Biomaterials 33 (2012) 7952–7958

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Surface localisation of photosensitisers on intraocular lens biomaterials for prevention of infectious endophthalmitis and retinal protection

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ARTICLE INFO

Article history:
Received 4 July 2012
Accepted 24 July 2012
Available online 11 August 2012

Keywords:
Bacterial adhesion
Copolymer
Endophthalmitis
Hydrogel
Intraocular lens
Photosensiter

ABSTRACT

Cataract surgery is one of the most commonly-practiced surgical procedures in Western medicine, and, while complications are rare, the most serious is infectious postoperative endophthalmitis. Bacteria may adhere to the implanted intraocular lens (IOL) and subsequent biofilm formation can lead to a chronic, difficult to treat infection. To date, no method to reduce the incidence of infectious endophthalmitis through bacterial elimination, while retaining optical transparency, has been reported. In this study we report a method to optimise the localisation of a cationic porphyrin at the surface of suitable acrylate copolymers, which is the first point of contact with potential pathogens. The porphyrin catalytically generates short-lived singlet oxygen, in the presence of visible light, which kills adherent bacteria indiscriminately. By restricting the photosensitiser to the surface of the biomaterial, reduction in optical transparency is minimised without affecting efficacy of singlet oxygen production. Hydrogel IOL biomaterials incorporating either methacrylic acid (MAA) or methyl methacrylate (MMA) co-monomers allow tuning of the hydrophobic and anionic properties to optimise the localisation of porphyrin. Physiochemical and antimicrobial properties of the materials have been characterised, giving candidate materials with self-generating, persistent anti-infective character against Gram-positive and Gram-negative organisms. Importantly, incorporation of porphyrin can also serve to protect the retina by filtering damaging shortwave visible light, due to the Soret absorption (λ_{max} 430 nm).

1. Introduction

Due to increasing life expectancies and changes in population structure, cataract surgery has become one of the most prevalent surgical procedures practiced in Western medicine [1]. Mature-onset cataracts develop when protein aggregation within the lens results in opacification and gradual loss of vision. Cataract surgery restores vision by removal of the natural crystalline lens and replacement with an implanted polymeric intraocular lens (IOL).

Following cataract extraction and IOL implantation, post-operative endophthalmitis (inflammation of the intraocular cavities) incidence in the UK is estimated conservatively to be between 0.1 and 0.2% [1–3]. Although occurring infrequently, endophthalmitis following cataract surgery is associated with significant postoperative morbidity. The main complication affecting at least 30% of patients is major visual loss, with blindness resulting in up to 18% of patients. Further negative implications for the patient include prolonged hospitalisation, and potential further surgery [4,5].

Bacterial adherence to an implanted IOL, and subsequent biofilm development, has been implicated in the pathogenesis of endophthalmitis. The Gram-positive coagulase-negative micrococcus *Staphylococcus epidermidis* is the most frequently cultured microorganism, however *Staphylococcus aureus* and anaerobic species have also been implicated [6]. The development of bacterial biofilm has negative implications on the outcome of treatment as bacteria in a biofilm are approximately 10–1000 times more resistant to antibiotics and biocides than their planktonic counterparts [7–9]. Currently, no IOLs are marketed which have strong, persistent anti-infective character.

Light-mediated antimicrobial strategies employing photosensitisers have been previously investigated for a number of pathogens including *Staphylococci* [10–12], viruses [13], and fungi [14]. Photoactivation of photosensitisers, typically porphyrins, results in the generation of a range of cytotoxic species, principally singlet oxygen ($^1O_2$), but also superoxide, hydroxyl and other radicals. The cytotoxicity of $^1O_2$ arises from indiscriminate oxidative reaction with any accessible macromolecule of the bacterial cell, so the development of bacterial resistance mechanisms is very

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http://dx.doi.org/10.1016/j.biomaterials.2012.07.052

difficult [15,16]. As the process of generation of the cytotoxic species is catalytic, the photosensitiser is not consumed in the process of cell killing, and can persist in providing a bacteriocidal effect for a prolonged period. While photosensitisers have proven antimicrobial efficacy, they have been used primarily in solution, and there is a compelling argument to develop methods to translate this activity to the surfaces of biomaterials.

Unlike previous work on PACT, uptake of the porphyrin by bacterial cells is not required. Rather, the $^1O_2$ generated by the porphyrin acts directly against bacteria adhering to the polymer surface, preventing subsequent bacterial adherence, the first stage of bacterial biofilm formation. As the lifetime of $^1O_2$ is in the range of 10^{-5}–10^{-6} s, the effective distance between the initial excitation event and cytotoxic damage is limited to a few micrometres, therefore preventing toxicity to normal tissue [5].

Recently, photoactivated porphyrin-impregnated hydrogels based on poly(2-(hydroxyethyl) methacrylate)-*co*-(methacrylic acid) copolymers have been reported [5,17]. To date, however, no studies have demonstrated localisation of photosensitiser on a polymer host whilst maintaining both antimicrobial activity and optical transparency to allow application in IOL biomaterials. In this paper, we describe methods to incorporate a tetracationic porphyrin, (tetrakis(4-*N*-methylpyridyl)porphyrin) (TMPyP), which binds electrostatically with methacrylate groups of the copolymer, in thin surface layers of a range of acrylate copolymers suitable for fabrication of IOLs using control of both hydrogel porosity and degree of electrostatic interaction between the biomaterial and the photosensitiser. This serves to give high surface concentrations of $^1O_2$ on excitation with visible light and allows the overall optical transparency to remain high for IOL applications. In addition to antimicrobial activity, an added benefit of TMPyP incorporation onto the surface of IOLs is the ability of this porphyrin to strongly absorb light in the blue/violet region of the visible spectrum (Soret band λ_{max} 430 nm). Much attention has been given to IOLs incorporating chromophores with the ability to absorb a certain amount of UV and/or blue light (<500 nm) in order to protect against photoreceptive retinal damage [18]. Exposure to such short wavelength light may be associated with an increased risk for age-related macular degeneration (AMD) and severe retinal damage [19,20]. Early IOLs allowed all UV and visible light to pass to the retina unrestricted, however UV-blocking lenses have been in use since the mid 1980s, and recently there has been support to increase the absorption spectrum of IOLs to reduce blue/violet light reaching the retina; such lenses show similar transmittance characteristics as a natural crystalline lens [18,21].

Here we have characterised the physicochemical and antimicrobial properties of TMPyP-incorporated acrylate hydrogel copolymers, and present candidate anti-infective, blue/violet-blocking, intraocular lens materials to improve patient outcomes in cataract surgery.

2. Materials and methods

2.1. Polymer preparation

Methyl methacrylate (MMA), methacrylic acid (MAA), 2-(hydroxyethyl)methacrylate (HEMA), benzoyl peroxide (BPO) and ethylene glycol dimethacrylate (EGDMA) were obtained from Aldrich.

Random copolymers composed of varying ratios of MMA:MAA:HEMA were prepared by free radical polymerisation, employing benzoyl peroxide (0.4% w/w) as an initiator and EGDMA (1% w/w) as a crosslinker as described previously [15]. Two series of copolymers were prepared; the first varies the MMA content at the expense of HEMA, while maintaining a constant MAA component, and the second varies the MAA content at the expense of HEMA, while maintaining a constant MMA component. The composition of copolymers prepared in this way is detailed in Tables 1 and 2. Included in Tables 1 and 2 are code definitions by which these copolymers are hereinafter identified.

Table 1
Polymers prepared by varying MMA content at the expense of HEMA, whilst MAA was maintained at 10%. Denoted hereafter as MMA series, with abbreviation codes as listed. EGDMA maintained constant at 1%. Percentages in table reflect the percentage composition of co-monomers in the remaining material.

MMA	MAA	HEMA	Abbreviation code
0	10	90	0MMA
5	10	85	5MMA
10	10	80	10MMA
15	10	75	15MMA

Impregnation of the hydrogels with TMPyP was performed by immersing samples into solutions of TMPyP (1 μg/ml) in phosphate buffered saline at pH 7.4 for 2 min, followed by washing with, and soaking in deionised water for seven days.

2.2. Thermal analysis of untreated polymers

Glass transition temperatures of untreated polymers were determined using a TA instruments 100 Modulated Differential Scanning Calorimeter (TA Instruments Ltd, Crawley, West Sussex, UK) with an attached refrigerated cooling system unit. A modulation amplitude of ±0.70 °C every 50 s was applied, with a 2 °C min^{-1} underlying heating rate. Heat flow was calibrated using an indium standard, and the heat capacity by means of a sapphire standard. Nitrogen was used as the purge gas, with a flow rate of 50 mL min^{-1} through the DSC cell. Standard aluminum pans were used, with the mass of each empty sample pan matching the mass of the empty reference pan to ±0.1 mg.

2.3. Determination of equilibrium water content

Coplymer samples were dried to a constant weight and then placed in distilled water at room temperature. Swelling of the samples was monitored by weighing at regular time intervals, after removing any surface water by blotting on tissue paper, until samples reached a constant hydrated weight. The equilibrium water content was calculated using Eq. (1):

$$EWC = [(W_2 - W_1)/W_2] \times 100 \quad (1)$$

where EWC is the equilibrium water content (%), W_2 is the mass of the sample after water absorption, and W_1 is the mass of dried gel.

2.4. Confocal laser scanning microscopy

Distribution of TMPyP in the hydrogels was characterised by visualising a cross-section of the relevant material using a Lecia TCS SP2 Confocal Laser Scanning Microscope (Lecia Microsystems, Wetzlar, Germany). The wavelength of excitation was 514 nm, generated by an Argon/Argon Krypton laser, and emission was monitored between 600 and 720 nm. 514 nm was selected as the wavelength of excitation due to its proximity to a Q band of TMPyP. The pinhole size and photomultiplier voltage were kept constant throughout the experiments.

2.5. UV–visible spectroscopic analysis

UV–visible absorbance measurements were obtained using a Perkin Elmer Lambda 650 UV–visible spectrometer coupled with UVWinLab software (Perkin Elmer, USA), enabling characterisation of total uptake of TMPyP. Spectra were recorded between 390 and 700 nm to permit visualisation of the Soret and Q bands of TMPyP. TMPyP loading was determined through measurement of absorbance at the peak maximum of the Soret band (430 nm). This was translated into quantitative TMPyP content per unit area by rearrangement of the Beer Lambert law. Assessment was made in quintuplicate for each material.

Table 2
Polymers prepared by varying MAA content at the expense of HEMA, whilst MMA was maintained at 5%. Denoted hereafter as MAA series, with abbreviation codes as listed. EGDMA maintained constant at 1%. Percentages in table reflect the percentage composition of co-monomers in the remaining material.

MAA	MMA	HEMA	Abbreviation code
0	5	95	0MAA
10	5	85	10MAA
20	5	75	20MAA
30	5	65	30MAA