Using Bioprinting to Study Cardiac Vascularization in Tissue Constructs

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Introduction: The biggest problem facing tissue engineering via 3D bioprinting is the aspect of vascularization[1]. Tissues that successfully survive in the human body rely on a network of blood vessels and capillaries to supply the tissue with nutrients and oxygen[1]. Vascularization usually happens naturally, however, natural vascularization happens too slow for the un-vascularized tissue to survive long enough to eventually become vascularized[1]. This period of insufficient vascularization will lead to nutrient deficiencies and might harm the tissue that is being implemented[1]. We are researching a novel technique that involves the formation of vascular tissue through printing sheets of endothelial cells within a printed fibroblast structure. We hypothesize that the sheet of endothelial cells that are printed within the larger structure of fibroblast cells, under appropriate conditions, will grow to form a vascular tube in incubation. We have developed the technique needed to carry out the study.

Materials and Methods: A collagen solution is needed to act as an appropriate media for the cells to grow and flourish. The materials needed to prepare the collagen media are: filter packs, 150mL distilled water, 15 and 50 mL tubes, a magnetic stirrer, pH meter, a container with ice, 2.381g HEPES (Sigma, St. Louis, MO, Cat. #H-4034), 3g sodium hydroxide (NaOH), 1 mL 10x minimum essential medium (MEM), and 8 mL Vitrogen 100 purified collagen. This collagen gel will serve as the media in which our cells will be printed in. Three-day old neonatal rats are used to obtain cardiomyocyte fibroblast cells. The protocol for this dissection is as follows: after the rats have been euthanized, the hearts are removed and minced into 1 mm^3 pieces using scissors[3]. The minced tissue was then digested with an enzyme solution of (0.14 mg/ml trypsin-NO EDTA in PBS solution) overnight and then was shaken at 75 rpm in a collagenase solution (PBS with 1 mg/ml collagenase II) for 1.5 hours[3]. Fibroblast cells were then removed from the solution using an adhesive assay. This was done by incubating the solution in a 150 cm^2 flask with culture medium for 2 hours at 37°C[3]. The cardiomyocytes were then diluted to a concentration of 100,000 cells/mL[3]. Endothelial cells are obtained by using differential cell attachment. The endothelial cells are differentially attach to laminin. After the cells are cultured, each type of cell is loaded into one of the two printing nozzles. Then g-code is used to power the printer to print sheets of the fibroblast cells into the collagen media. Endothelial cells are then incorporated into the fibroblast sheets. The completed print is then placed in an incubator and vascularization is taken note of.

Results and Discussion: There is literature that shows angiogenesis that originates from the placement of endothelial cells during tissue production[3]. This result as well as the many other citations in literature lead us to believe that 3D printing endothelial cells within layers of fibroblast layers, under the right conditions, will result of angiogenesis of the tissue printed. So far, we have engineered the methods that will be used to conduct the project as well as the methods to produce the biomaterials and cells needed to conduct the experiment.

Conclusions: If the findings of this research project yield the anticipated results, it will put the field of bioengineering one step closer to a fully external engineered vascular network. This will, in turn, put the field of regenerative medicine one step closer to fully functioning implementation of the externally grown tissue.

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