Three Dimensional poly-N-isopropylacrylamide Constructs for Culture and Harvesting of Muscle Fibers

Samuel DuPont[1], Kranthi Kumar Elineni[2], Nathan D. Gallant[2], and Ryan Toomey[1].
[1] University of South Florida Department of Chemical and Biomedical Engineering
[2] University of South Florida Department of Mechanical Engineering

Statement of Purpose: Confinement of swellable polymer gels on a rigid substrate leads to the formation of surface instabilities in the swollen gel [1]. The form and scale of the instability can be modulated by the geometry and chemistry of the patterned polymer surface. When gel structures are composed of an environmentally sensitive polymer, such as poly-N-isopropylacrylamide (pNIPAAm), slight changes in an environmental variable such as temperature, pH, and ionic strength can have an extensive effect on the solubility of the polymer gel in an aqueous environment [2].The tunable properties of these materials provide a unique platform for cell culture and non-enzymatic harvest of multicellular structures [3]. Preliminary studies on the behavior of fibroblasts on 3D constructs fabricated out of pNIPAAm forms the basis for a more intense investigation on fabricating skeletal muscle constructs from myoblasts. Formation of myotubes, an important structure in skeletal muscle, requires the differentiation of myoblasts. Alignment of myoblasts during differentiation results in the formation of long myotubes which have promising applications in regenerative medicine. Previous studies have shown that alignment of myoblasts on a culture surface, by means of topographical or chemical cues, leads to the formation of continuous, aligned myotubes [4]. Our objective is to develop a novel platform for the growth and harvest of engineered skeletal muscle assemblies using pNIPAAm structures.

Methods: Rectangular surface-extrusions (RSE) were fabricated atop glass slides with a covalent binding layer using standard soft lithography techniques. The nature of confinement induced swelling instabilities was observed and measured by confocal microscopy (Figure 1).

Figure 1: pNIPAAm structures in the collapsed state (A) and the swollen state (B) as imaged by confocal micrscopy.

To test these substrates for cell culture purposes, NIH3T3 fibroblasts were seeded on these substrates at a cell density of 500 cells/mm^2. Cells in complete growth medium were incubated for 16 hr and then imaged for cell attachment before and after swelling of the polymer using phase contrast microscopy. To determine the viability of myoblasts (C2C12) on pNIPAAm surfaces for long culture periods, cells were seeded at the same density on a pNIPAAm film and formation of myotubes was observed by phase contrast microscopy.

Results: At steady state adhesion, the cells were completely spread on top of the pNIPAAm structures. Observed under phase contrast microscopy, the cells were mostly aligned along the longitudinal axis of the unswollen RSEs when the temperature was above the lower critical solution temperature (LCST) (>32°C). As the temperature decreased to below LCST, the structures began to swell and buckle. The aligned cells, which were initially attached to the pNIPAAm surface and to adjacent cells, took the shape of the RSEs and soon detached from the surface (Figure 2). Contrary to expectations, the cell-cell attachment was intact and the 'cell fiber' was detached from the substrate in all locations except the inflection points in the buckled gel (Figure 3). This architecture could be useful for aligning myoblasts to facilitate the formation of homogenous myotubes which can then be harvested by switching the ambient temperature.

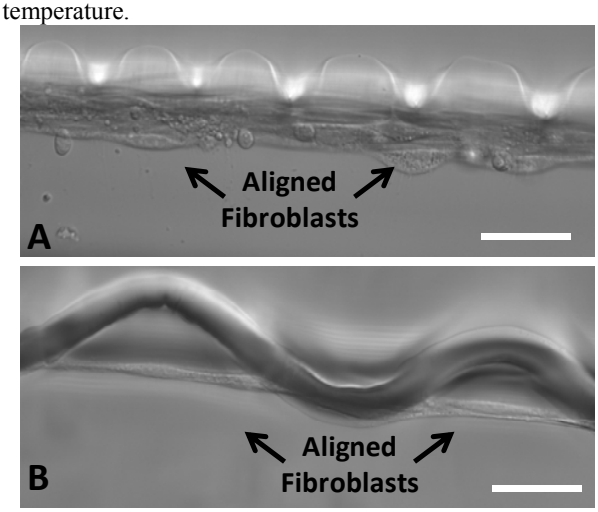

Figure 2: NIH3T3 fibroblasts aligned atop swollen pNIPAAm constructs of varied widths. (bar = 50μm)

Conclusions: This work provides the basis for using 3D structures fabricated out of pNIPAAm for cell culture purposes. It can be deduced from these results that cells sense the topography of this polymer and align themselves along the longitudinal axis due to contact guidance. Moreover, these multicellular structures can be released from the surface by osmotic swelling-induced buckling for higher order assembly processes. Current work is focused on engineering a variety of dimensions and spacing of these RSEs to enable alignment and differentiation of myoblasts into myotubes with controlled architectures. Three-dimensional structures fabricated out of pNIPAAm provide a promising future in engineering scaffolds for culturing and harvesting aligned, cellular monolayers, which can be organized into 3D muscle constructs.

References:

[1] DuPont SJ. Soft Matter. 2010;6:3876-3882.
[2] Gil ES. Prog Polym Sci. 2004;29:1173-1222.
[3] Cooperstein MA. Langmuir. 2010;26:7695-707.
[4] Zhao Y. Biotechnol Bioeng. 2009;102:624-31.