-day loaded sterile coated samples). Different exposure times (12 h and 24 h) and dilutions were tested (Fig. 9). The results show that the solutions are non-toxic, since the observed cell viability levels were well above the 70 % threshold.

3.9. Antibacterial activity

The antibacterial activity of the coating was evaluated for the uncoated and coated samples loaded in PBS and in DCF. Fig. 10 shows the optical density values obtained after 24 h incubation of the samples incubated in solutions containing *S. aureus*, *P. aeruginosa, S. pneumoniae and B. cereus*.

In the absence of drug, it was verified that the presence of the coating led to an inhibition of growth of both *S. aureus* ($p = 0.0041$) and less markedly of *P. aeruginosa* ($p = 0.0436$) (Fig. 10 A and B). However, no statistically significant effect was observed for *S. pneumoniae and B. cereus* (Fig. 10 C and D).

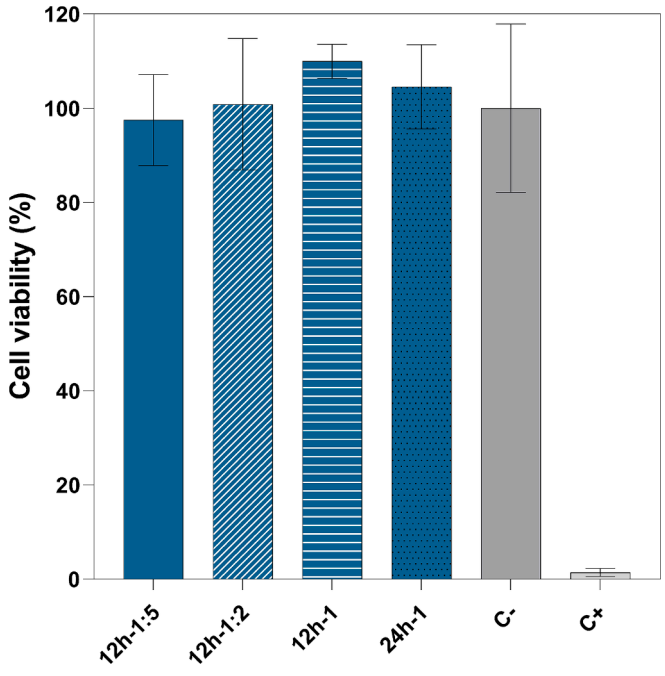

Fig. 9. Human corneal epithelial cell viability (%) after 12 h and 24 h of exposure to 0.1 mg/mL of DCF solution (12 h 1 and 24 h 1, respectively). Dilutions of 1:2 and 1:5 were also used in experiments performed for 12 h. The negative and positive controls are also shown. Error bars are the ± standard deviations ($n = 8$).

Regarding the drug-loaded samples. a significant decrease was observed in the optical density values for *S. aureus* (Fig. 10 A, both $p < 0.0001$), but in this case, the effect of the coating in the inhibition of the bacterial growth was not so accentuated as for the unloaded samples. In the case of *P. aeruginosa* (Fig. 10B), although the uncoated samples presented some antibacterial effect ($p = 0.0091$), this was more significant for the coated ones ($p < 0.0001$). The same tendency was observed for *B. Cereus* (Fig. 10D), but the antibacterial effect was much more marked for the coated samples ($p = 0.0319$ for uncoated samples and $p < 0.0001$ for the coated). Finally, for *S. pneumoniae* (Fig. 10C), the presence of the drug did not improved the antibacterial efficacy of the materials, independently of being or not coated.

DCF proven its antibacterial effect against several gram-positive and gram-negative bacteria [118]. As for the coating, CHI has been extensively reported by its notable properties, namely its antimicrobial activity that greatly depends on its molecular weight and acetylation degree [119]. The biomolecular mechanism through which HA exhibits antimicrobial activity is still a subject of study [120]. Previous works proved that depending on the conditions, it can impair or stimulate the bacteria growth [121]. When combined with antimicrobial agents like CHI, its antimicrobial properties are further enhanced. Additionally, its high viscosity and ability to form a barrier can protect tissues from microbial contamination.

3.10. Comparison with other systems

A comparative analysis of the obtained results with those of other studies or commercial products is essential to identify the potential advantages and drawbacks of the developed system.

In terms of drug delivery performance, the coated samples are able to ensure a sustained DCF release with concentrations above therapeutic levels for at least 24 h, when tested in hydrodynamic conditions similar to those found in the eye. This represents a significant improvement relatively to the conventional eye drops that, as referred above, usually lead to a low drug bioavailability (1–5 % [16]), due to rapid tear clearance and short retention times on the ocular surface. Comparison with other DCF loaded CLs materials whose development is reported in literature is not straightforward, since the drug release profiles are strongly affected by the drug release conditions, which are not standardised. Several examples can be found where DCF release from the hydrogels is complete from a few hours [17,122], till weeks [16]. The most important thing to retain in the present case is that DCF release profile of the system here proposed meets the requirements for the production of daily disposable CLs effective for treating inflammation.

Moving to the tribological behaviour, commercial CLs often have issues with friction due to tear film instability and protein deposits, which can lead to discomfort during prolonged wear. Some lenses incorporate lubricating agents like polyvinylpyrrolidone (PVP) to maintain moisture and provide long-lasting comfort (e.g., Acuvue Oasys with Hydraclear Plus or Acuvue Moist from Johnson & Johnson) or polyvinyl alcohol (PVA) to provide continuous lubrication (e.g., Dailies Aqua Comfort Plus from Alcon). Others are submitted to plasma treatments to enhance surface wettability, reduce friction, and improve wearer comfort (e.g., Air Optix from Alcon or Purevision2 from Baush + Lomb). In the present study, the CHI/HA coating significantly reduced μ, providing enhanced lubrication even when using more complex lubricating media (i.e., in the presence of albumin, lysozyme and cholesterol). The coating also increased the material's hydrophilicity, contributing to improve the interaction with the tear film, which shall be particularly beneficial for those experiencing dryness or discomfort with regular lenses.

Another feature of the developed CHI/HA-coated hydrogel and loaded with DCF that must be highlighted has to do with the inherent antimicrobial properties against several ocular pathogens. In fact, it showed some efficacy against some of the most common ocular bacteria,

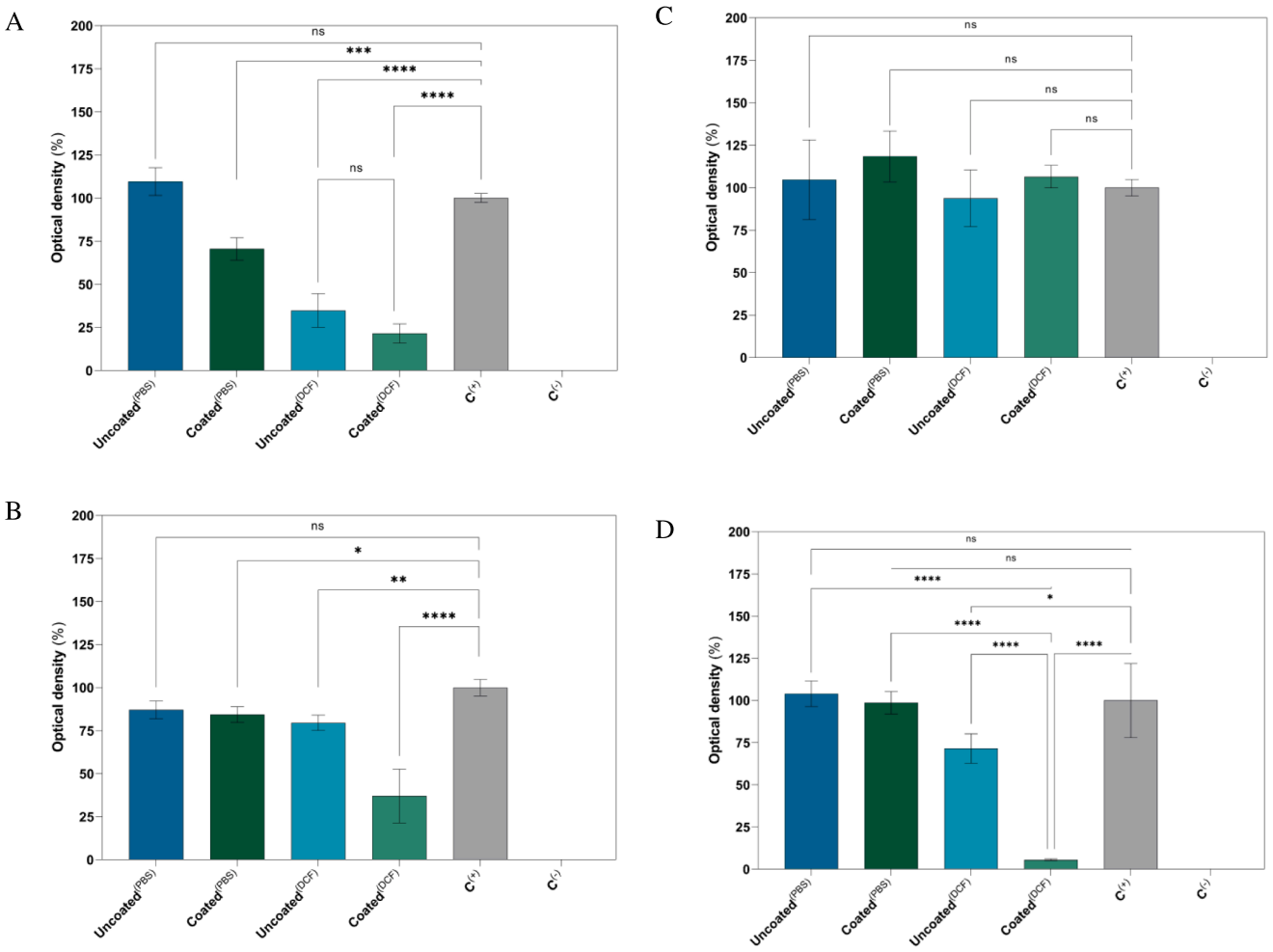

Fig. 10. Optical density (%) of solutions containing (A) *S. aureus*, (B) *P. aeruginosa* (C) *S. pneumoniae,* and (D) *B. cereus* after 24 h of incubation with the uncoated and coated samples, loaded in PBS and DCF. The results for negative (medium) and positive (medium + inoculum) controls are also shown. The error bars are the ± standard deviations ($n = 4$).

that may lead to eye infections, e.g. *S. aureus*, *P. aeruginosa*, and in particular against *B. Cereus,* which despite being more rare is known to cause quite dangerous infections that evolute very quickly and may lead to significant vision loss, or even loss of the eye, within hours [48]. This effect shall be further enhanced by the lysozyme adsorption tendency. Such characteristic is an advantage when compared with many commercial CLs, including silicone hydrogels or daily disposable lenses, which are not antimicrobial. However, in the last years an effort has been made to find strategies to confer to CLs materials this characteristic. For example, the impregnation of silver metal has been widely investigated, and Ag-impregnated CLs are already commercially available [13], but other alternatives are under study.

The main limitations of the lenses proposed in this work have to do with the lack of antifouling behaviour and the sterilisation method scalability. In fact, the antifouling limitation could lead to protein buildup that over time might foster bacterial colonisation. However, since the lenses are intended for daily use, this shall not be a critical issue. Concerning HPP sterilisation, while effective, it may face challenges in widespread adoption, when compared to more traditional sterilisation methods. Indeed, it is not yet implemented in commercial lenses, so scaling up may involve regulatory and practical challenges in industrial adaptation.

To conclude, it is important to highlight that the multifunctionality of the developed coated contact lenses is a significant advance. They offer simultaneously a sustained release of an anti-inflammatory agent, low μ and antimicrobial properties. This combination of features sets them apart from many commercial contact lenses, which typically focus on only one of these aspects, making the developed system a unique and promising solution for managing dry eye and improving overall comfort.

4. Conclusion

In this work, a CHI/HA coating was plasma-grafted onto a HEMA-based hydrogel, which was further loaded with DCF and sterilised by HPP, aiming to develop therapeutic CLs to manage DED. *In vitro* drug release tests performed in hydrodynamic conditions showed that the hydrogels pre-loaded with the drug for 30-days (to simulated storage time), maintained DCF concentrations well above the IC_{50} values of COX-1 and COX-2 for at least 24 h. The coating significantly reduced μ, both in the absence and in the presence of lacrimal biomolecules (cholesterol, albumin, lysozyme). Contrarily to what was expected, the CHI/HA film did not present antifouling properties. Viscoelastic films were formed upon adsorption of the studied proteins. In terms of physical properties, the coating did not impair those required for CL application. The hydrogels presented high transmittance to visible light, hydrophilic surface, considerable water content, and suitable mechanical properties. The DCF loaded coated samples showed antibacterial properties against *S. aureus*, *P. aeruginosa* and *B. Cereus,* this last one a quite feared bacteria, since it may cause serious damages to the eye. Moreover, the coating favoured the adsorption of lysozyme which also

has antimicrobial properties. Despite the high concentration of DCF released, the coated samples did not induce any irritation on the CAM or cytotoxicity in human corneal epithelial cells.

Overall, the coating CHI/HA improved the drug release behaviour, decreased friction and conferred antimicrobial properties to the hydrogels, demonstrating that this strategy can be a simple way of making CLs more suitable for individuals experiencing dry eye symptoms. In fact, the proposed approach has the potential to address a critical need in clinical practice by providing a dual-functional therapeutic CLs that not only improves lubrication but also delivers anti-inflammatory treatment directly to the ocular surface. This sustained drug release system can enhance patient comfort by reducing the need for frequent eye drops, which are often poorly retained on the eye and lead to significant drug loss. Furthermore, the reduction in friction and enhanced antimicrobial properties of the coated lenses may decrease the risk of infection and inflammation associated with prolonged CLs wear. By improving comfort and mitigating inflammation, this approach could lead to better management of dry eye symptoms, improved patient outcomes, and greater acceptance of CLs as a therapeutic option for DED sufferers.

These final remarks cannot be concluded without acknowledging some study's limitations and suggesting areas for further research. The lack of antifouling properties could be considered a main concern. However, as these lenses are designed for daily use, this is unlikely to become a critical issue. Another limitation could be related to the chosen sterilisation method. Although HPP proved effective, it poses challenges in terms of scalability and industrial adaptation, as it is not yet widely adopted in commercial lenses and may face regulatory hurdles. Regarding future research, it would be interesting to approach the real conditions, e.g., by studying the behaviour of the developed CLs in the presence of other tear biomolecules (or a combination of them that better mimicked that fluid) or performing long-term studies that allowed to infer about the stability of the CLs for longer storage times.

To advance toward clinical translation, further preclinical studies will be required to assess sustained drug release *in vivo*. Tests with animal models should focus on the efficacy of reducing dry eye symptoms and preventing bacterial colonisation over extended periods of lens use. Clinical trials will need to establish safety, efficacy, and patient comfort, while also comparing the lenses to current commercial alternatives. Regulatory approval will be demanding since the lenses combine both a medical device (the CL) and a drug (DCF), and thus may be classified as a combination product. Finally, commercialisation may face obstacles in scaling production and achieving cost-effectiveness, but these can be addressed with continued collaboration between academic research, regulatory bodies, and industry partners.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymeth.2024.11.015.

Data availability

Data will be made available on request.

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