Acta Biomaterialia 9 (2013) 5040–5051

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

The influence of biomimetic topographical features and the extracellular matrix peptide RGD on human corneal epithelial contact guidance

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ARTICLE INFO

Article history:
Received 29 June 2012
Received in revised form 18 September 2012
Accepted 5 October 2012
Available online 13 October 2012

Keywords:
Cornea
Epithelial cell
RGD peptide
Nanotopography

ABSTRACT

A major focus in the field of tissue engineering is the regulation of essential cell behaviors through biophysical and biochemical cues from the local extracellular environment. The impact of nanotopographical cues on human corneal epithelial cell (HCEC) contact guidance, proliferation, migration and adhesion have previously been demonstrated. In the current report we have expanded our study of HCEC responses to include both biophysical and controlled biochemical extracellular cues. By exploiting methods for the layer-by-layer coating of substrates with reactive poly(ethylene imine)/poly(2-vinyl-4,4-dimethylazlactone)-based multilayer thin films we have incorporated a single adhesion peptide motif, Arg–Gly–Asp (RGD), on topographically patterned substrates. This strategy eliminates protein adsorption onto the surface, thus decoupling the effects of the HCEC response to topographical cues from adsorbed proteins and soluble media proteins. The direction of cell alignment was dependent on the scale of the topographical cues and, to less of an extent, the culture medium. In EpiLife® medium cell alignment to unmodified-NOA81 topographical features, which allowed protein adsorption, differed significantly from cell alignment on RGD-modified features. These results demonstrate that the surface chemical composition significantly affects how HCECs respond to topographical cues. In summary, we have demonstrated modulation of the HCEC response to environmental cues through critical substrate and soluble parameters.

1. Introduction

The extracellular matrix (ECM) serves as a natural scaffold for cells, acting as a mechanical support as well as creating a distinct microenvironment to which cells can respond. Within our tissue of interest, the human cornea, as well as other epithelial tissues, lies a specialized matrix referred to as the basement membrane (BM). The BM is a thin and highly specialized ECM component found between the epithelium and stromal layers which plays a critical role in the organization, maintenance and integrity of the overlying corneal epithelium [1–3]. This specialized matrix is highly complex, with both biophysical and biochemical components [4,5]. Cells integrate these external cues to trigger a cascade of intercellular mechanistic pathways that ultimately results in the modulation of specific cell phenotypes, including proliferation, migration, adhesion, differentiation and apoptosis [6]. Each of these properties is essential for the formation and maintenance of epithelial tissue. Current tissue engineering approaches seek to take advantage of these biophysical and biochemical features through the fabrication of substrates that mimic specific aspects of the native in vivo microenvironment of the ECM in order to promote or inhibit specific epithelial behaviors and improve the likelihood of success of an engineered tissue. Currently available corneal prosthetics have focused mainly on the stromal and not the epithelial component of the tissue, which may explain the poor re-epithelialization and ultimate failure of the replacements. With this approach we believe that incorporation of ECM elements, specifically nano- and micron-scale topographical cues, as well as biochemical components in the form of adherent peptides from the basement membrane, will provide the missing elements in the current design and improve the future design of tissue replacements, specifically for the human cornea.

The complexity of the corneal BM presents a significant problem in regenerative medicine due to the limited information available regarding the biophysical and biochemical factors that dictate complex biological processes, such as the formation of epithelial tissue. The specific biophysical and biochemical elements of the BM that are critical in promoting the creation and maintenance

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http://dx.doi.org/10.1016/j.actbio.2012.10.007

of the epithelial component within an engineered corneal tissue have yet to be clearly defined. Therefore, our strategy over the course of the last decade has focused on the systematic characterization and quantitation of biophysical cues from the native BM of several different species and tissues, including the human cornea, with the goal of incorporating some of these components in a corneal prosthetic [7–11]. We have successfully quantified several biophysical characteristics, including the size range of complex topographical features, such as intertwining fibers and pores of varying nanoscale and submicron dimensions and the compliance of the BM on which human corneal epithelial cells (HCECs) reside [5,12]. These characterized properties have been utilized as a guide for substrate design to establish the impact of specific BM biophysical cues on essential HCEC behaviors. We have successfully demonstrated and confirmed the scale-dependent impact that nano- to micron-sized cues have on adhesion [13], proliferation [14], migration [15] and contact guidance of HCECs [16–18].

In addition to topography, the BM also presents bioactive adhesive molecules as well as soluble growth factors that are sequestered by the matrix. Systematic control of each set of cellular cues is critical in order to decouple the biophysical and biochemical cues and elucidate how each signal has an impact on HCEC behavior. For example, the HCEC response to topographical cues is influenced by several external environmental factors that include soluble biochemical factors within the tissue culture medium. Specifically, the extent and preferred orientation of HCEC alignment is dependent on whether the cells are cultured in serum-containing or serum-free medium. In previous experiments cells were found to interact with a topographical surface through an irreversible adsorbed layer of protein that was deposited upon immersion of a silicon- [16–20] or polyurethane-based [14,15] substrate in culture medium. HCECs plated on biomimetic nanoscale ridge/groove structures align either parallel or perpendicular to the underlying substrate topography, depending on the cell culture medium [16–18,20,21].

More recent studies have investigated the combined impact of biochemical responses that modulate HCEC contact guidance and responses to topographical cues through the exposure of HCECs to varying soluble factors using different growth media [17]. Although it is a reasonable assumption that the surface chemistry is homogeneous, the chemical composition of the adsorbed protein layer depends on several conditions, which include the substrate material, the components within the culture medium and the scale of the substrate topography [22–24]. Previous work using topographical features that allow protein adsorption have been unable to separate the HCEC responses to topographical cues due to surface chemical heterogeneities.

The objective of the current study was to incorporate ECM peptides onto topographically patterned substrates to control both topographical and adherent biochemical cues and demonstrate the influence on essential HCEC behaviors. The peptide selected for incorporation onto topographically patterned substrates is Arg–Gly–Asp (RGD). The RGD peptide sequence is located within major ECM components, including the proteins laminin and fibronectin, serves as an adhesive ligand to integrins, and has been demonstrated to have an impact on HCEC behavior [25–30]. We have recently demonstrated successful control of the adherent biochemical environment through the immobilization of RGD on planar surfaces coated with reactive polymer multilayers fabricated by the layer-by-layer deposition of poly(ethylene imine) and poly(2-vinyl-4,4-dimethylazlactone) (PEI/PVDMA) [31–33]. The resulting PEI/PVDMA films allow the stable and covalent integration of our peptide of interest (RGD), resulting in the control of cell–substrate attachment and the inhibition of non-specific protein absorption from the tissue culture medium to the nano and micron scale substrates without disruption of the underlying topographical features [33]. HCECs were exposed to varying soluble factors using two distinct culture media, serum-free EpiLife® and serum growth factor-rich epithelial medium. HCEC contact guidance to the RGD and control D-glucamine-modified topographical features provides a measurable end-point to demonstrate that topography, surface chemistry and soluble components within the medium cue cell behaviors both independently and in combination. Contact guidance of cells is highly correlated with the direction of cell migration [15], and provides an efficient end-point to determine whether the cells integrate the physical and chemical cues of the material. The methods developed here also provide a means for high throughput analysis of the cellular response to interactions with controlled surface chemistris, topographies and soluble factors.

2. Materials and methods

2.1. Materials

Branched poly(ethylene imine) (PEI) (M_n 10,000 g mol^{-1}, M_w 25,000 g mol^{-1}) and solvents were purchased from Sigma–Aldrich (Milwaukee, WI). 2-Vinyl-4,4-dimethylazlactone (VDMA) monomer was a kind gift from Steve Heilmann at 3 M (Minneapolis, MN). Poly(2-vinyl-4,4-dimethylazlactone) (PVDMA, M_n 18,200, PDI 3.1) was synthesized according to methods described in Buck and Lynn [34]. The peptides GGGRGDSP (RGD) and GGGRDGSP (RDG) were synthesized at the University of Wisconsin Biotechnology Center (Madison, WI). D-Glucamine was purchased from TCI America (Portland, OR). Silgard-184 silicone elastomer base and the curing agents were purchased from Dow Corning (Midland, MI). NOA81 optical adhesive was purchased from Norland Products (Cranbury, NJ). Cell culture and staining reagents were purchased from Invitrogen (Carlsbad, CA) unless otherwise noted.

2.2. Fabrication of micro- and nanoscale ridge/groove features

Silicon chips containing six regions of ridge/groove features (400, 800, 1200, 1600, 2000 and 4000 nm in pitch) and flat control areas were fabricated using X-ray lithography as described previously [13,16]. The silicon chips were used as masters for soft lithography reproduction in NOA81 optical adhesive. PDMS stamps were generated as previously described [14,35]. The resulting composite stamp consisted of a "hard" poly(dimethylsiloxane) (PDMS) layer, to retain the topographical features, and a pliable PDMS layer, for easy removal and handling of the stamp. An ~18 mm diameter blob of NOA81 optical adhesive was deposited on an oxygen plasma-treated glass slide. Slides were placed in a spin coater for 40 s at 4000 r.p.m. The topographically patterned PDMS stamp was placed on the NOA81-coated slides and cured in a XL-1500 UV cross-linker under 365 nm light for 100 min, after which the stamp was carefully removed.

2.3. Fabrication of PEI/PVDMA films on topographically patterned substrates

Reactive PEI/PVDMA multilayer films were fabricated on glass substrates containing NOA81 molded topographical features using a layer-by-layer approach, as previously described [32]. Substrates were rinsed with acetone, ethanol, methanol, and deionized water, and then (1) submerged in a solution of PEI in acetone (20 mM with respect to the molecular weight of the polymer repeat unit) for 30 s, (2) submerged for 30 s in two consecutive acetone rinse solutions, (3) submerged in a solution of PVDMA in acetone (20 mM with respect to the polymer repeat unit) for 30 s, and, finally, (4) submerged in two more subsequent acetone rinse baths