Degradable Elastin-Based Biomaterials as Scaffolds for Multilayered, Aligned Myoblasts

Debanti Sengupta[1], Sarah C. Heilshorn[2]
[1]Department of Chemistry, [2]Department of Materials Science and Engineering, Stanford University, CA

Statement of Purpose: Our aim is to produce transplantable, multi-layered sheets of aligned myotubes that are capable of therapeutic force generation. Human diseases such as cardiac infarct, muscular dystrophy, and cancer as well as physical trauma can result in the loss of muscle tissue [1]. One potential therapeutic strategy is to tissue engineer sheets of aligned, mature muscle fibers consisting of multi-nucleated myotubes that facilitate synchronized muscular contraction as found *in vivo*. We present a new strategy to create 3D multilayered sheets of aligned cells using "on-demand" biomaterial degradation. To enable this strategy, we utilize a family of protein-based biomaterials comprised entirely of amino acids that are biocompatible and cell-adhesive and undergo tunable enzyme-mediated degradation by urokinase plasminogen activator (uPA) [2]. To generate free-standing, transplantable sheets of aligned myotube fibers, myoblasts are seeded onto a cell-adhesive, elastin-like biomaterial with a linear surface topography with a sacrificial, degradable layer underneath (Fig 1A). Upon myoblast alignment with the surface pattern and fusion into multi-nucleated myotube fibers, the biomaterial-cell sheet construct is released by uPA-induced degradation of the sacrificial protein sub-layer.

Methods: Protein biomaterials are synthesized using recombinant DNA technology and plasmid expression in *Escherichia coli*. Structural integrity is ensured by the inclusion of a peptide sequence that mimics elastin, a protein found in mammalian connective tissue. A peptide derived from the RGD sequence of fibronectin is included to induce cell adhesion while several uPA-degradable peptide sequences are included to induce proteolytic degradation at tunable rates [2]. Elastin-like proteins are purified by thermal cycling and characterized by gel electrophoresis and amino acid analysis. Glass coverslips are aminated using 3-aminotriethoxysilane. A thin film of the uPA-degradable, elastin-like biomaterial is covalently crosslinked to the aminated glass coverslip using THPP. On top of this thin film, a second layer of uPA-inert, elastin-like biomaterial is patterned using a soft lithographic PDMS mold. The surface topography includes a series of channels (10 μm wide by 5 μm deep). C2C12 myoblasts are seeded onto the constructs at a density of 25 cells/μm^2 and cultured in DMEM with 10% fetal bovine serum for 4 days. To induce myoblast fusion into myotubes, differentiation medium (DMEM with 2% horse serum) is added for 4 days. Cultures are characterized using time-lapse phase contrast microscopy and immunocytochemistry followed by image analysis to determine degree of alignment, cell area, and cell ellipticity. The secretion of uPA from C2C12 myoblasts and myotubes is quantified using ELISA. Degradation kinetics of the biomaterial is monitored by fluorimetry to detect fluorophores released from the sacrificial sub-layer.

Results: C2C12 myoblasts were found to align onto topologically patterned elastin-like substrates after 24 hours. While substantial cell alignment was observed on substrates lacking the RGD cell-adhesive domain, increasing the RGD domain density resulted in greater pattern alignment (Fig. 1B). These cells maintain their myoblast phenotype as evidenced by positive smooth muscle myosin heavy chain staining (Fig. 1C) and the ability to fuse into multi-nucleated fibers. As myoblast fusion occurred, the myotubes retained their alignment with the surface pattern (Fig. 1D). Covalent attachment of the biomaterial to the glass coverslip was required to withstand the contractile forces exerted by the myotubes on the biomaterial and to prevent premature release of the cell-biomaterial construct. Although uPA secretion was found to be significantly upregulated following myotube fusion, the addition of exogenous uPA was required to induce bulk degradation of the sacrificial sub-layer. This strategy enables precise control of cell-biomaterial release at the desired state of cell maturation. The released cell-biomaterial constructs can be successfully transferred to another culture dish and retain their aligned phenotype.

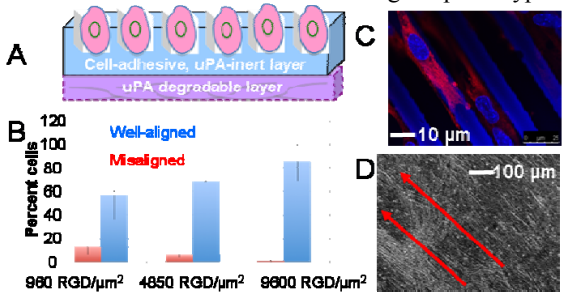

Figure 1: ***A.*** *Schematic of cell-biomaterial construct, B. Percentage of well-aligned and misaligned cells on patterned substrates of varying RGD density, C. C2C12 myoblasts (red-smooth muscle myosin heavy chain) on a patterned biomaterial (blue), D. Patterned C2C12 multi-nucleated myotubes (arrow shows direction of alignment).*

Conclusions: We have demonstrated that surface topography in conjunction with specific, localized degradation induced on demand is a viable tissue engineering strategy to organize cells into transplantable aligned sheets. Current efforts are underway to align and stack multiple cell-sheets, resulting in the formation of an *ex vivo* multilayer construct. This strategy is also being extended to create layered co-culture constructs.

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References:

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