Interpenetrating Collagen-Fibrin Hydrogels for Skeletal Muscle Regeneration

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STATEMENT OF PURPOSE: Biomimetic hydrogel scaffolds have been used extensively for *in vitro* investigation and to create synthetic grafts for wound healing applications, especially for soft tissues such as skeletal muscle[1]. The use of biologically ubiquitous extracellular matrix proteins such as collagen I and fibrin provides the necessary biocompatibility and biodegradability to scaffolds. Previously, we performed an *in-vitro* screening of natural hydrogels, evaluating collagen I, agarose, alginate, fibrin and collagen-chitosan. This included 14 day cell studies with both L6 rat cells (ATCC, Manassas, VA) and rat satellite cells (Fig. 1).

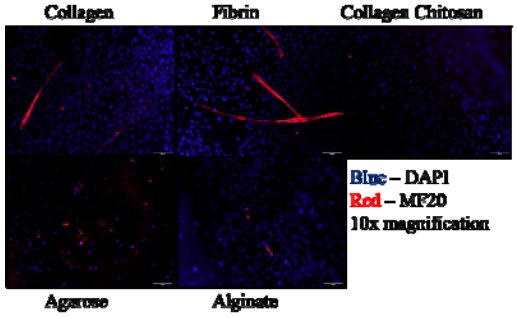

Figure 1: Immunostaining for satellite cell myofiber development MF20 (red) with a nuclear DAPI stain (blue). An MF20 pixel analysis histogram showed fibrin and collagen with the most MF20 staining.

The results of this study showed that collagen and fibrin were more suited as myogenic scaffolds than the other tested groups, with fibrin as the most promising. Further investigation into blends of collagen I and fibrin may indicate that blends of the two might be better suited for myogenesis. Previous studies have investigated collagen:fibrin blends with skeletal muscle growth [2,3]; however, these studies were focused primarily on *in vitro* cell studies rather than mechanical characterization of the scaffolds. *The purpose of this study is to mechanically characterize the Collagen:Fibrin scaffolds prior to in vitro testing in order to identify the underlying mechano-biology that affects skeletal muscle regeneration.*

METHODS: Collagen I and Fibrin gels of 4 mg/ml concentration were prepared using the rat tail Collagen I, and bovine fibrinogen and thrombin. The following collagen:fibrin ratios were made: 100:0 (100c), 75:25 (75c25f), 50:50 (50c50f), 25:75(25c75f), and 0:100 (100f). Material stability was evaluated over 14 days: 1 mL gels (n=4) were made in Teflon molds and then immersed in PBS with either normal physiological pH (~7.35) or a wound environment simulating pH (~6) and kept in an incubator (~37°C). The solutions were changed and the gels were weighed daily. The biomechanical properties were further characterized by rheological evaluation and uniaxial tensile testing. Statistical differences were determined using a two-way ANOVA with Tukey's post hoc test ($p<0.05$) (n=6/group/time).

RESULTS: Fig. 2 shows the results of the stability study for groups under normal physiological pH solution (trends in wound pH were similar). The main effect of significantly greater degradation at normal compared to wound pH($p<0.001$) was observed and each of the gel compositions was found to degrade significantly differently from one another irrespective of pH ($p<0.001$) with a trend of faster degradation with more fibrin content. The results to the rheological testing are shown in Fig. 3. Tensile testing showed no statistically significant differences with elastic moduli being tightly regulated: 14.3 ± 3.7 kPa (ranging from 12.3 kPa to 17.5 kPa).

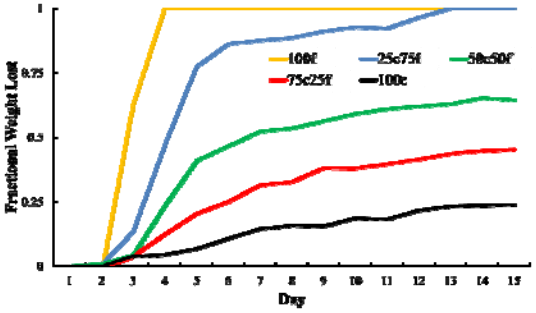

Figure 2: Average fractional weight loss of each group (n=4) over the 14-day period for the groups in normal physiological solution.

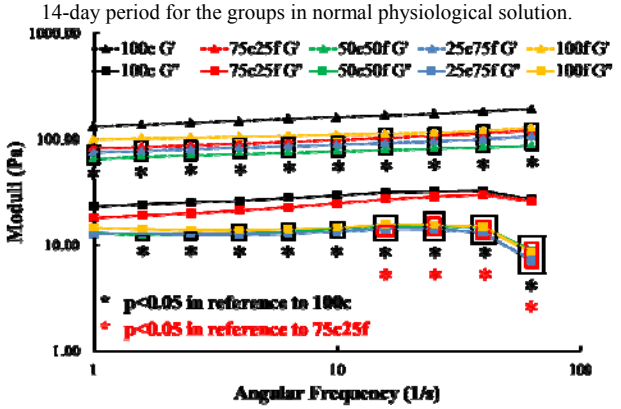

Figure 3: Rheological storage (G') and Loss (G'') moduli.

CONCLUSIONS: The stability test indicates that the groups have different levels of degradation. The ability to control scaffold degradation by controlling composition allows us the ability to tune the rate of scaffold degradation to match the rate of extracellular matrix synthesis by skeletal myoblasts, using the scaffolds as delivery platforms. The rheological data shows that all groups have predominantly elastic behavior rather than viscous. Further, the similarity in elastic moduli between groups indicates that all groups have mechanical potential to perform as myogenic scaffolds. *In vitro* testing of the gels will thus allow determination of particular blend suitability for myogenesis and muscle regeneration.

REFERENCES: [1] Sell et al. Adv Drug Deliv Rev (2009) 61(12): 1007-1019. [2] Bian and Bursac. Biomaterials (2009) 30(7): 1401-1412. [3] Beier et al, BMC Biotechnology (2009) 9(1): 34.

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