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Syndecan-1 in mechanosensing of nanotopological cues in engineered materials

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ABSTRACT

The cells of the vascular system are highly sensitive to biophysical cues from their local cellular microenvironment. To engineer improved materials for vascular devices and delivery of cell therapies, a key challenge is to understand the mechanisms that cells use to sense biophysical cues from their environment. Syndecans are heparan sulfate proteoglycans (HSPGs) that consist of a protein core modified with heparan sulfate glycosaminoglycan chains. Due to their presence on the cell surface and their interaction with cytoskeletal and focal adhesion associated molecules, cell surface proteoglycans are well poised to serve as mechanosensors of the cellular microenvironment. Nanotopological cues have become recognized as major regulators of cell growth, migration and phenotype. We hypothesized that syndecan-1 could serve as a mechanosensor for nanotopological cues and can mediate the responsiveness of vascular smooth muscle cells to nanoengineered materials. We created engineered substrates made of polyurethane acrylate with nanogrooves using ultraviolet-assisted capillary force lithography. We cultured vascular smooth muscle cells with knockout of syndecan-1 on engineered substrates with varying compliance and nanotopology. We found that knockout of syndecan-1 reduced alignment of vascular smooth muscle cells to the nanogrooves under inflammatory treatments. In addition, we found that loss of syndecan-1 increased nuclear localization of Yap/Taz and phospho-Smad2/3 in response to nanogrooves. Syndecan-1 knockout vascular smooth muscle cells also had elevated levels of Rho-associated protein kinase-1 (Rock1), leading to increased cell stiffness and an enhanced contractile state in the cells. Together, our findings support that syndecan-1 knockout leads to alterations in mechanosensing of nanotopographical cues through alterations of in rho-associated signaling pathways, cell mechanics and mediators of the Hippo and TGF-β signaling pathways.

Cell therapies have great potential to improve the quality of life of countless patients with incurable diseases or severe injury. By their active nature, cell therapeutics can adapt and integrate into host tissues to perform functions not possible with drugs or protein therapeutics. A major component of enabling effective cell therapies is the use of scaffolds or delivery systems that support the function and phenotype of the cells once delivered [1]. In such engineered scaffolds, a key challenge is the development of engineered materials that can potentiate the curative effects of endogenous or delivered cells to enable the maximal therapeutic benefit from the implanted material [2]. Such instructive scaffolds or templates can recapitulate the cell-cell, cell-ECM, and cell-soluble factor signaling to orchestrate a therapeutic effect [3]. Only when facilitated by such an appropriate scaffold or carrier can cell-based therapies, such as those used in osteoarthritis [4] or in cardiac or vascular repair [5], truly transform the landscape of tissue engineering [6,7]. While there is much information on the use of chemical and biochemical signals to enhance engineered biomaterial function, the effects of physical forces and the mechanisms of mechanosensing of nanomechanical cues remain poorly

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defined and have begun to emerge as critical parameters in nanomaterial design.

In particular, vascular tissue engineering has made great strides towards recreating artificial blood vessels to treat a variety of cardiovascular conditions, ranging from myocardial infarction and ischemia to peripheral vascular disease and wound healing after vascular injury [8–11]. In the bench to bedside translation of engineered blood vessels, the importance of vascular integrity and the ability of vascular smooth muscle cells (vSMCs) to impart long-term stability to the engineered vessels is a key factor [12–14]. The vSMCs populate the medial layer of blood vessels and are present in multiple layers embedded in the basement membrane, which consists of fibrillar collagens and elastin [15,16]. The cells are organized in an aligned helical pattern around the blood vessel circumference with the successive vSMC layers demonstrating alternating pitch directions [17]. Within this structure, organized extracellular matrix (ECM) molecules provide mechanical stability to the cells as well as biophysical and biochemical cues within the vascular "niche." [18] On a biophysical level, the underlying ECM in the blood vessel provides nanotopological cues with feature sizes from tens to several hundreds of nanometers [19–23]. The nanotopographical cues can regulate the function of many cells types [24,25]. We and others have shown that vSMCs respond to nanotopographical cues that can regulate their alignment and phenotype [26–30]. While the effects of these physical cues are now widely accepted, the mechanisms of nanotopographically-stimulated regulation remain largely unknown. Previous studies have implicated Rac1 GTPase as a sensory mechanism and basis for directional alignment of cells on nanopatterned surfaces [31]. In addition, knockdown of focal adhesion kinase leads to increased cell alignment of corneal epithelial cells with nanopatterns and regulated gene expression of nesprin-1 and -2 [32]. In vSMCs, nanopatterning led to a significant increase in RhoA ROCK1 and ROCK2, implicating these pathways as potential mediators of mechanotransduction to nanopatterned surfaces [26]. Further, several studies have also found regulation of integrins, Src, p130Cas and actin reorganization in response to nanopatterned substrates [33–35].

In this work, we examined the role of syndecan-1 (SDC-1) in mechanosensing of nanopatterned substrates and material rigidity by vSMCs. Syndecan-1 (SDC-1) is a transmembrane cell surface proteoglycan that is found on endothelial cells [36], vSMCs [37] and macrophages [38] in the vascular system, as well as on many other cell types. Our group has recently shown that SDC-1 regulates the inflammatory state of endothelial cells in response to shear stress [36] and several studies have implicated heparan sulfate proteoglycans as key molecules in mechanosensing [39–41]. In this study, we created materials with nanoscale contact guidance cues using ultraviolet (UV)-assisted capillary force lithography (CFL), a versatile molding technology for imparting nanoscale architecture to tissue scaffolds over large surface areas [42]. We grew vSMCs with genetic knockout of SDC-1 and wild type phenotype on nanopatterned materials to explore mechanosensing and activation of the cells by nanotopographical cues. Our analyses support that SDC-1 is a key molecule in regulating the vSMC response to nanopatterned substrates and alters mechanosensitive signaling pathways including those involving Yap/Taz, Smad2/3 and cytoskeletal regulators Rock1, Rac-1 and integrin-linked kinase (ILK).

1. Materials and methods

1.1. Nanopatterned materials

Nanopatterned substrates were fabricated from a polyurethane acrylate (PUA) precursor, as described previously [43]. Briefly, an adhesion promoter (Minuta Tech) was applied to the glass coverslips by spin coating at 2000 rpm for 20 s and the coverslips were then baked at 65 °C for 20 min. A PUA-based polymer of commercially characterized stiffness (6.7 MPa or 2.4 GPa) was then spin coated onto the glass substrate and a PDMS mold with nanogrooves having an 800:800 nm groove:gap ratio with a 400 nm groove depth was placed on top of the PUA precursor layer. For nonpatterned surfaces, a mold with no features was placed on the polymer layer. Through capillary action, the polymer fills the mold and a nanopatterned surface is obtained. The patterns were UV cured using a wavelength of 365 nm for 60 s. The substrates were then placed into well plates and plasma coated to facilitate protein adsorption. The substrates were then coated with a solution of 11% (w/v) type I collagen overnight to facilitate cell attachment.

1.2. Cell isolation and culture

Aortae were harvested from 6 to 10 week old male syndecan-1 knockout (S1KO) and wild type (WT) mice. Following harvest, the aortae were minced and a glass coverslip was placed over the tissue fragments. The cells were then cultured in MCDB-131 culture medium (Life Technologies) with 20% fetal bovine serum (FBS), L-glutamine and antibiotics. The vSMCs migrated out of the tissue and were allowed to proliferate. After the first passage, the cells were grown in MCDB-131 with 10% FBS, L-glutamine and antibiotics. The vSMCs were seeded onto the substrates at 50% confluence. For long-term drug treatment experiments, cells were seeded onto the substrates and treated 24 h later with 1% DMSO, 10 μM Y-27632 (Sigma), or 10 μM Verteporfin (Tocris) in culture medium for 48 h. For short-term drug treatment experiments, cells were seeded onto the substrates and treated 24 h later with 0.1% DMSO, 10 μM Y-27632 (Sigma), 10 μM Verteporfin (Tocris), 1 μM Latrunculin A (Abcam) or 10 μM Nocodazole (Abcam) in culture medium for 2 h.

1.3. Immunostaining and image analysis

Following the treatments, the cells were washed with PBS at 37 °C and fixed with 4% paraformaldehyde for 10 min. The cells were then washed three times with PBS and permeabilized with 0.2% Triton X-100 for 5 min. The cells were blocked with 1% BSA in PBS for 40 min and stained with primary antibody diluted in PBS with 1% BSA overnight. The primary antibodies and dilution used in the study are shown in Supplemental Table 1. The cells were then rinsed PBS for 10 min three times. Secondary antibodies conjugated to fluorophores were added at 1:1000 dilution in 1% BSA in PBS containing 1 μg/ml of DAPI for nuclear staining. After 75 min of incubation at room temperature, the cell were rinsed extensively with repeated PBS washes. The cells were mounted and coverslipped in anti-fade mounting media (Vector Laboratories, Inc.).

The cells were imaged using an epifluorescent microscope (Axio Observer microscope, Carl Zeiss, Inc.). For each coverslip, ten images were taken at randomly selected areas. The same imaging exposure time and illumination intensity was used for all groups that were compared to each other following analysis. The cells and nuclei were selected and analysis was performed using computer-assisted morphometry with Metamorph 7.0 (Molecular Devices, LLC) or ImageJ. Measurements were made of the elliptical form factor (EFF; defined as the major axis divided by minor axis) and shape factor (defined as $4\pi[Area]/[Perimeter]^2$). For the quantification of cell alignment, the perimeters of the cells were traced and the angle of the cells calculated from the major axis of the cell area and compared to the angle of the nanogrooves in the substrate. For measurements of cell intensity, mean immunofluorescence intensities were measured in the selected areas using ImageJ.