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Research paper

Surface modification of an intraocular lens material by plasma-assisted grafting with 2-hydroxyethyl methacrylate (HEMA), for controlled release of moxifloxacin

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ABSTRACT

Endophthalmitis, an inflammation of the eye due to perioperative infection, may occur after cataract surgery. Intraocular lenses (IOLs) loaded with an antibiotic have been proposed as an alternative to the conventional postoperative endophthalmitis prophylaxis, since the antibiotic is delivered directly to the target site. In this work, an IOL-based antibiotic releasing system was prepared from a copolymer used in the production of IOLs and a fluoroquinolone used in endophthalmitis prophylaxis (moxifloxacin, MFX). Argon plasma-assisted grafting with 2-hydroxyethyl methacrylate (HEMA) in the presence of MFX was the approach selected for surface modification, with MFX loaded both by entrapment in the grafted polyHEMA coating and by soaking. Surface and bulk properties were evaluated before and after surface modification and the MFX release profiles were obtained both in batch mode (sink conditions) and under hydrodynamic conditions, employing a purpose-built microfluidic cell, which simulated the hydrodynamic conditions around the eye lens. The effect of storage on the release profile of the best system was also assessed. The best system released MFX for ca. 15 days above the minimum inhibitory concentration for *Staphylococcus aureus* and *Staphylococcus epidermidis*. The released MFX showed antimicrobial activity against these bacteria and was non-cytotoxic against corneal endothelial cells.

1. Introduction

Cataracts, the opacification of the eye lens or of the anterior or posterior part of the lens capsule, are the leading cause of visual impairment. In the United States of America (USA), it accounts for 46% of visual disability in people aged 75 to 85 years while, in developing countries such as India, it shows a dramatic rate of 82% among people aged 75 to 83 years [1]. Treatment requires the removal of the natural lens of the eye and implantation of a polymeric intraocular lens (IOL). IOLs are also used in intraocular refractive surgery, where vision correction is addressed employing an IOL with the adequate refractive power. Postoperative endophthalmitis, a purulent inflammation of the aqueous and vitreous humours of the eye, may occur after cataract surgery or after any other type of surgical intervention in the eye [2]. It is usually due to perioperative infection caused by bacteria, fungi or, more rarely, parasites that have entered the eye. It has an incidence up

Abbreviations: AFM, Atomic Force Microscopy; *ca.*, *circa*; CEC, corneal endothelial cells; EWC, equilibrium water content; FBS, Fetal bovine serum; HBSS, Hank's balanced salt solution; HEMA, 2-Hydroxyethyl methacrylate; HPLC, high-performance liquid chromatography; IOL, Intraocular lenses; ISO, International Organization for Standardization; K-, negative control; K+, positive control; m_d, mass of dry disc; MEM, Eagle's Minimum Essential Medium; MFX, moxifloxacin; MH, Mueller Hinton; MIC, Minimum Inhibitory Concentration; m_s, Mass of disc at swelling equilibrium; MTS, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium, inner salt; *n*, refractive index; NADH, Nicotinamide adenine dinucleotide (reduced form); NADPH, Nicotinamide adenine dinucleotide phosphate (reduced form); *p*, Probability of rejecting the null hypothesis; PES, Phenazine ethosulfate; PHEMA, PolyHEMA; PolyHEMA, Poly(2-hydroxyethyl methacrylate); R_a, roughness; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; SD, standard deviation; SEM, Scanning Electron Microscopy; UV, ultraviolet; Vis, visible; v/v, Volume to volume; WCA, water contact angle

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to 0.2% [3] and, if left untreated, irreversible damage to photoreceptor cells of the retina may occur, with partial or complete loss of vision, frequently within a few days [2,4]. Prophylaxis by biocide and antibiotic administration is carried out in every surgical intervention, with protocols which vary with country. In general, preoperative use of povidone-iodine (a topical antiseptic) and preoperative and postoperative topical, subconjunctival or intracameral administration of antibiotics, such as vancomycin, cefuroxime and fluoroquinolones, are employed [5]. In Europe, a survey which involved nine countries revealed that, in general, preoperative antisepsis employing topical application of povidone-iodine or chlorhexidine, associated with postoperative topical antibiotic prophylaxis for up to 2 weeks, or intracameral cefuroxime injection at the end of surgery, are the most common approaches [6]. Another survey found that most European surgeons now favour intracameral administration of antibiotics over subconjunctival administration or topical antibiotics alone [7]. In the USA, a different approach is taken: the most common form of prophylaxis has been the use of topical fluoroquinolones prescribed 1 to 3 days preoperatively and resumed immediately postoperatively for 1 week [3,8]. Intracameral administration has the advantage of providing far higher antibiotic concentrations at the target site than subconjunctival injection or topical administration (eye drops) [7]. However, it is an invasive procedure. Eye drops are a simple ocular drug administration method but suffers from: (i) low bioavailability, due to anatomical and physiological barriers (blinking, lacrimation, flow through the nasolacrimal duct, rapid absorption into the bloodstream and poor corneal penetration), and (ii) poor compliance, due to inadequate postoperative use and timing by patients who, in most cases, show some age-related physical impairment. As such, less than 5% of the administered drug enters the eye [9], and the required therapeutic concentration may not be attained.

IOLs loaded with an antibiotic have been proposed as an alternative to both intracameral injection and eye drops in endophthalmitis prophylaxis, since drug delivery directly to the target site is achieved. In the 90′s, Nishi et al. [10] and Tetz et al. [11] were among the pioneers who proposed and showed the efficacy of IOLs as drug delivery systems, obtaining reduced eye inflammation and reduced posterior chamber opacification (a complication of cataract surgery), employing IOLs loaded with anti-inflammatory and anti-proliferative drugs. In the following decade, Shimizu et al. [12] and Kleinmann et al. [13] were among the first to propose the use of antibiotic-loaded IOLs for endophthalmitis prophylaxis. In the first study, it was shown that polymethacrylate IOLs loaded with fluoroquinolones had a preventive effect against *in vivo* bacterial biofilm formation (employing a rabbit endophthalmitis model) [12] and, in the second study, which employed a fluoroquinolone (moxifloxacin; MFX) and polymethacrylate IOLs, *in vivo* concentrations above the Minimum Inhibitory Concentration (MIC) for bacteria associated with endophthalmitis were sustained for 14 to 16 h by drug release from IOLs soaked for 24 h in a MFX solution at 5 mg/mL [13]. A study with the rabbit model, which compared the use of an antibiotic-loaded hydrophilic acrylic IOL to intracameral antibiotic injection for endophthalmitis prophylaxis with fluoroquinolones, showed that the effect against bacterial proliferation was similar for both drug administration types [14].

IOLs have been loaded with different types of drugs, including antibiotics [15], anti-inflammatory [10] and anti-proliferative drugs [11]. The drugs were located (i) in the IOL itself, (ii) in a coating applied onto the IOL or (iii) in a separate reservoir attached to the IOL [16]. However, in spite of more than 20 years of research, drug-loaded IOLs have not become commercially available and currently, there are no clinical trials in the european [17], american [18] or japanese [19–21] clinical trials registers.

The objective of this work was to develop antibiotic releasing systems for use in prophylaxis of postoperative endophthalmitis, employing discs made from a hydrophilic, polymethacrylate-based copolymer used in the production of IOLs. These systems aim to release an antibiotic at a concentration above its MIC for bacteria commonly associated with endophthalmitis, such as *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) [2], during the required period of time (10 days, at least [3,5,22]). Moxifloxacin (MFX), a fourth generation fluoroquinolone used in endophthalmitis prophylaxis [23], was the antibiotic selected, due to its potency and use in the context of endophthalmitis prophylaxis [24,25], its antifungal activity [26], and its good photochemical [27] and thermal [28] resistance. This last characteristic is of utmost importance, since autoclaving is the method employed industrially to sterilize IOLs prepared with the material used in this work. Approaches based on surface modification by coating were adopted, since thin coatings (i) do not compromise bulk IOL properties, (ii) can act as a barrier to the release of the drug loaded in the IOL, and (iii) can be loaded with extra drug by entrapment. Surface modification of IOLs started probably in the 1970s, when dip coating of polymethacrylate IOLs with polyvinylpyrrolidone [29] and both plasma-assisted and gamma radiation-assisted grafting with 2-hydroxyethyl methacrylate (HEMA) and vinylpyrrolidone [30] were employed as a means to create lubricious coatings, in the context of prevention of corneal endothelial damage. In this work, the approach selected for surface modification was argon plasma-assisted grafting with HEMA, with MFX loaded both by entrapment in the grafted polyHEMA (PHEMA) coating and by soaking. PHEMA, a hydrophilic polymer, was selected, since hydrophilic IOLs tend to show lower incidence of both bacterial adhesion and endophthalmitis than hydrophobic IOLs [31]. Additionally, HEMA is a monomer adequate for use in IOLs, since it is one of the monomers present in the formulation of the material used in this work. Plasma-assisted grafting copolymerization presents the following advantages: (i) plasma only penetrates a few nanometers beyond the surface of samples [32], keeping the bulk of the sample unchanged, (ii) surface modification by grafting can be achieved without the use of polymerization initiators, which may be difficult to extract from the modified IOL, and (iii) plasma technology is already used industrially for surface modification of ophthalmic lenses, such as contact lenses and IOLs [33,34]. We used low-pressure plasma as a means to create reactive species on the material's surface (in particular, free radicals, anions and cations), which can initiate graft copolymerization from the surface after contact between the plasma-activated surface and a vinyl-type monomer in the liquid phase.

The surface and bulk properties of the material were evaluated before and after surface modification, namely wettability, equilibrium water content (EWC), topography/morphology, transmittance, refractive index and coating thickness. The release profiles of MFX-loaded discs were obtained both in batch mode (sink conditions, i.e., employing a volume of release medium that was at least 3 to 10 times the saturation volume) and in a microfluidic cell which simulated the hydrodynamic conditions around the eye lens. The effect of storage on the release profile of the best system was assessed, as well as the best system's antibacterial activity against relevant bacteria (*S. aureus* and *S. epidermidis*) and its cytotoxicity towards relevant cells (rabbit corneal endothelial cells).

2. Materials and methods

2.1. Materials

Discs made from a hydrophilic poly[(2-hydroxyethyl methacrylate)-*co*-(methyl methacrylate)]-based material (EWC: 26%) used in the production of IOLs were provided by PhysIOL S.A. (Liège, Belgium). They contained a proprietary yellow chromophore which acts as a blue light filter [35]. The discs, which had a diameter of 1.6 cm and a thickness of 1 mm, were Soxhlet-extracted with distilled water (*ca.* 60 extraction cycles) before use, and were cut to the required diameter with a cork borer. 2-Hydroxyethyl methacrylate (HEMA) was supplied by Sigma-Aldrich (St. Louis, USA) and moxifloxacin hydrochloride (MFX) was supplied by TSZCHEM/BioTang (Lexington, USA). Hank's