

Patterned Tissue Precursors Microcontact Printed from Shape-Changing Hydrogel Stamps

Olukemi O. Akintewe, Ryan G. Toomey, Nathan D. Gallant
University of South Florida, Tampa, Florida 33620

Statement of Purpose: Reconstructions of damaged tissues or organs using a “bottom up” approach may enable the formation of functional tissues with complex structural features such as vascular network, thereby enhancing tissue regeneration capacity [1]. Recently, we demonstrated that intact tissue modules are non-destructively released from a shape-changing hydrogel construct when a lateral strain of at least 25% is applied [2]. Herein, we show that the tissue modules released can be printed via microcontact printing on multiple surfaces while still preserving cell viability and organization.

Methods: Patterned poly-N-isopropylacrylamide (pNIPAAm) shape-changing supports were fabricated from a PDMS (Sylgard®184, Dow Corning) master mold using a micromolding process in which the prepolymer was irradiated with UV light (Figure 1a) [3]. NIH-3T3 fibroblasts were cultured on shape-changing constructs (Figure 1b). Tissue module detachment was initiated by a mechanical strain of at least 25% induced via reducing the medium temperature below the hydrogel’s transition temperature of 32°C. Glass target surfaces were coated with poly-l-lysine prior to contact printing to promote cell adhesion. NIH ImageJ software was employed for cell image analyses. Tissue precursor viability was examined by staining cells with LIVE/DEAD assay kit (Life Technologies). Automated microscopy (Nikon NIS Elements) was used to visualize and analyze patterns of fluorescently stained cells.

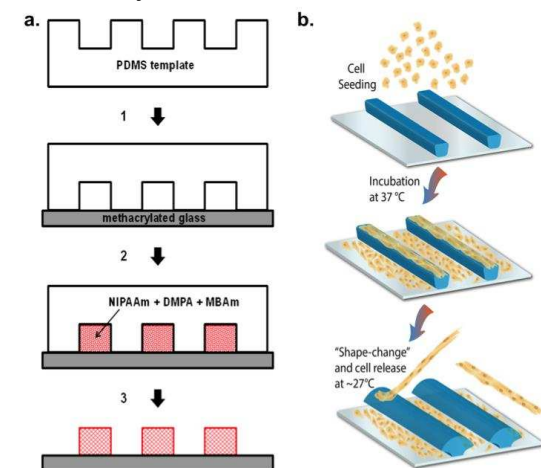


Figure 1: “Shape-changing” hydrogel microbeams for tissue module culture and harvest [2]. (a) Schematic of microbeam fabrication steps. (1) A PDMS template is placed on a methacrylated coverslip and (2) prepolymer solution is flowed into the recesses. (3) The hydrogel networks are polymerized with 350 nm UV light before the PDMS template is removed. (b) Schematic of formation and release of tissue modules from “shape-changing” hydrogel microbeams. (1) Cells adhered to and conformed to the shape of the microbeams, organizing into geometric tissue modules. (2) Tissue modules released from microbeams upon expansion beyond a critical lateral strain.

Results: Variation in the crosslink density of the hydrogel varies its extent of strain; fewer crosslinks results in greater surface expansion. Cells seeded on a 1% cross linker polymerized hydrogel forms an aligned orientation on the array of hydrogel protrusions (Figure 2a). The pressure applied during conformal microcontact printing regulates cell viability and transfer efficiency. Tissue modules printed on a target surface with loads $\ll$ 15 psi were found to retain cell viability during transfer ($\sim$ 90%) (Figure 2b). These shape-changing constructs also support cell printing onto a second cell layer with preserved alignment from the transferred tissue precursors (Figure 3). Tissue printing occurs by contacting the stamp and target surfaces for 15 min followed by reducing the temperature to $\sim$ 27°C for 5 min. Cells stained with Live/DEAD assay kit showed no cell death for 48 hrs after printing. The formation of intact tissue modules after printing suggests that this tissue microcontact printing technique maintains spatial organization directed through physical guidance cues and causes minimal cell damage.

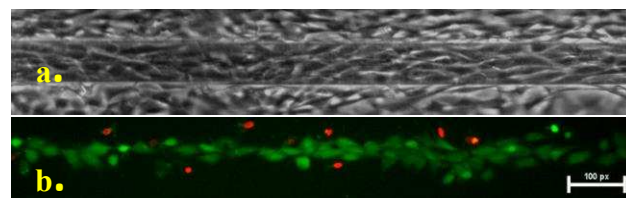


Figure 2: Contact printing of micro-tissues from isolated shape-changing hydrogel. (a) Cells adhere and align atop arrays of shape changing hydrogel microbeams. (b) Tissue modules maintain organization and viability (green=LIVE) after printing on poly-l-lysine coated glass surfaces. Scale bar = 100µm.

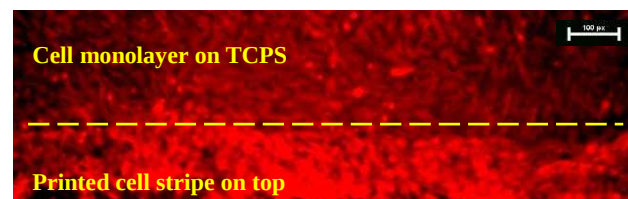


Figure 3: Patterned tissue precursors printed on a confluent layer of fibroblasts. Cells were stained with cell tracker red, CMTMR. Scale bar = 100µm.

Conclusions: This simple microcontact printing method can be used to form the diverse building blocks required for building robust multilayered tissues that are complex in architecture. As a result, thicker tissue grafts formed in vitro may be possible for organ repair or replacement by incorporating support structures such as vascularization. Future work includes sequential printing of three-dimensional tissues of multiple cell types.

References:

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