Laser Micromachining of Differentially-Adherent Substrates to Direct Cellular Growth for Functional Tissue Engineering

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Statement of Purpose: A bottom-up approach to tissue engineering can provide a scaffold-free method to engineer and build tissue with fiber-level architecture and functionality. By directing cellular growth, and guiding the production of their native extracellular scaffold, we aim to exploit the natural behavior of musculoskeletal derived cells to develop a bottom-up engineering approach for customized tissue replacements. We have adapted a laser micromachining method to create differentially-adherent growth channels that provide the geometric cues and constraints necessary to direct cell proliferation and encourage 3-D fiber formation. We can utilize the CAD/CAM nature of this approach to create fibers with specific geometry to mimic the structure (e.g., biomimetic) or function (e.g., idealized for performance) of the native tissue.

Methods: A pulsed UV excimer laser (ArF, 193 nm, TeoSys, Crofton MD) was used to micromachine 3-D features in 2% agarose gel (prepared by mixing 1 g of electrophoresis-grade agarose powder with 50 ml of Dulbecco's Modified Eagle's Medium) using an ablation-based technique; subtractive matrix assisted pulsed laser evaporation (MAPLE). When coupled with CAD/CAM capabilities and a motorized stage with 1-μm resolution, patterns are created rapidly and with high precision. Through adjusting the intra-cavity variable aperture and irises, the near Gaussian beam can be varied from approximately 2 μm up to 400 μm. Customized patterns, or growth channels, can be rapidly fabricated for various biomaterial studies and cell types (Fig. 2).

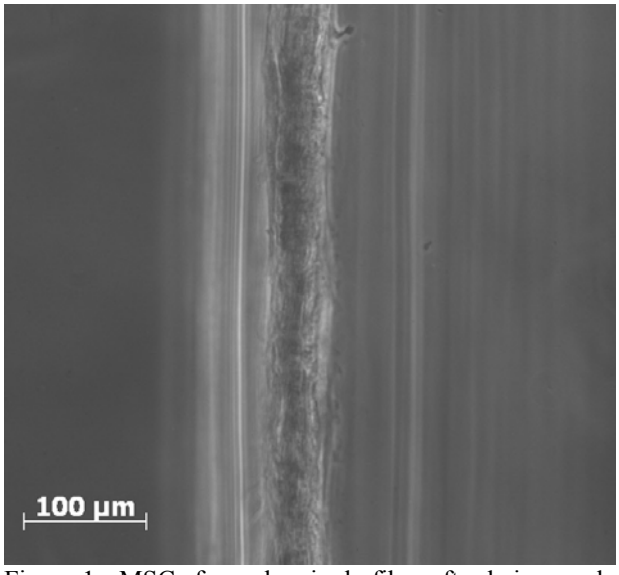

Figure 1. MSCs formed a single fiber after being seeded into a laser micromachined channel. Similar fibers have also been created using fibroblasts and myoblasts

Selective cell adhesion is achieved by adding extracellular matrix protein suspensions (e.g., fibronectin, laminin) into the micromachined channels, and drying them in a laminar flow hood for one hour. Human dermal fibroblasts, mouse C2C12 myoblasts, and adult bone-marrow derived mesenchymal stem cells (MSCs), were grown in standard cell culture media and conditions, seeded into the channels, and their behavior was observed microscopically.

Results: Within 24 hours, all cells types (fibroblast, myoblast, MSC) began to align and form 3-D fibers that spanned the entire length of a 1-cm long channel with a 100-μm width (Fig. 1).

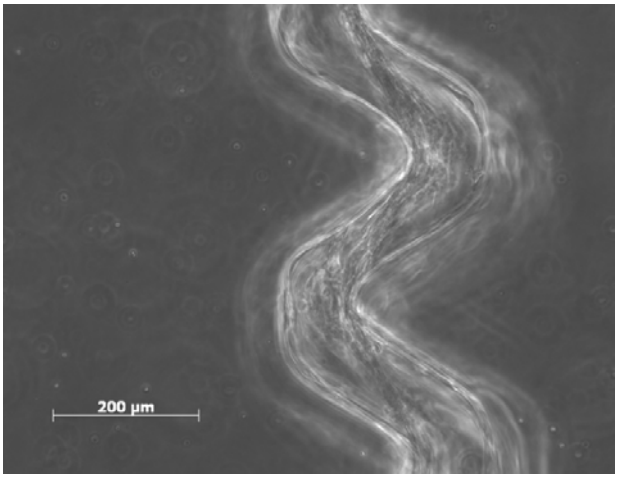

Figure 2. Fiber grown with a specific crimp pattern, using a CAD-designed growth channel precisely laser micromachined into an agarose gel, coated in fibronectin, and seeded with fibroblasts.

Conclusions: Laser micromachining proves to be a successful method to create growth channels with 3-D features that provide the necessary geometric cues and constraints to direct fiber formation from a variety of cells, including fibroblasts, myoblasts, and MSCs. This scaffold-free approach represents a departure from traditional tissue engineering (TE), in that we harness the cells' natural abilities to differentiate, organize, align, create extracellular matrix, and form fibers. Further, we can utilize the CAD/CAM nature of this bottom-up approach to create fibers with specific geometry (e.g., length, CSA, taper, crimp) to mimic the structure or function of the native tissue. To further customize mechanical properties and cellular alignment, we are currently investigating providing mechanical, electrical, and chemical stimulation to the fiber-based constructs during development.