Computer Aided Design and Manufacturing of Soft, Three-Dimensional, Multi-Layer, Biological Constructs Via Laser Printing Onto Laser Machined Composite Biopapers

Russell Kirk Pirlo,[1] Peter Wu,[2] and Brad Ringeisen[1]; 1. U.S Naval Research Laboratory, Chemistry Division, 4555 Overlook Ave. SW Washington, DC 20375 and 2. University of Southern Oregon, Department of Physics and Engineering, 1250 Siskiyou Blvd Ashland, OR 97520

Abstract

Engineering a tissue construct which mimics a native tissue's form and function requires mimicry of the material, chemical, and morphological properties of the target tissue. However, depending on the target tissue, matching these very properties make fabrication of a composite structure challenging. Here we discuss a collection of techniques which allow us to create three-dimensional soft tissue constructs comprised of multiple layers of hydrogels with high inter-layer registration. These techniques include a custom bioreactor platform integrated with NRL's Biological Laser Printing (BioLP™) stage and CAM application which allows for quick and reproducible printing, culturing and stacking of individual biopaers. The Bioreactors are designed for culturing and mounting laser machined biopapers, and the laser machined biopaper frames which allow for handling and high resolution (less than 100 micron) registration of otherwise difficult to handle 500Pa – 5kPa hydrogels selected for their mechanical and chemical resemblance to in vivo soft tissues. We demonstrate printing of immortalized endothelial cells (EC) onto biopapers and their subsequent self-assembly into capillary-like structures or tubulogenesis. Our composite stainless steel framed - collagen membrane supported – hydrogel biopapers enable asynchronous printing, cell development and delayed stacking of the soft substrates required for EC tubulogenesis.