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Shape-changing hydrogel surfaces trigger rapid release of patterned tissue modules

Olukemi O. Akintewe [a,1], Samuel J. DuPont [a,1], Kranthi Kumar Elineni [b], Michael C. Cross [c], Ryan G. Toomey [a,*], Nathan D. Gallant [b,*]

[a] Department of Chemical and Biomedical Engineering, University of South Florida, Tampa, FL 33620, USA
[b] Department of Mechanical Engineering, University of South Florida, Tampa, FL 33620, USA
[c] Department of Physics, University of South Florida, Tampa, FL 33620, USA

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ABSTRACT

The formation and assembly of diverse tissue building blocks is considered a promising bottom-up approach for the construction of complex three-dimensional tissues. Patterned shape-changing materials were investigated as an innovative method to form and harvest free-standing tissue modules with preserved spatial organization and cell–cell connections. Arrays of micro-scale surface-attached hydrogels made of a thermoresponsive polymer were used as cell culture supports to fabricate tissue modules of defined geometric shape. Upon stimulation, these hydrogels swelled anisotropically, resulting in significant expansion of the culture surface and subsequent expulsion of the intact tissue modules. By varying the network crosslink density, the surface strain was modulated and a strain threshold for tissue module release was identified. This mechanical mechanism for rapid tissue module harvest was found to require inter- and intra-cellular tension. These results suggest that the cell–matrix adhesions are disrupted by the incompatibility of surface expansion with tissue module cohesion and stiffness, thus providing a novel method of forming and harvesting tissue building blocks by a mechanism independent of the thermal stimulus that induces the biomaterial shape change.

1. Introduction

An emerging field known as modular tissue engineering employs a bottom-up approach that utilizes tissue building blocks as modular units to construct biological tissues with specific architectural features. As an example, modular tissues can be created using cell sheets and assembled through stacking of layers to enhance formation of complex microstructural functional units such as microvascular networks, thereby augmenting integration and facilitating recovery [1–4]. This bottom-up approach aims to develop biomimetic engineered tissues that effectively recapitulates native tissues [5,6].

Recently, responsive materials have been used as a platform for generating tissue modules due to their convenient manipulation of cell–surface interactions on culture supports induced by an environmental cue such as temperature, pH, ionic strength, solvent, salt, surfactant, electric or magnetic field [7–9]. In particular, grafted films of poly(*N*-isopropylacrylamide) (pNIPAAm), a thermally responsive polymer, have attracted a great deal of attention for use as cell culture platforms for cell sheets because this polymer undergoes a sharp volume-phase transition due to thermally mediated changes in the hydrophilic and hydrophobic interactions around its lower critical solution temperature (LCST) of 32 °C [9–11]. In comparison to conventional enzymatic release of cells with trypsin and ethylene diamine tetraacetic acid (EDTA), the hydration of grafted pNIPAAm provides a slow, but non-destructive, approach for tissue harvest so that intact monolayers of cells can be formed [12–14].

Rapid production, versatility and scalability are important factors for in vitro construction of tissues and organs. Herein, a shape-changing hydrogel cell culture platform that demonstrates these properties is described. This platform is based on patterned arrays of microscale protrusions (or microbeams) of cross-linked pNIPAAm [15]. Lateral swelling of the microbeams occurs upon thermal activation, expanding and distorting the surface of microbeams. Although several studies have shown release of tissue modules from two-dimensional (2-D) grafted thin pNIPAAm films, the release of tissues from shape-changing three-dimensional (3-D)

* Corresponding authors. Tel.: +1 813 974 9164; fax: +1 813 974 3651 (R.G. Toomey). Tel.: +1 813 974 5840; fax: +1 813 974 3539 (N.D. Gallant).
E-mail addresses: toomey@usf.edu (R.G. Toomey), ngallant@usf.edu (N.D. Gallant).
[1] These authors contributed equally to this work.

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pNIPAAm hydrogels is unexplored. While thin films can be used to generate layered tissues from stacked cell sheets [16–21], such substrates provide a limited range in tissue geometries and slower cell release times.

Hence, developing an alternative method that enables faster cell detachment and allows for the fabrication of geometrical patterns of the hydrogel could facilitate the organization and rapid release of tissue modules. We hypothesized that rapid tissue module release occurs on shape-changing surfaces via mechanical mechanisms that are unique to patterned shape-changing cell culture supports. Furthermore, it is speculated that this simple method can be used to form the diverse building blocks required for building robust multilayered tissues that are complex in architecture and may, for example, be used to enhance vascularization in thicker tissue grafts for organ repair or replacement.

The objective of this work is to demonstrate the feasibility of fabricating and harvesting tissue modules via a strain-mediated process and to study the mechanism that regulates rapid release from shape-changing biomaterials. We fabricated 3-D pNIPAAm microbeams having various swelling ratios to investigate the effect of swelling-induced strain on tissue module release. We also examined the effect of cell density on cell detachment; and to understand the mechanism of tissue release from these shape-changing surfaces, we investigated the roles of metabolic activity and cytoskeletal contractility by probing the adhesive interface. Finally, we evaluated the viability of cells within the released tissue modules. The results described here provide the first steps toward fabricating and harvesting living tissue building blocks with intact organization and cell–cell connections that may ultimately be used to build complex 3-D tissues via the assembly of diverse tissue modules.

2. Materials and methods

2.1. Materials

NIH/3T3 mouse embryonic fibroblast cells were purchased from the American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), newborn calf serum (NCS), 0.25% trypsin EDTA (1×), calcein AM, ethidium homodimer, penicillin and streptomycin were all obtained from Life Technologies. *N*-isopropylacrylamide (NIPAAm), 2-dimethoxy-2-phenylacetophenone (DMPA), N,N′-methylenebisacrylamide (MBAm), 3-(trichlorosilyl) propyl methacrylate (TPM), sodium azide (NaN_3), Rho-associated protein kinase (ROCK) inhibitor Y-27632, acetone and carbon tetrachloride were all purchased from Sigma-Aldrich. Methacryloxyethyl thiocarbonyl rhodamine B (polyfluor® 570) was purchased from Polysciences. 3,3′-dithiobissulfosuccinimidylpropionate (DTSSP) was obtained from Thermo Scientific. Silicone elastomer (PDMS) kits (Sylgard®184) were obtained from Dow Corning.

2.2. Preparation of dynamic pNIPAAm hydrogel arrays

Patterns of crosslinked pNIPAAm hydrogel (50–100 μm width × 25 μm height × 5 mm length) microbeams were fabricated as described previously [15,22] on 22 mm × 22 mm glass coverslips (#1.5) using PDMS molds by employing the micromolding in capillaries (MIMIC) technique (Fig. 1a). Briefly, the glass cover slip was surface modified with TPM in carbon tetrachloride. 1–4% MBAm crosslinker (5 mg ml^{-1}), 10% DMPA photo initiator (20 mg ml^{-1}) and 1% polyfluor® 570 (0.5 mg ml^{-1}) were added to a 250 mg ml^{-1} solution of NIPAAm in acetone. The resulting solution was introduced to the PDMS molds and polymerized with ultraviolet light (350 nm) for 4 min. The fabricated surfaces were sequentially rinsed with acetone, ethanol and water to remove unpolymerized monomer.

2.3. Cell culture

NIH/3T3 mouse embryonic fibroblast cells were cultured in 10% NCS growth medium containing 1% antibiotics (10,000 units ml^{-1} penicillin and 10,000 microgram ml^{-1} streptomycin stock solution) at 37 °C in a humidified atmosphere of 5% CO_2. To prepare tissue modules, trypsinized fibroblasts were seeded onto the fabricated responsive hydrogel arrays at a density of 500–750 cells mm^{-2} and cultured at 37 °C until confluence (24–48 h). Studies of release from low cell density (100 cells mm^{-2}) were cultured for 24 h.

2.4. Release of tissue modules from shape-changing hydrogels

Rapid release of tissue modules was induced by thermally initiated swelling of the hydrogel beams. 2 ml of cold PBS (4–10 °C) was introduced 1 ml at a time into the seeded dish containing 2.5 ml of 37 °C medium resulting in cooling to ~27 °C. Cell release was monitored via time-lapse image acquisition on a microscope for at least 70 s.

2.5. Viability of cells released

A cell viability assay was performed on released cells by using a LIVE/DEAD kit (containing calcein AM and ethidium homodimer) following the commercially recommended protocol. Once the cells reached confluence on the hydrogel, the tissue modules were released with fresh cold medium and plated onto a new tissue culture polystyrene (TCPS) dish. Following incubation for 24 or 48 h at 37 °C, the cells were stained with 300 μl of 20 μM calcein AM and 40 μM ethidium homodimer-1 solution. After 30 min, the dish was rinsed twice with warm PBS and replenished with fresh medium prior to imaging.

2.6. Mechanism of tissue module detachment studies

The mode of cell release from hydrogel surfaces was examined by separately treating seeded samples with agents that modulate metabolism, contractility or adhesion. 24 h after attachment to microbeams, cells were exposed to sodium azide, a compound known to block ATP production via the inhibition of cytochrome C oxidase in mitochondria [13,23], Y-27632, a selective inhibitor of Rho-associated protein kinases [24,25], or DTSSP, a homobifunctional crosslinker that fixes only integrins bound to the extracellular matrix [26,27]. Briefly, samples were exposed to 2 mM sodium azide for 60 min or 50 μM Y-27632 or 2 mM DTSSP for 30 min prior to initiating tissue module release. To investigate the effect of surface strain on attached cells, the concentration of the network crosslinker (MBAm) in the prepolymer solution was varied from 1 to 4% before MIMIC processing. The one-dimensional width-wise strain in each microbeam was calculated from phase contrast micrographs as follows:

$$\varepsilon = \frac{\Delta w}{w_{collapsed}} = \frac{w_{swollen} - w_{collapsed}}{w_{collapsed}} \quad (1)$$

where ε is the Cauchy strain, $w_{collapsed}$ is the width of the hydrogel beam in the collapsed state and $w_{swollen}$ is the width of the hydrogel beam in the swollen state. Cell detachment was calculated as the per cent of cells released from the microbeams within 3 min after thermal stimulation.