Biofabrication of a Vascular Network: Applying AC Electrospinning to 3D Printing for Tissue Engineering

Victoria Gilchrist, Ryan Bailey, Scott Baskerville, Ian McClure, and Michael Weng Kwan
Florida Institute of Technology

Introduction: Additive manufacturing, or 3D printing, has already made an impact on commercial manufacturing, and now the biomedical community has started taking advantage of this remarkably useful technology. Already in use for medical devices and prosthetics, 3D printing will play a key role in the next major breakthrough in the healthcare industry through biofabrication, or the printing biological tissue and organ replacements. These replacements will be made of a patient's own cells, be printed on-demand, and will integrate with the body with limited adverse effects. Currently the need for replacement organs far outnumbers the available donors. Additionally, the transplants have limited success due to biocompatibility issues, cost, and the risk of rejection. In order to generate a replacement organ with a patient's own cells, the printed tissue must incorporate a working vascular network in order to supply nutrients to the organ so that it is viable and can integrate with the body. Current methods of 3D printing have not accomplished this feat. After all, printing a structure with the dimensions of a vascular network is difficult, let alone one that mimics the mechanical properties. Which is why printing the structure may not be the answer. By introducing alternating current (AC) electrospinning, the cells in the printed tissue can form the vascular network without the need to print microstructures.

Materials and Methods: A tissue incubation enclosure system was designed and built to house a novel 3D printing system designed to produce a tissue sample with its own vascular network. Advanced 3D printing software uses a 3D model of the desired sample's various tissue layers, vascular components, and dimensions, and provides a detailed print plan. A custom-built rack-and-pinion system, powered by programmable high torque stepper motors, extrudes bioink from three syringe cartridges. The bioinks - sodium alginate hydrogel embedded with human umbilical endothelial cells (HUVECs) at less than 30,000 cells/mL, porcine gelatin, and polylactic acid dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol – are directed onto the printing platform via a tri-tip luer nozzle designed specifically for this project. A layer of calcium chloride solution on the printing surface allows crosslinking of the alginate and helps maintain the shape of the printed sample. The gelatin, which is solid at room temperature, liquefies at 37 degrees Celsius and leaves open channels in the printed tissue. AC electrospinning produces a mesh of PLA that mimics the natural extracellular matrix and stimulates the endothelial cells to make arterial walls. The sample is printed directly inside the enclosure, which utilizes environmental sensors and controls, providing the optimal conditions for mammalian cell survival of 37 degrees Celsius, 5% CO_2, and 90% relative humidity. After incubating for a period of 36 hours, the sample is treated with DAPI (4',6-diamidino-2-phenylindole), a fluorescent stain that binds to DNA in living cells, and observed under a fluorescent microscope.

Results and Discussion: The extruded cell-seeded hydrogel is integrated with an electrospun fiber network that provides the desired conditions for endothelial cells to form a vascular network. After 36 hours of incubation, the cells migrated through the hydrogel and aggregated around the electrospun fibers. These results confirmed that the cells are attracted to the fibers and that this biodegradable scaffold leaves a tube of cells that may be capable of perfusion.

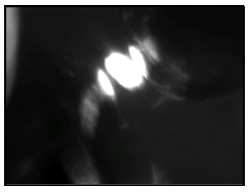

Figure 1. Fluorescent microscopy image of the printed sample after 36 hours of incubation shows the HUVECs have migrated from within the hydrogel to aggregate around the electrospun fibers.

Conclusions: The future of tissue engineering is printing on-demand replacements for damaged tissue and organs. To do this, the printed tissue must incorporate its own vascular network, a feat that may be accomplished with the use of AC electrospinning. By providing an optimum environment for cells to aggregate, the printed tissue may form its own vascularization. This disovery shows promise to revolutionize tissue engineering with the ultimate goal of 3D printing a transplantable organ.