Experimental Cell Research 346 (2016) 17–29

Contents lists available at ScienceDirect

Experimental Cell Research

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

Research Article

Collective epithelial cell sheet adhesion and migration on polyelectrolyte multilayers with uniform and gradients of compliance

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ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form
6 May 2016
Accepted 5 June 2016
Available online 9 June 2016

Keywords:
Polyelectrolyte multilayer (PEMU)
Collective cell migration
Durotaxis
Poly(acrylic acid) (PAA)
Poly(allylamine hydrochloride) (PAH)
Myosin II
Modulus gradient
Photocrosslinking

ABSTRACT

Polyelectrolyte multilayers (PEMUs) are tunable thin films that could serve as coatings for biomedical implants. PEMUs built layer by layer with the polyanion poly(acrylic acid) (PAA) modified with a photosensitive 4-(2-hydroxyethoxy) benzophenone (PAABp) group and the polycation poly(allylamine hydrochloride) (PAH) are mechanically tunable by UV irradiation, which forms covalent bonds between the layers and increases PEMU stiffness. PAH-terminated PEMUs (PAH-PEMUs) that were uncrosslinked, UV-crosslinked to a uniform stiffness, or UV-crosslinked with an edge mask or through a neutral density optical gradient filter to form continuous compliance gradients were used to investigate how differences in PEMU stiffness affect the adhesion and migration of epithelial cell sheets from scales of the fish *Poecilia sphenops* (Black Molly) and *Carassius auratus* (Comet Goldfish). During the progressive collective cell migration, the edge cells (also known as 'leader' cells) in the sheets on softer uncrosslinked PEMUs and less crosslinked regions of the gradient formed more actin filaments and vinculin-containing adherens junctions and focal adhesions than formed in the sheet cells on stiffer PEMUs or glass. During sheet migration, the ratio of edge cell to internal cell (also known as 'follower' cells) motilities were greater on the softer PEMUs than on the stiffer PEMUs or glass, causing tension to develop across the sheet and periods of retraction, during which the edge cells lost adhesion to the substrate and regions of the sheet retracted toward the more adherent internal cell region. These retraction events were inhibited by the myosin II inhibitor Blebbistatin, which reduced the motility velocity ratios to those for sheets on the stiffer PEMUs. Blebbistatin also caused disassembly of actin filaments, reorganization of focal adhesions, increased cell spreading at the leading edge, as well as loss of edge cell-cell connections in epithelial cell sheets on all surfaces. Interestingly, cells throughout the interior region of the sheets on uncrosslinked PEMUs retained their actin and vinculin organization at adherens junctions after treatment with Blebbistatin. Like Blebbistatin, a Rho-kinase (ROCK) inhibitor, Y27632, promoted loss of cell-cell connections between edge cells, whereas a Rac1 inhibitor, NSC23766, primarily altered the lamellipodial protrusion in edge cells. Compliance gradient PAH-PEMUs promoted durotaxis of the cell sheets but not of individual keratocytes, demonstrating durotaxis, like plithotaxis, is an emergent property of cell sheet organization.

1. Introduction

Collective cell migration is crucial for normal tissue development and wound healing. Injury to skin, for example, triggers activation of various cells that release cytokines, remodel ECM, sprout blood vessels, and close the wound through epithelial cell sheet migration [1]. As epithelial cell sheets migrate to close the wound, unified contractile forces within the sheet help pull the skin tissue together [1–3]. Cells in these migrating multilayer sheets remain connected to each other through cadherin-containing cell-cell adhesions, which are stabilized by the cortical actin cytoskeleton and intermediate filaments. The interconnectedness of the cells and their traction as they move along the underlying substratum maintain robust mechanical tension throughout the migrating epithelial cell sheet [2,3].

Cells can sense a variety of cues from their microenvironment, including surface modulus (stiffness), and respond to changes in microenvironment stiffness, stress, and elasticity by remodeling their cytoskeleton [4] and altering their morphology, substrate adhesion, and migration [5–8]. Some individual cells migrate directionally along a modulus gradient through a process known as durotaxis [9–13].

Cells migrating collectively as a sheet through a process known as plithotaxis and cells migrating independently share certain

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http://dx.doi.org/10.1016/j.yexcr.2016.06.002
0014-4827/

characteristics but differ in their ability to affect each other mechanically and through signaling. Plithotaxis, an emergent property of cell sheets, requires coordination of cell motility that subjects individual cells within the collective cell sheet to additional regulation and restraints that control cell morphology and motility guidance [2,3,14,15]; forces transmitted across cell-cell junctions direct individual cells to migrate along the local orientation of maximal principal stress, or equivalently, minimal intercellular shear stress [16]. Edge cell formation of robust vinculin-containing focal adhesions in highly active lamellipodia, in which actin filament turnover is controlled by Rac-dependent signaling pathways, contributes to the traction at the sheet leading edge and development of tension across the sheet that is necessary for plithotaxis [2,3,17–20]. Myosin II-dependent force production, controlled by Rho-dependent processes, maintains tensile forces on the focal adhesions in cells and across sheet cell-cell junctions [21–24]. Focal adhesions and stress fibers are components of mechanotransduction signaling pathways that enable a cell to sense physical cues from its environment such as surface modulus (stiffness) and intracellular shear stress [8,25–32].

During collective cell plithotaxis, intercellular response to extracellular signals is processed by edge cells and subsequently transmitted via chemical or mechanical signaling to internal cells, eliciting a larger and more expanded response throughout the collective sheet. Polarity is established in both individual edge cells and through the collective mass, forming a front and back end in the migrating cell sheet. Guidance signals distributed throughout the sheet regulate individual cell responses [3]. Collectively, sheet cells can sample cues from a larger area than can a single cell [2,3]. Because every cell of the collective provides input regarding environmental guidance cues, the sheet responds as a coordinated unit to global guidance cues rather than to more local differences in cues [2].

Epithelial tissue isolated from fish scales provides an excellent model cell system for studying migration of individual cells and epithelial cell sheets of the type observed during wound healing [20,33,34]. In this study, we used primary explants of epithelial cell sheets associated with scales of the fish *Poecilia sphenops* (Black Molly) and *Carassius auratus* (Comet Goldfish) to investigate collective cell interaction with polyelectrolyte multilayers (PEMUs).

PEMUs are thin film coatings built layer by layer with alternating pairs of polyelectrolytes (PEs) [35,36]. Varying parameters such as the specific types of PE used, number of layers, and degree of layer crosslinking yields PEMUs with a wide variety of bulk chemical and physical properties [35–43]. Ability to tune PEMU properties to mimic aspects of natural cell microenvironments that affect cell behavior such as adhesion makes PEMUs potentially useful in biomedical applications [44–46].

PEMUs used in this investigation were built with alternating layers of the polyanion PAA-PAABp [PAA (poly(acrylic acid)) modified with a photosensitive 4-(2-hydroxyethoxy) benzophenone (PAABp)], and the polycation PAH (poly(allylamine hydrochloride)). Photoactivation of the PAABp generates covalent crosslinks between the layers, increasing surface modulus [47]. Varying the time of crosslinking generates surfaces with varied uniform moduli. Photoactivation through an optical gradient mask generates modulus gradients [47]. Because only 5% of the PAA side groups were modified with the photosensitive benzophenone group, surface modulus manipulation by crosslinking has little effect on net surface charge. This investigation is the first to characterize fish scale cell sheet behavior on PEMUs and demonstrates sheet durotaxis on a compliance gradient and the importance of myosin II activity on sheet integrity and motility.

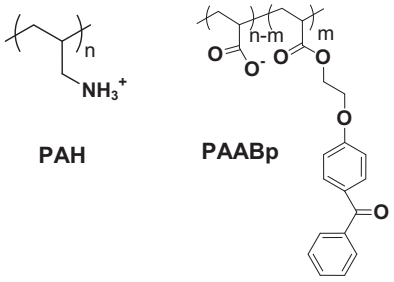
2. Materials and methods

2.1. Polyelectrolytes and reagents

Poly(acrylic acid) (PAA, molar mass 100,000 g mol^{-1}) and poly (allylamine hydrochloride) (PAH, molar mass 56,000 g mol^{-1}) from Sigma-Aldrich, and poly(ethyleneimine) (PEI, molar mass 70,000 g mol^{-1}) from Polysciences, Inc. (Warrington, PA) were used as received. PAA grafted with photosensitive benzophenone (5 mol%) was synthesized as described previously [47]. Unless noted otherwise, all other reagents were used as received from Sigma-Aldrich. For myosin II, Rho kinase (ROCK), and Rac1 inhibition, cell sheets were treated with low and high concentrations of inhibitors as follows: 5 μM and 50 μM Blebbistatin (myosin II inhibitor), 1 μM and 50 μM of Y27632 (Rho Kinase [ROCK] inhibitor), and 5 μM and 50 μM NSC23766 (Rac1 inhibitor). Cell sheets were observed for 1 h before treatment and for 3 h after treatment.

2.2. Polyelectrolyte structures

The PAABp benzophenone side group (m) is 5% of the total monomer units (n). (n=1; m=0.05; n−m=0.95).



2.3. Polyelectrolyte multilayer preparation

The 31-layer PAH-terminated PEI(PAABp/PAH)$_{15}$ PEMUs used in this investigation were built using 10 mM (with respect to the repeat unit) PE solutions made in 0.15 M NaCl, 25 mM Tris–HCl, pH 7.4, as described previously [47]. The PEMUs were built on Fisherbrand Cover Glass no. 1 (Fisher Scientific) 22 × 22 × 0.17 mm coverslips for uniform modulus and steep modulus gradient assays, and on 50 × 22 × 0.17 mm coverslips for shallow modulus gradient assays. Prior to PEMU coating, the glass coverslips were cleaned by soaking in 70% ethanol overnight, rinsed extensively with H_2O, dried with a stream of N_2, and exposed to an air plasma for 1 min. All surfaces were first primed with a layer of PEI by dipping into PEI solution for 30 min and rinsing with water. This initial PEI layer promoted a more uniform coating by subsequent PE layers.

The remainder of the PEMU was built layer by layer with the aid of a robot (StratoSequence V, nanoStrata Inc., Tallahassee, FL). The PEI-coated surfaces were mounted in the robot on a shaft that rotated at 300 rpm for 15 cycles of dipping in alternating baths of PAABp and PAH for 5 min each, with three 1-min water rinsing steps between each PE bath. Before use in cell culture, the PEMU-coated coverslips were incubated overnight in 0.15 M NaCl, 25 mM Tris–HCl, pH 7.4. All PE solutions used for building the cell culture PEMUs contained 0.02 wt% sodium azide to prevent microbial contamination. PEMUs were handled and stored using sterile laboratory practices. Microbial contamination of polyelectrolyte solutions lacking sodium azide was rare, and its inclusion was only precautionary and unnecessary if PEMUs were handled following sterile practices. Azide was rinsed out of PEMUs in the final rinsing