Micro-contact Printing of Viable Tissues via Geometrically Patterned Shape-Shifting Supports

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Statement of Purpose: Scaffold based tissue reconstruction inherently limits regenerative capacity due to inflammatory response and inadequate vascularization [1]. In contrast, scaffold free methods promise formation of functional tissues with both an intact basal membrane and vascular network. Herein, we present a new method of μ-contact printing organized tissue precursors without a scaffold. This method is based on a shape-shifting poly-*N*-isopropylacrylamide (pNIPAAm) construct, (Figure 1) which supports cell adhesion and directs organization. These constructs are used as stamps to print tissues onto a target surface. Spontaneous release of aligned viable tissues occurs upon thermal shift around the volume phase transition temperature of the polymer.

Methods: Patterned pNIPAAm shape-shifting supports were fabricated from a PDMS (Sylgard®184, Dow Corning) master mold using a micromolding in capillaries (MIMIC) process while the hydrogel was irradiated with UV light [2]. NIH-3T3 fibroblast cells were cultured on shape-shifting substrates. Reduction in cell culture temperature below its liquid critical solution temperature (LCST) facilitates cell detachment. Glass target surfaces were coated with fibronectin prior to contact printing of aligned tissues. NIH Image J software was employed for cell alignment and orientation analyses. Cell viability was assessed by staining cells with Calcein AM (Life Technologies). Automated microscopy (Nikon NIS Elements) was used to visualize and analyze patterns of fluorescently stained cells.

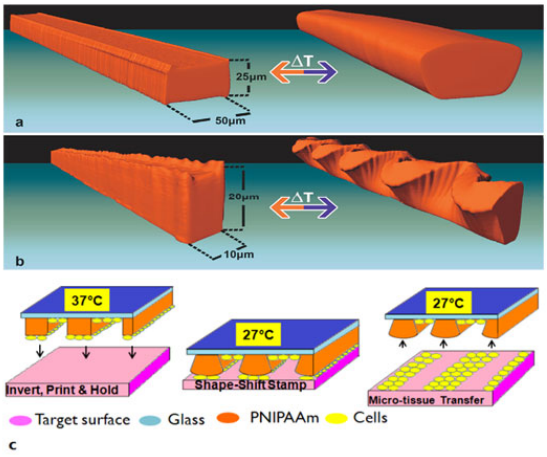

Figure 1: Schematic diagrams of shape-shifting platform and contact printing technique. (a) Arrays with low aspect ratio (e.g. 0.5) undergo lateral swelling upon thermal shift; while (b) high aspect ratios (e.g. 2) result in buckled structures; (c) Contact printing technique: cells are cultured on shape-shifting stamp; the stamp is inverted and placed in contact with a target surface to facilitate cell adhesion; a thermal shift induces swelling which causes rapid detachment of the μ-tissues from the stamp, transferring the pattern to the target.

Results: Surface confinement of pNIPAAm hydrogels restricts isotropic swelling. The resulting 3D swelling depends on the aspect ratio of the structure [3]. Confined pNIPAAm structures with equivalent crosslink densities swell into a bulbous shape if the aspect ratio is < 0.93 or buckles if > 0.93 (Figure 1). These shape-shifting structures support cell culture and are used as stamps for μ-contact printing of aligned tissues (Figure 1c). A combination of thermal shift near 27°C and low pressure (~1mN/mm^2) induces confluent tissue transfer within 5 minutes (Figure 2). Cells were stained with Calcein AM and the dye did not leak out from the cells for 24 hrs after tissue printing. Angular histograms, indicating cell orientation, shows well aligned μ-tissue formation on closely-spaced shape-shifting supports compared to cells seeded on plain glass (Figure 3). The formation of intact tissues suggests that the geometric patterning of pNIPAAm directs spatial organization through physical guidance cues.

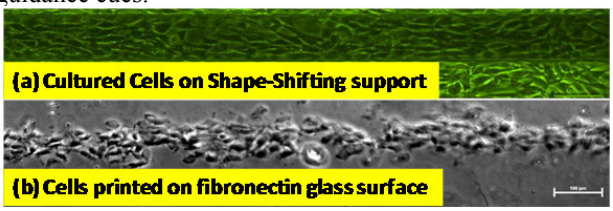

Figure 2: Contact printing of micro-tissues from isolated shape-shifting supports (a). Cells cultured on pNIPAAm support show alignment (b). Complete tissue transfer is achieved on fibronectin surface. Scale bar = 100μm.

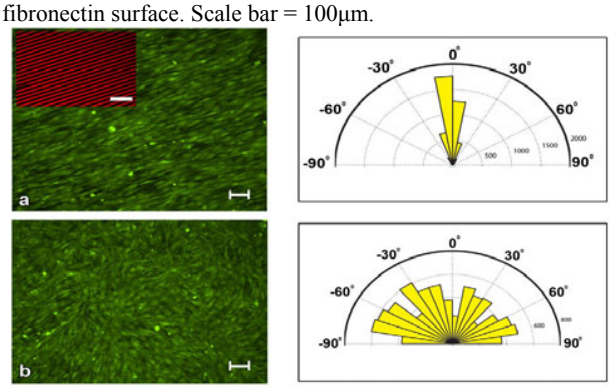

Figure 3: Micro-tissue formation maintained alignment on shape-shifting platform. (a) Closely-spaced shape-shifting arrays with low aspect ratio of 0.1 (b) Cells cultured on glass surface. Scale bar = 100μm.

Conclusions: Rapid fabrication of spatially defined μ-tissues and their transfer to a target while still preserving local and global organization is achievable via a μ-contact printing technique. Future work includes sequential printing of multilayered 3D tissues for reconstruction of highly vascularized tissues such as cardiac muscle.

References:

1. Curtis, M., J Cardiovasc Nurs. 2009; 24:87-92
2. Xia Y., Annu Rev Mater Sci. 1998; 28:153-84.
3. DuPont Jr, S. Soft Matter, 2010; 6:3876-3882.