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Cell sheet mechanics: How geometrical constraints induce the detachment of cell sheets from concave surfaces

Tadahiro Yamashita [a,b], Philip Kollmannsberger [a,1], Kazuma Mawatari [c,d], Takehiko Kitamori [c,d,*], Viola Vogel [a,*]

[a] Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Vladimir-Prelog Weg 4, 8093 Zurich, Switzerland
[b] Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656 Tokyo, Japan
[c] Department of Applied Chemistry, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656 Tokyo, Japan
[d] CREST, JST, 4-1-8 Honcho, Kawaguchi-shi, Saitama 332-0012, Japan

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ABSTRACT

Despite of the progress made to engineer structured microtissues such as BioMEMS and 3D bioprinting, little control exists how microtissues transform as they mature, as the misbalance between cell-generated forces and the strength of cell-cell and cell-substrate contacts can result in unintended tissue deformations and ruptures. To develop a quantitative perspective on how cellular contractility, scaffold curvature and cell-substrate adhesion control such rupture processes, human aortic smooth muscle cells were grown on glass substrates with submillimeter semichannels. We quantified cell sheet detachment from 3D confocal image stacks as a function of channel curvature and cell sheet tension by adding different amounts of Blebbistatin and TGF-β to inhibit or enhance cell contractility, respectively. We found that both higher curvature and higher contractility increased the detachment probability. Variations of the adhesive strength of the protein coating on the substrate revealed that the rupture plane was localized along the substrate-extracellular matrix interface for non-covalently adsorbed adhesion proteins, while the collagen-integrin interface ruptured when collagen I was covalently crosslinked to the substrate. Finally, a simple mechanical model is introduced that quantitatively explains how the tuning of substrate curvature, cell sheet contractility and adhesive strength can be used as tunable parameters as summarized in a first semi-quantitative phase diagram. These parameters can thus be exploited to either inhibit or purposefully induce a collective detachment of sheet-like microtissues for the use in tissue engineering and regenerative therapies.

Statement of Significance

Despite of the significant progress in 3D tissue fabrication technologies at the microscale, there is still no quantitative model that can predict if cells seeded on a 3D structure maintain the imposed geometry while they form a continuous microtissue. Especially, detachment or loss of shape control of growing tissue is a major concern when designing 3D-structured scaffolds. Utilizing semi-cylindrical channels and vascular smooth muscle cells, we characterized how geometrical and mechanical parameters such as curvature of the substrate, cellular contractility, or protein-substrate adhesion strength tune the catastrophic detachment of microtissue. Observed results were rationalized by a theoretical model. The phase diagram showing how unintended tissue detachment progresses would help in designing of mechanically-balanced 3D scaffolds in future tissue engineering applications.

* Corresponding authors at: Department of Applied Chemistry, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656 Tokyo, Japan (T. Kitamori); Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Vladimir-Prelog-Weg 4, 8093 Zurich, Switzerland (V. Vogel).
E-mail addresses: kitamori@icl.t.u-tokyo.ac.jp (T. Kitamori), viola.vogel@hest.ethz.ch (V. Vogel).
[1] Current address: Center for Computational and Theoretical Biology, University of Würzburg, Campus Hubland Nord 32, 97074 Würzburg, Germany.

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1. Introduction

Cells and extracellular matrix (ECM) in living tissues are highly organized over multiple length scales which is a prerequisite for proper organ function. To repair organs, tissue engineers have traditionally utilized mostly *top-down* approaches where cells were seeded into artificial [1,2] or bio-derived scaffolds [3,4] to provide them with a structural guidance to derive the desired tissue organization, and more recently explore the *bottom-up* assembly of cells to induce the formation of organoids [5–7] with various microengineered geometries such as spheroids [8], cell sheets [9], hydrogel blocks [10] and fibers [11], either under static conditions or in microfluidic devices [12–16]. Alternatively, direct fabrication of microtissues has been realized, for example via bioprinting approaches where cells encapsulated in matrix gels are spatially positioned on demand [17,18], for example to produce microtissues with vascular-mimetic structures [17]. A typical assumption underlying these approaches is that the spatial organization initially imposed by artificial environments or by the bioprinting process is basically preserved throughout the tissue remodeling phases, yet their morphologies can often not be maintained over longer time periods. From basic studies in development and morphogenesis it is evident that tissue growth and remodeling are highly dynamic processes driven by cell-generated forces, whereby mechanical signal transduction through cell-ECM and cell-cell contacts are integrated to give a global response [19–36]. Although these basic studies have shown the significance of the interplay between cell-generated forces and ECM remodeling in tissue morphogenesis, little quantitative insight exists how the cell generated forces acting on the geometrical constraints affect the morphogenesis of such multicellular systems.

To gain a good quantitative perspective of how large multicellular collectives interact with predefined scaffold geometries or confinements, and to learn how to best tweak tunable parameters on demand for tissue engineering applications, our goal here was to develop a quantitative understanding of the underpinning mechanisms. As an *in vitro* model system whose geometrical features are motivated by vascular engineering, we focused here on the mechanical interplay between contractile smooth muscle cells (SMCs) interacting with mesoscale curved scaffold geometries. Typical vascular tissues consist of several layers of different cell types embedded in ECM [37,38]. A monolayer of endothelial cells (ECs) line the internal surface of vessels, surrounded by several layers of smooth muscle cells (SMCs) whose major function is to regulate the vascular diameter and hence the blood pressure by contraction. While various microdevices have been used for culturing ECs to construct vascular models [14,39,40], or tissue grafts [11,41,42] on the μm-scale, there are few reports on SMC culture in microdevices [43,44]. Regarding the interaction between SMCs and microdevices, we previously reported the unexpected phenomenon that SMCs do not form a stable confluent monolayer on curved substrates of several 100 μm scale [45], even though they form tubular vascular walls *in vivo*. With the goal to develop a first phase diagram, we investigated here the causes that lead to this failure by probing how submillimeter curvature of the imposed geometry, together with cellular contractility and adhesive strength of ECM to substrate of SMC culture modulate this catastrophic tissue rupture.

2. Experimental

2.1. Microfabricated model systems

Semicircular microchannels were fabricated from glass substrates with well-defined radii of curvature. These glass substrates are too rigid to be deformed by contractile tissues for maintaining the defined curvature. The radii of curvature of the semichannels were in the range of several 100 μm, similar to vascular tissues that have a contractile layer of SMCs. Using this model system, human aortic SMCs (SMCs) were cultured in microchannels of different curvatures, while modulating cell contractility and the surface treatment of the channels. Finally, the cell sheets were fixed, immunostained wherever indicated, and imaged together with the surrounding ECM by confocal microscopy. Image processing was utilized to quantify the data.

2.2. Cell culture

Human aortic EC (Kurabo, Osaka, Japan) and human aortic SMC (Kurabo, Osaka, Japan) were maintained using similar cell culture protocols. Endothelial Growth Medium-2 Bullet Kit (EGM2) (Lonza, Basel, Switzerland) and Smooth Muscle Growth Medium-2 Bullet Kit (SmGM2) (Lonza, Basel, Switzerland) were used as cell culture medium for human aortic EC and SMC, respectively. These cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. 3 days after seeding, those cells had reached confluence and were washed with 1 mL of HEPES buffered saline solution and detached from the culturing surface by exposure to 1 mL of 0.05% trypsin for 3 min. The cell suspension was added to 5 mL of medium, followed by centrifugation at 1100 rpm for 5 min. Centrifuged cells were re-suspended in medium at defined concentrations for either subculture or each experiment described as follows. Cells between passages 6 and 10 were used in this study.

2.3. Collagen I coating of the glass surfaces

Prior to cell culture, collagen I was adsorbed onto substrates based on a previously published protocol [46] using poly-L-lysine (PLL) to stabilize protein adsorption via electrostatic interactions. Briefly, a glass substrate containing curved semichannels was cleaned by ethanol, acetone and distilled water followed by plasma treatment for 60 s (RF-Level: Hi, PDC-32G, Harrick Plasma, Ithaca, NY, USA). Then, the substrate was immersed in 0.001% PLL (Sigma-Aldrich, St. Louis, MO, USA) in PBS for at least one hour. After washing with distilled water, the substrate was immersed into 0.3 mg/mL rat tail collagen type I (BD Biosciences, San Jose, CA, USA) solution at 4 °C overnight to allow them physisorb onto the substrate. Next, the surface was neutralized by 0.1 N NaOH solution for 10 min. Then, the substrate was sterilized by 70% ethanol. After washing with PBS, the collagen-adsorbed substrate was kept in PBS at 4 °C until the experiment was performed. All substrates for cell culture were pretreated according to this protocol, unless otherwise noted.

Alternatively, collagen I was covalently crosslinked to the glass substrate. The substrate was cleaned by ethanol, acetone and water, followed by a plasma treatment as described above. The substrate was then sealed in a Teflon box with a small vial containing 200 μL of 3-(Trimethoxysilyl)propylmethacrylate (MPTMS, Sigma-Aldrich, St. Louis, MO, USA). Next, the box was baked at 80 °C for two hours. Then, the vial of MPTMS was removed and the substrate was baked at 80 °C again for one hour. After washing with distilled water, the substrate was covered by 1 mM sulfosuccinimidyl 6-(4′-azido-2′-nitrophyenylamino)hexanoate (SulfoSANPAH) (Thermo Fisher Scientific, Waltham, MA, USA) diluted in PBS (pH = 8.5) and exposed to UV (0.039 mW/cm^2) for 4.5 min. After replacing the Sulfo SANPAH solution, UV exposure was repeated once again. After washing with PBS, the substrate was immersed into 0.3 mg/mL collagen type I solution at 4 °C overnight. Finally, the substrate was neutralized, sterilized and preserved as described above.