Material Screening for Skeletal Muscle Regeneration

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STATEMENT OF PURPOSE:

While skeletal muscle demonstrates the ability to heal small injuries and strains, there is limited regeneration of severe injuries associated with significant volumetric muscle loss[1]. With few standard alternatives available beyond muscle flaps, which are severely limited by donor site morbidity and functional loss, developing synthetic scaffolds for skeletal regeneration is of great interest. Various scaffold materials have been used in skeletal muscle regeneration[2]. These materials generally fulfill several pre-conditions that relate to the success of a synthetic implant, such as: biocompatibility, degradability and suitable elasticity to provide contractile functionality. Multiple materials have been evaluated individually, but limited data exists on relative performance with regards to cell engraftment, functional compatibility and material survival. In this study, we run a materials screen *in vitro* to compare and contrast the suitability of 5 hydrogels as suitable muscle tissue engineering matrices.

METHODS:

Five skeletal muscle substrates were evaluated to assess their mechanical and biological properties. Collagen(CO)[3], agarose(AG)[4], alginate(AL)[5], fibrin (FB)[6] & chitosan-collagen (CC)[7] have all previously been utilized as tissue engineering scaffolds and each has shown *in vitro* biocompatibility through cell culture experiments. Each gel was fabricated and its elastic modulus evaluated in tensile testing. 3D gels were then fabricated, seeded with L6 rat myoblasts (15,000 cells/mL) and cultured for 14 days. Cell number was measured using Quantiflor, and myogenic differentiation was measured using myosin light chain and creatine kinase-mm activity (via ELISA) at 3, 7 and 14 days. The groups were compared using 2-way ANOVA across time & materials with Tukey's post hoc test ($p<0.05$).

RESULTS:

Table 1. *Calculated elastic modulus for each gel.*

Gel	*Collagen*	*Agarose*	*Alginate*	*Fibrin*	*Collagen Chitosan*
Modulus (MPa)	*3.7±1.2*	*87.3±32.6*	*N/A*	*3.3±0. 5*	*2.7±1.2*

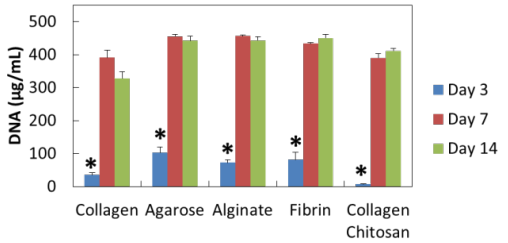

Figure 1. *Cell number at 3, 7 and 14 days. (* indicates significantly less DNA compared to Day 7 and 14, p<0.001)*

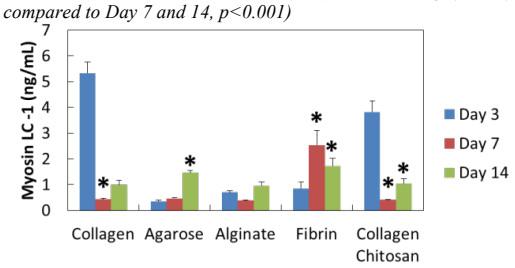

Figure 2. *Myosin light chain-1 activity at 3, 7, and 14 days. (* indicates significant difference from Day 3 value, p<0.001)*

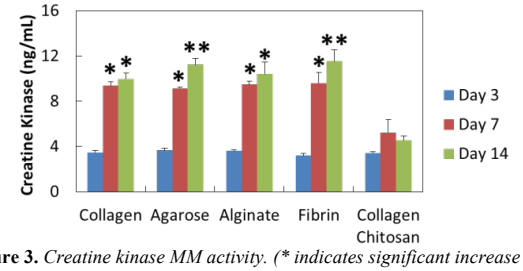

Figure 3. *Creatine kinase MM activity. (* indicates significant increase from Day 3 p<0.001, ** indicates significant increase from Day 7, p<0.05)*

DISCUSSION:

AG exhibited the highest elastic modulus; was also the most brittle (max stretch 20%). CO, CC and FB all demonstrated high elasticity and 100% stretch without failure. AL could not be tested due to decreased handleabilty.

There was a significant increase in cell number (Fig 1) from day 3 to both day 7 and day 14 across all materials. The materials containing collagen (CO and CC) have a lower cell number on day 3; however, they have a significantly higher amount of early myosin light chain-1 activity (Fig 2) compared to all other materials which may be an indication of earlier cell cycle arrest and/or myogenic differentiation. Whereas CO and CC decreased myosin light chain between days 3 and 14, AG and FB had an increase in myosin light chain expression between days 3 and day 14.

CO and AL showed a significant increase in creatine kinase-mm expression from day 3 to days 7 and 14 (Fig 3). FB and AG also showed the same trend, but demonstrated a significant increase from day 7 to day 14, indicating continued myogenic activity. The only material that didn't demonstrate an increase in creatine kinase was CC.

CONCLUSIONS:

- All materials were shown to support cell growth.
- The presence of collagen seems to induce early myosin light chain activity.
- Collagen, Fibrin and Agarose are found to be best suited for skeletal muscle graft development among materials tested when considering their superior handleability, myosin light chain and creatine kinase expression.

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ACKNOWLEDGEMENTS:

This study was supported in part by DoD funds, Orthopaedic Extremity Trauma Research Program (TG), the US Army Institute for Surgical Research Graduate Fellowship and the University Teaching Fellows Program UTSA (BP).