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Approaches to improving the biocompatibility of porous perfluoropolyethers for ophthalmic applications

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Abstract

Porous perfluoropolyether (PFPE) membranes for ophthalmic applications were prepared with a zwitterion monomer, 3-[[2-(methacryloxy) ethyl](*N*,*N*-dimethyl)ammonio]-propane-1-sulphonate, copolymerized in weight ratios of 0–10%. The polymer samples were assessed for a range of physical properties, including equilibrium water content, bovine serum albumin permeability, transparency, refractive index and the ability to support corneal epithelial cell and tissue attachment, growth and migration. In vitro assessment of the polymers using bovine corneal epithelial cells and tissue showed that a zwitterion incorporation level of between 0% and 6% in the PFPE membranes supported the migration of an intact sheet of epithelial tissue without compromising epithelial cell attachment and growth, with 4–6% being the optimal level for these properties. Binding patterns of the cell adhesion glycoprotein fibronectin were also found to reflect the cell and tissue response. Effective nutrient permeability, refractive index and optical transparency were also maintained by the porous PFPE polymers containing this concentration of zwitterionic monomer. The presence of amounts of zwitterion greater than 6% was inhibitory to both tissue migration and cell growth and was associated with increased optical haze. These results demonstrated that it is possible to achieve the potential for increased biocompatibility in zwitterion-containing PFPE polymers without compromising existing beneficial characteristics.

Keywords: Perfluoropolyether; Zwitterion; In vitro test; Cornea; Epithelium

1. Introduction

Perfluoropolyethers (PFPEs) are a class of fluoropolymers with an oxygen linked fluorocarbon structure that, like other perfluorinated polymers, possess a number of favourable physical properties that render them suitable for use in biomedical applications. Such characteristics include a low coefficient of friction and surface tension, good thermal and chemical stability, a demonstrated biocompatibility [1] and high oxygen transmission [2]. Additionally, PFPEs have been synthesized with refractive indices [3] close to that of the cornea–tear film interface (1.3375) [4] As a consequence, versions of these polymers have found potential use in ophthalmic applications such as contact lenses [3,5] and are currently under investigation by our group for use as an implantable corneal lens [6,7].

The preparation of porous PFPE membranes from perfluoropolyether macromonomers has been patented [8–10] and these materials have delivered promising results in vivo as an ophthalmic device [6,7,11]. During the early stages of development, the inherent hydrophobicity of a typical perfluorinated polymer such as PFPE was recognized as a potentially limiting factor with regard to the capacity of this material to support cell and tissue growth. Studies on non-porous versions of PFPE membranes clearly demonstrated that less hydrophobic versions provided better support for corneal epithelial cell and tissue attachment

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and growth [12]. This was consistent with reports showing that surfaces with a more hydrophilic nature can be expected to provide superior cell and tissue interactions, provided that the surface chemistry is not too mobile [13–15].

A commonly used method for increasing the wettability of a material is to incorporate a hydrophilic species into the pore structure of the polymer or alternatively to coat the surface with a hydrophilic species. Zwitterionic compounds such as 2-(methacryloyloxyethyl) phosphorylcholine (MPC) and 3-[[2-(methacryloxy) ethyl](*N*,*N*-dimethyl)ammonio]-propane-1-sulphonate have been successfully used to modify the interaction between biological material and the substrate, both in vitro [16–19] and in vivo [20–22] These modifications demonstrated that the biocompatibility of a polymer could be altered by effectively reducing the level of non-specific protein and lipid adsorption. However, one of the fundamental requirements for a corneal onlay polymer is to maintain a capacity for supporting corneal epithelial tissue attachment, growth, migration, and appropriate differentiation status. To facilitate this, some interaction is still required to potentiate these events via the adsorption of cell adhesion molecules [23–25] and other necessary components for the successful reestablishment of a functional tissue. The challenge then for enhancing the biocompatibility of our PFPE-based corneal onlay polymer is to attain a balance between increasing levels of hydrophilicity and the retention of sufficient substrate–tissue interaction to support and maintain tissue integrity. In attaining this balance one must also be mindful of the impact on corneal tissue migration arising from alterations to the polymer topography as a consequence of changes in the chemical composition. Increases in surface roughness beyond certain limits can severely impair, or totally inhibit corneal tissue migration [26].

In this study we report on the in vitro optimization of the zwitterion-containing PFPE polymer as a means of improving the long-term efficacy of porous PFPE polymers in vivo.

2. Materials and methods

2.1. Reagents and materials

Perfluoropolyether diols (Fomblin Z-Dol^{TM}) of average molecular weight 1000 and 2000 were supplied by Ausimont S.p.A (Milan, Italy). 2-Isocyanatoethyl methacrylate was obtained from Showa Denko (Tokyo, Japan). Dibutyltin dilaurate, 1, 3-propane sultone, 2,2,3,3-tetrafluoro-1-propanol and iso-propyl acetate were purchased from Aldrich (Milwaukee, USA) and used as supplied. *N*,*N*-dimethyl aminoethyl methacrylate was purchased from Acros Organics (Glee, Belgium) and purified by vacuum distillation prior to use. HFE-7100^{TM} was purchased from 3M Specialty Materials (St Paul, USA). All other solvents were of analytical grade and were used without further purification. Ciba Specialty Chemicals (Basel, Switzerland) supplied Darocur 1173 photoinitiator. Polypropylene moulds for PFPE polymer fabrication were obtained from Ciba Vision Surgical Corp (Atlanta, USA).

2.2. Monomer synthesis

The zwitterionic monomer (Fig. 1a) was prepared as previously described in the literature [27,28]. The PFPE macromonomers (Fig. 1b) were synthesized from the reaction of the PFPE diols, Z-Dol^{TM} 1000 (average MW 1030) or Z-Dol^{TM} 2000 (average MW 1908), respectively, with two molar equivalents of 2-isocyantoethyl methacrylate in the presence of a catalytic amount of dibutyltin dilaurate (DBTDL) according to literature procedure [3,10].

2.3. Perfluoropolyether (PFPE) membrane preparation

The polymer membranes were prepared from the copolymerization of the zwitterionic monomer with the PFPE macromonomers according to procedures reported by Chaouk et al. [29]. In the current study, the zwitterionic monomer was copolymerized in weight ratios of 0–10%. SEM images showing typical PFPE membrane topographies obtained at various zwitterion concentrations are shown in Fig. 2 and the ratios of components in the formulations are detailed in Table 1.

The monomeric components and solvents were mixed together and stirred for 5 min at room temperature. Darocur 1173 was then added, and the mixture covered and stirred for a further 5 min at room temperature. The mixture was then cast without degassing, into polypropylene moulds, and polymerization initiated by exposure to UV light (Philips BLE-1800B long-wave UV-A lamps with an output of 1 mW/cm^2, at a distance of 25 cm) for 2 h. The polymers thus formed were porous circular membrane discs with an approximate thickness of 0.2 mm and a diameter of 20 mm. The porous polymer membranes were subjected to a lengthy extraction procedure in HFE-7100 (24 h), iso-propyl acetate (24 h), methanol (24 h), ethanol (24 h), 75% ethanol (24 h), 50% ethanol (24 h) and 25% ethanol (24 h) at 37 °C to remove residual unreacted material. The membranes were then equilibrated with MilliQ water (37 °C, 24 h).

2.4. Equilibrium water content

Equilibrium water content (EWC) evaluation and calculation was performed according to a previously reported procedure [10].

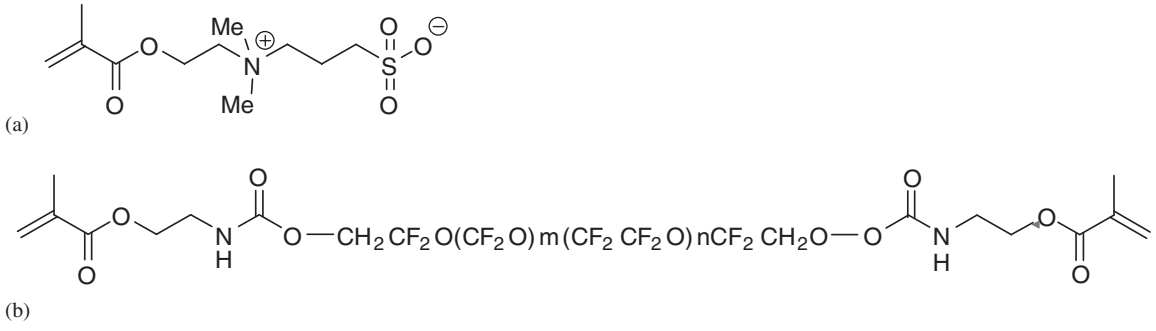

Fig. 1. (a) Structure of zwitterions and (b) structure of macromonomers.