Development of a Graft for Volumetric Muscle Loss using Bioreactor Technology

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STATEMENT OF PURPOSE:

Bioreactors can provide a physiologically relevant environment (mechanical, electrical and biological) that can readily collect data and enable more complex functions to be regulated throughout a group of samples. Bioreactors are of particular importance for skeletal muscle regeneration as they can simulate contractile functions and allow the pre-seeding of progenitor cells within a three dimensional scaffold[1]. Ensuring that a graft can maintain initial mechanical integrity along with predicting the structural architecture of a volumetric muscle defect are challenging assignments for skeletal muscle engineered scaffolds. Most grafts are developed as transport vehicles with the material itself developed for protected cell delivery. Fibrin has been investigated for the development of skeletal muscle grafts, but limited data exists on their relative performance with regards to myogenic capacity, and functional material survival[2]. In this study, we've developed a fibrin graft that addresses these issues and used a bioreactor system to simulate the *in vivo* conditions of loading and flow on myoblast engraftment.

METHODS:

Fibrin has previously been utilized[3] as a tissue engineering scaffold and has shown *in vitro* biocompatibility through cell culture experiments evaluating cell proliferation and differentiation with L6 myoblasts. Through material degradation, tensile testing, rheological testing and structural analysis, a density of 5.6 mg/mL fibrin gel was determined to have the necessary handeability and myogenic capabilities to survive *in vivo*. Using a Bose ElectroForce BioDynamic bioreactor, fibrin gels with L6 myoblasts (2x 10^5 /sample, n=4) were stimulated at 10% longitudinal cyclic stretch and with dynamic flow to characterize myogenic differentiation against samples held in unconstrained conditions. Loading was performed at a rate of 2 Hz for 2 hours daily and flow was at 60 mL/min. Substrates were examined over 7 days. The samples were then evaluated for change in tensile strength, elastic modulus, pH, CO_2 levels, and O_2 levels. Myoblast development was assessed for each gel using immunofluorescent staining for muscle marker MF20. The groups were compared using 1-way ANOVA and Tukey's post hoc test ($p<0.05$).

RESULTS:

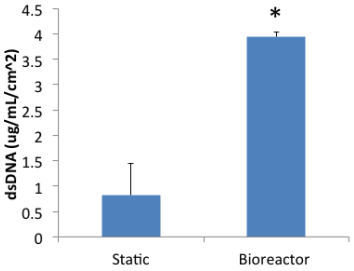

Figure 1. *dsDNA levels between the dynamic and static samples over 7 days of culture indicative of increased cell proliferation with bioreactor conditioning.*

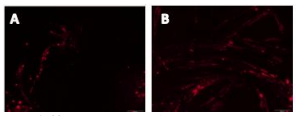

Figure 2. *Myogenic differentiation (MF20 staining) at 7 days between static (A) and bioreactor (B) samples.*

Table 1. *Dynamic samples changes in pH, CO_2 and O_2 levels.*

	pH	CO_2	O_2
Day 0	7.3	4.9	20.72
Day 7	6.32	7.2	4.63

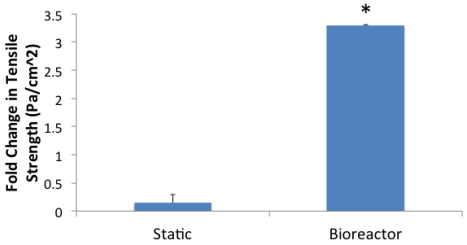

Figure 3. *Bioreactor conditioning resulted in an increase in tensile strength over 7 days.*

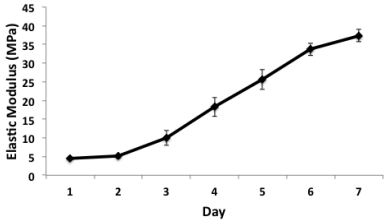

Figure 4. *Changes in elastic modulus over 7 days in the bioreactor.*

DISCUSSION:

The number of cells, the strength of the scaffold, and the amount of myogenic differentiation all significantly increased over 7 days for samples in the bioreactor compared to the static samples. This may be explained by the changes recorded in pH, CO_2 and O_2 (Table 1). The fibrin itself is considerably strengthened over time with stimulation from the bioreactor. The tensile strength (Fig 3) and elastic modulus (Fig 4) of the three dimensional fibrin gel with L6 myoblasts increased significantly over 7 days. This could be due extracellular matrix (ECM) deposition by myoblasts along with the increase in cell number (Fig 1) and beginning of fusion of the myoblasts as they begin to differentiation. This is validated by the MF20 staining which shows the beginning of multinucleated myofibers forming at day 7 in the bioreactor group (Fig 2).

CONCLUSIONS:

- Cells in a stimulated environment show increased levels of metabolic activity over time through pH, CO_2 and O_2 levels.
- Use of cyclic and laminar shear stress significantly increased strength, proliferation and differentiation over 7 days.

REFERENCES:

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2. Rossi, CA et al. Organogenesis, 2010, 6:1-6.
3. Huang YC et al. J Appl Physiol, 2005, 98:706-713.

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