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Epithelialization of hydrogels achieved by amine functionalization and co-culture with stromal cells

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Abstract

The aim of this study was to develop a hydrogel which would be suitable for corneal cell re-epithelialization when used as a corneal implant. To achieve this, a series of hydrogels were functionalized with primary amines by post-polymerization reactions between amine compounds and glycidyl ether groups attached to the hydrogels. We report a strong correlation between the structure of the amine and the viability of stromal cells and epithelial cells cultured on these hydrogels. Subsequent co-culture of epithelial and stromal cells on the amine modified hydrogels allowed successful expansion of epithelial cells on surfaces functionalized with alkyl α–ω diamines with carbon chain lengths of between 3 and 6. Analysis of variance showed that corneal epithelial cells had a strong preference for surfaces functionalized by the reaction of excess 1,3 diaminopropane with units of glycidyl methacrylate compared to the reaction products of other amines (ammonia; 1,2-diaminoethane; 1,4-diaminobutane or 1,6-diaminohexane). We suggest this approach of amine functionalization combined with stromal/epithelial co-culture offers a promising new approach to achieving a secure corneal epithelium.

Keywords: Hydrogels; Amines; Cornea; Epithelial cells

1. Introduction

Synthetic hydrogels containing high densities of hydroxyl groups are generally known as non-fouling biomaterials that are not conducive to the adhesion of most mammalian cell types. On the other hand, they have distinct advantages over other potential materials when used as supports or scaffolds in tissue engineering. In particular, their non-adhesive nature allows for modification with molecules that can guide cell growth [1] and their swollen state allows diffusion and release of essential biomolecules throughout the construct [2]. In this connection we previously showed that mammalian cells can adhere and proliferate on amphiphilic co-networks [3–5]. In that work, the polymer co-networks, formed from co-polymerization of pre-formed oligomers [6] and low molecular weight monomer, phase separated from the reaction medium during synthesis to give porous structures and cell adhesion and proliferation was dependant both on overall polymer composition and on morphology.

In this study we address the problem of developing hydrogel surfaces for corneal implants. The use of phase-separated materials in ophthalmic applications is problematic because of light scattering from either water/polymer interface (in porous materials) or micro-phase separated polymer domains. Careful optimization of random copolymer amphiphilic networks, which do not phase separate and are often optically clear, can provide materials for cell

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culture scaffolds but considerable improvements are needed if they are to be of use in clinical situations [7]. One strategy for further improvement of the cell-adhesive properties of hydrogels is to incorporate specific cell-adhesive peptide sequences [8–18]. Other non-peptide chemical surface modifications, particularly creating charged surfaces, have also been shown to improve cell adhesion and proliferation on hydrogels. For example, it has been shown that neurite outgrowth and viability was enhanced [19,20] and proliferation of keratinocytes improved [21] as the amine content of chitin hydrogels increased. Also, Pokharna et al. [22] observed increased growth of fibroblasts on poly(hydroxyethyl methacrylate) hydrogels containing amine functionality. These authors also suggested that the length of the branch from the polymer chain to the amine group might have a critical effect on biocompatibility. Hydrogels containing quarterniary ammonium functionality have also been reported to be good substrates for some cell types [23].

In bioengineering of hydrogel-based corneal implants it is necessary to have a surface that promotes attachment, migration and proliferation of corneal epithelial cells. This has not yet been achieved to the best of our knowledge. In this paper we describe how we have combined amine modification of the surface of a hydrogel with a knowledge of epithelial/stromal cell dependency (as detailed in [24–28]) to control and optimize epithelialization on hydrogel surfaces. In brief our prior experience with stromal cell/epithelial cell co-culture has shown that it is possible to achieve epithelial cell growth (both primary human keratinocytes [25–28] and primary human corneal epithelial cells—HCECs) [29] on a range of substrates providing one co-cultures them with stromal cells. Cell culture conditions can be achieved where stromal cells act as initial support/feeder cell for epithelial cells so that epithelial cells can be grown serum free on substrates where they would not previously grow unaided [25–29].

In this study we first modified the hydrogel surfaces with amines of different chain lengths and showed that the carbon chain length of these amine modifications is a critical parameter in the attachment of stromal cells or epithelial cells. We then combined this with corneal epithelial cell/stromal cell co-culture to provide the crucial epithelial–fibroblast interactions which then enabled the epithelial cells to survive and proliferate on the hydrogel surface.

2. Methods

2.1. Materials

Unless stated otherwise all reagents and chemicals were obtained from Aldrich (UK) Ltd. Laurylmethacrylate (DM) and ethyleneglycol dimethacrylate (EGDMA) were passed through a column of basic alumina to remove the inhibitors. Glycerol monomethacrylate (GM) (Rohm Haas) was stored at −18 °C and used as received. Glycidylmethacrylate (GME) was distilled in vacuo prior to use. All amines were obtained from Fluka (UK), stored under nitrogen and used without further purification. Photoinitiator, 2-hydroxy-2-methylpropiophenone (Irgacure 1173), was stored in the dark at −18 °C and used as supplied. Dioxane (“sureseal”, Aldrich (UK)) and 2-propanol (IPA), HPLC grade, (Fischer) were used as received.

2.2. Photopolymerization

The monomers, DM, GM, EGDMA and GME in varying ratios (total 9 g) were dissolved in IPA (4 ml) along with 2-hydroxy-2-methylpropiophenone (90 mg, 1wt% monomers). The mixtures were added to a polymerization mould and irradiated with 2″ arc 400 W mercury discharge lamp at a distance of 10 cm in a Dimax model Bondbox on a rotating table for 40 s on each side. The polymerization mould consisted of two 4 mm thick glass sheets covered with 100 μm PET film (hifi films pmx727, no slip) attached by the minimum amount of 3 M spray-mount® adhesive. The plates were separated with a rectangular 500 μm PTFE spacer. The monomer feeds used are shown in Table 1. The sheets were 125 mm × 70 mm × 0.5 mm when removed from the mould.

2.3. Reaction with diamines or ammonia

Amine functional polymers (10 g) were produced by soaking freshly prepared polymers in ethanolic solutions containing the required amine (250 cm^3, 5 vol%) in large excess for 24 h at room temperature. The polymers were modified with ammonia (**1**), 1,2-diaminoethane (**2**), 1,3-diaminopropane (**3**), 1,4-diaminobutane (**4**) or 1,6-diaminohexane (**5**). In the case of ammonia, concentrated ammonia was used. In all other cases pure amines were used as supplied. The use of a large excess of the amine ensures that only one amine of the diamine compounds reacted with the glycidyl ether group of the GMA residues and that all of the glycidyl ether groups were quantitatively converted. This operation was conducted in screw-top polypropylene bottles. Polymers were soaked twice overnight in absolute ethanol to remove excess amine.

2.4. Characterization of hydrogels

The equilibrium water contents (EWC) were determined gravimetrically in deionized water on cylindical samples cut using a cork borer. six sample disks per material were cut with a number 2 cork borer (6 mm diameter) and were hydrated over 24 h in ultrapure water prepared by filtration (Milli-Q Systems). The EWC were calculated as

$$\text{EWC} = \text{wet weight} - \text{dry weight}/\text{wet weight} \times 100. \quad (1)$$

Table 1
The composition of the hydrogels **A**, **B** and **C**

Hydrogel	Glycerol monomethacrylate (pbw)	Lauryl methacrylate (pbw)	EGDMA (pbw)	Glycidyl methacrylate (pbw)
A	75	12	8	5
B	75	14	6	5
C	85	0	10	5