Formation of Aggregated Alginate Constructs in a Tubular Perfusion System

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Statement of Purpose: A significant challenge in the implementation of cell based tissue engineering strategies remains the inability to successfully culture large constructs *in vitro*. To this end the goal of this work is to create a tissue engineering construct aggregated from many smaller scaffolds. This will allow for the *in vitro* development of tissue engineering constructs on size scales not easily possible with previous culture methods. The system involves the culture of human mesenchymal stem cells (hMSCs) in alginate beads in the tubular perfusion system (TPS) bioreactor. The TPS bioreactor has been shown to support the growth and osteoblastic differentiation of hMSCs as well as enhance late osteoblastic differentiation and calcium matrix production[1]. The objective of this study is to develop an aggregated alginate construct (AAC), evaluate its mechanical properties, and demonstrate the viability of hMSCs during the formation of this construct.

Methods: To form the AACs, alginate beads of diameters ranging from 2.15 ± 0.07 to 3.90 ± 0.09 mm are tightly packed in a tubular growth chamber within the TPS bioreactor (Figure 1). A 0.025M solution of ethyldiaminetetraacetic acid (EDTA) was flowed through the growth chamber at 1.8mL/min for five minutes. Following this the beads are perfused with 0.5 M calcium chloride for 5 minutes at 1.8 mL/min and 20 minutes at 10 mL/minute. Diameter of beads composing AACs is modified by changing the syringe gauge, and mechanical testing of AACs is completed to observe variation in mechanical strength in relation to bead diameter. To complete mechanical testing, constructs were loaded into custom fit clamps and the tensile strength was measured using a Tensilon RTF-1310 mechanical tester outfitted with a 50N load cell and MSAT0002 materials testing software. The AAC samples were stretched with a constant crosshead speed of 1 mm/min, with the software constantly recording the stress and strain. The test ended with sample fracture. Young's modulus, tensile strength, and yield strength of the AACs were calculated. To test cell response to AAC preparation beads were exposed to AAC treatment and metabolic activity measured using an MTT based *in vitro* toxicology kit (Sigma, St. Louis MO).

Results: Mechanical testing of these constructs revealed that smaller diameter beads resulted in stronger aggregated constructs (Figure 1). Aggregated constructs made from beads 2.15 mm in diameters had a Young's modulus of 85.6 ± 15.8 kPa, a tensile strength of 3.24 ± 0.55 kPa and a yield strength of 1.44 ± 0.27 kPa. These values were statistically similar ($p>0.05$) to constructs made from beads with 2.46 mm diameter. An increase in bead diameter to 2.65 mm resulted in a statistically significant change ($p<0.05$) in mechanical properties producing constructs with a Young's modulus of 39.2 ± 10.6 kPa, a tensile strength of 0.85 ± 0.25 kPa and a yield strength of 0.52 ± 0.15 kPa. These mechanical properties were statistically similar ($p>0.05$) to constructs made from beads with 2.97 mm and 3.90 mm diameters though beads with the largest diameter, 3.90 mm, exhibited the weakest mechanical properties. MTT toxicology assay indicated that the AAC treatment does not negatively affect cell metabolic activity (Figure 2).

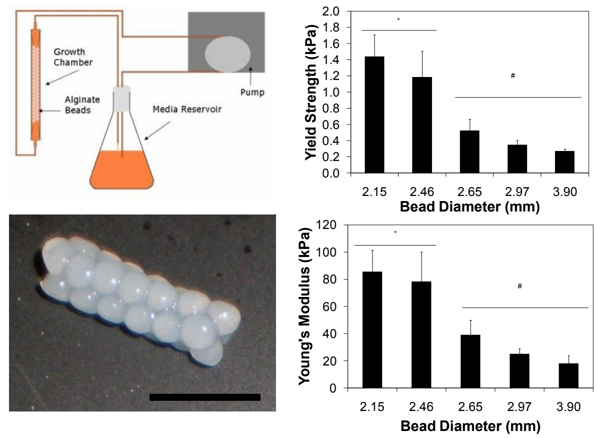

Figure 1. Schematic of TPS bioreactor (top left) Images of aggregated alginate construct (bottom left). Scale bar represents 5 mm. Yield strength (kPa) (top right) and Young's modulus (kPa) (bottom right) of AACs composed of beads of different diameters. The symbols (*, #) indicates statistical significance within a timepoint ($p < 0.05$).

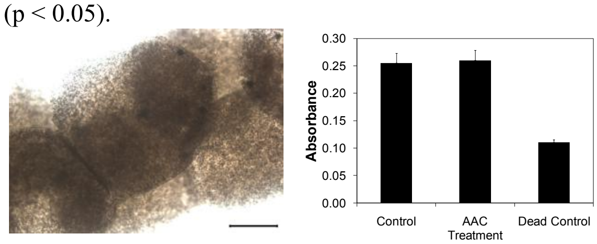

Figure 2. Image of cell containing AAC (left). Scale bar represents 1000 µm. Absorbance correlating to metabolic activity of cells in alginate bead exposed to control media, AAC treatment, or 70% methanol (right). Control and AAC treatment groups exhibit similar levels of metabolic activity.

Conclusions: Following completion of this study a protocol has been developed for the successful generation of aggregated alginate constructs. Mechanical testing indicates smaller bead sizes produce stronger aggregated constructs. Toxicology data reveals that the AAC treatment does not negatively affect cells, thus this process could be readily utilized with cell containing scaffolds to create aggregated constructs. This work represents an elegant method to create aggregated tissue engineering scaffolds from many smaller scaffolds cultured in a bioreactor system.

References: [1]Yeatts AB, Fisher JP. Tissue Eng. 2010. In Press.

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