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EGF-grafted PDMS surfaces in artificial cornea applications

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Abstract

Lack of epithelial cell coverage has remained a persistent problem in the design of an artificial cornea. In this work, polydimethylsiloxane (PDMS) surfaces were modified with epidermal growth factor (EGF) to improve the growth of corneal epithelial cells. The EGF was covalently tethered to PDMS substrates aminated by plasma polymerization of allylamine via a homobifunctional polyethylene glycol (PEG) spacer. Surface modification was confirmed by contact angle and X-ray photoelectron spectroscopy measurements. By varying the ratio of EGF to PEG from 1:50 to 1:5, EGF amounts from 40 to 90 ng/cm^2 could be bound, as determined by surface plasmon resonance (SPR) and ^{125}I radiolabelling. Human corneal epithelial cells on the various modified surfaces were cultured both in the presence and absence of EGF in the culture medium to determine the effect of covalently bound EGF on the cells. The results demonstrated that covalently bound EGF on the surfaces is active with respect to promoting epithelial cell coverage. This was significant when compared to unmodified controls.

Keywords: Artificial cornea; Epidermal growth factor; PDMS; PEO; Corneal epithelial cells

1. Introduction

An artificial cornea is a desirable alternative to donor graft transplantation for treatment of cornea blindness, which currently affects approximately 10 million people worldwide [1,2]. For successful integration into the host eye tissue, the device must interact appropriately with the different cellular layers of the cornea. In addition to the penetration of stromal keratocytes and deposition of extracellular matrix along the periphery for anchoring, the device should ideally support a layer of epithelial cells over the anterior surface [2–4]. An epithelial layer would provide a protective barrier, allow for maintenance of a precorneal tear layer and to prevent the downgrowth of epithelial cells that leads to stromal necrosis and spontaneous extrusion of the device. While significant advances in artificial cornea materials and design [1–5] have been made in recent years, particularly related to stromal cell anchorage [6–8], the lack of epithelialization remains a persistent problem and has contributed to the limited success of current devices in the long term [4,8].

As a result, improvement in corneal epithelialization has been the subject of several recent investigations. Epithelial cells must be able to migrate from the remaining corneal tissue over the implant surface, attach to the material and proliferate to restore complete coverage [9]. Several factors including surface hydrophilicity, porosity, topography and permeability to nutrients have been suggested to affect epithelialization, particularly under in vivo conditions [10–12]. Treatment of polymers with PHEMA [9,13] or argon [14] plasmas resulted in improvement in cell adhesion in some conditions; however other argon-treated substrate materials did not support cell growth [13]. Other work has focused on coating materials with extracellular matrix proteins, including collagen, laminin and fibronectin

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[3,12,15] and cell adhesion peptides [16,17], in order to promote cell adhesion and migration. While in vitro data have generally been encouraging, in vivo results have been less conclusive, and long-term stability data is lacking.

An alternative, less investigated method of promoting cell interactions with materials is with the use of growth factors that are present during normal wound-healing processes. Epidermal growth factor (EGF) is of particular interest for this application. A compact, globular, 6 kDa polypeptide, EGF is well-known to be a potent mitogen for epithelial cells including those of the cornea [18,19]. EGF and its receptor are present in corneal epithelium [20], where it inhibits terminal differentiation, increases proliferation and stimulates cell motility in a dose-dependent manner [21–25], and is a key factor in corneal wound-healing [26–28]. Additionally EGF has been shown to stimulate production of matrix proteins including fibronectin and hyaluronic acid [18,29]. It is therefore hypothesized that localization of EGF to the surface of a corneal prosthesis may be effective in promoting epithelial coverage of the device.

A number of different growth factors and cytokines have been covalently bound to substrates for use in biomaterials and tissue engineering applications. Kuhl and Griffith-Cima observed that tethering of EGF to glass substrates via flexible polyethylene glycol (PEG) spacers resulted in significantly improved activity over adsorbed EGF [30]. Others have covalently immobilized EGF [31], transforming growth factor-β [32,33], vascular endothelial growth factor [34] or nerve growth factor [35,36] for various applications. In corneal applications, Merrett et al. [37] tethered TGF-β to polydimethylsiloxane substrates via difunctional PEG in order to inhibit corneal cell downgrowth.

In this work, PDMS substrates, aminated by plasma polymerization of allylamine, were modified with pegylated EGF for enhancement of corneal epithelial cell growth. The EGF–PEG ratio and binding conditions had been previously optimized to maximize the amount of covalently bound growth factor [38]. The surfaces were characterized by various methods including surface plasmon resonance (SPR) and radiolabelling as well as by examining the interactions of human corneal epithelial cells with the surfaces in vitro.

2. Materials and methods

2.1. Surface preparation

PDMS surfaces (Sylgard 184, Dow Corning, Midland MI) were prepared according to the manufacturer's directions. A 10:1 (wt/wt) mixture of elastomer and curing agent were mixed in a Petri dish and placed under vacuum for 90 min to remove entrapped air. The PDMS was left to cure at room temperature for at least 48 h. Gold chips for SPR (Biacore SIA Kit Au, Piscataway NJ) and gold-coated silicon wafers for radiolabelling and ellipsometry experiments were cleaned in ethanol and potassium hydroxide, followed by ultrasonic cleaning in Milli-Q (18 MΩ) water. Surfaces were then immersed in 1.5 wt% (97–99)% dimethyl-(1–3)% mercaptopropylmethylsiloxane copolymer (Petrarch Systems, Bristol, PA) in tetrahydrofuran for 24 h to generate a thin PDMS layer. This was followed by rinsing with tetrahydrofuran and Milli-Q water to remove loosely bound polymer chains.

Subsequently, all surfaces were aminated by microwave frequency plasma polymerization of allylamine (Aldrich, Milwaukee WI) using a custom-built plasma reactor [39]. The reactor was evacuated to a pressure below 50 μm Hg and the surfaces exposed to an argon plasma (flow rate of 235 standard cubic centimeters per minute) for a period of 5 min. This was followed by the introduction of allylamine monomer at a flowrate of 0.9 scm, at which point glow discharge was initiated at a power of 20 W for 10 min. The reactor pressure during allylamine excitation was approximately 90 μm Hg. Following the reaction, the allylamine flow was stopped. Once the pressure had re-stabilized, the reactor was backfilled with argon and the samples removed. Surfaces were rinsed in Milli-Q water and dried under vacuum.

2.2. EGF modification of surfaces

Lyophilized recombinant human EGF (Upstate Cell Signaling Solutions, Lake Placid, NY) was reconstituted to 100 μg/mL in 150 mM phosphate buffered saline (PBS) (pH 7.4). The EGF was pre-reacted with a molar excess of homo bifunctional *N*-hydroxysuccinimide ester of PEG-butanoic acid (SBA_2-PEG, referred to as PEG in this paper), MW 3400 (Nektar Therapeutics, Huntsville, AL) in PBS at room temperature for various times. EGF–PEG molar ratios examined were 1:50, 1:10 and 1:5, corresponding to EGF concentrations of 1, 5 and 10 μg/mL, respectively, with PEG concentration kept constant. Surfaces modified by SPA–PEG-methoxy, MW 5000 (Nektar), were used as a control (referred to as mPEG). Following pre-reaction, the aminated surfaces were exposed to the EGF–PEG solutions for various durations at room temperature and subsequently rinsed three times in PBS. The modification process is summarized in Fig. 1.

2.3. Ellipsometry measurements

PDMS and allylamine layer thickness on the gold-coated silicone wafers were determined ellipsometrically using an Exacta 2000 instrument (Waterloo Digital Electronics, Waterloo, ON). Refractive indices of 1.4 and 1.6 for PDMS and polyallylamine, respectively, were used.

2.4. Surface analysis

2.4.1. Protein-surface binding: SPR experiments

Binding of pegylated EGF to aminated Au-PDMS surfaces was monitored by SPR, using a Biacore 2000 instrument (Biacore, Piscataway, NJ). This technique monitors the optical properties of the surface layer adjacent to a gold film upon binding of a biomolecule [40]. The modified gold chips were