Temporal Dynamics of Interpenetrating Collagen I:Fibrin Hydrogels in Supporting Musculoskeletal Remodeling

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Statement of Purpose

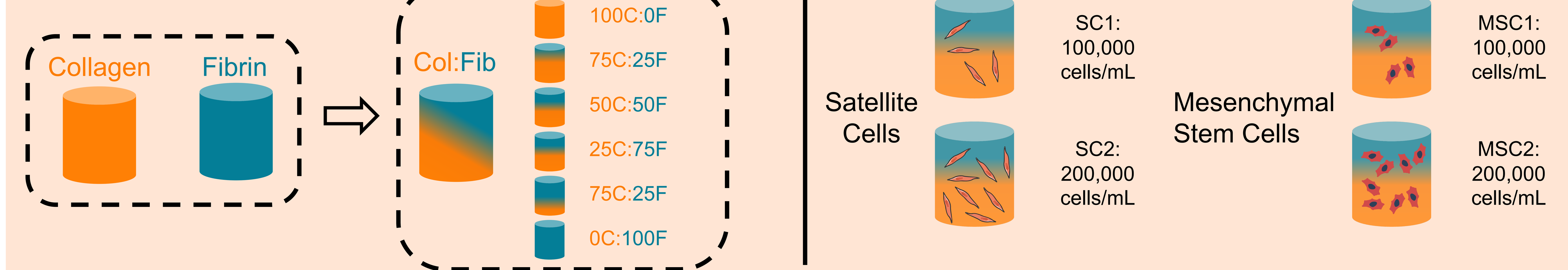
The temporal development of the tissue extracellular matrix is necessary for sustained and successful tissue regeneration. While there are vast differences among tissues in regard to gross composition, function, and mechanical characteristics, the local matrix of these tissues contains some ubiquitous proteins, such as collagen Type I in musculoskeletal tissues.. In tissues that have undergone injury, fibrin clots are the first substantive scaffolds that support cell infiltration and remodeling and provide initial stability. Thus, to investigate the temporal remodeling of the tissue matrix, we investigate collagen I:fibrin (Col:Fib) interpenetrating network blend hydrogels composed of differing ratios of Col:Fib to be able to support the remodeling of the tissue during regeneration.

Objective

To evaluate the impact of musculoskeletal progenitor cells on the mechanical stability of Col:Fib blended hydrogels and to evaluate if the matrix degradation rate and cellular remodeling rate equilibrated.

Methods

Hydrogel Synthesis: Briefly, fibrin gels were prepared with a fibrinogen concentration of 20 mg/mL and combined with thrombin at a ratio of 2:5, respectively. Collagen type I gels were formed at a concentration of 4 mg/mL according to manufacturer's protocol. Samples were allowed to gel at 37° C for 30 minutes. After gelation, appropriate growth media was added. Col:Fib gels were formed at the follow ratios: 100C:0F, 75C:25F, 50C:50F, 25C:75F, 0C:100F. Additionally, gels of 50C:50F were seeded with both satellite cells (SC) and mesenchymal stem cells (MSC) were isolated from rats and seeded at 2 densities: 100,000 cells/mL (SC1 or MSC1) and 200,000 cells/mL (SC2 or MSC2).



Sample Characterization: Samples were characterized using an ElastoSens Bio (Rheolution, Quebec, Canada) daily over a period of 14 days or until complete sample degradation for storage modulus and sample height.

Statistical Analysis: Since the same hydrogel is measured over time, paired statistical modeling was used for improved power. A two-way ANOVA (across time and cell concentration or material composition) for n=3/group/time and Holm-Sidak post hoc test for significance determined at $p<0.05$.

Results

Increasing amounts of fibrin content within gels lead to increased rates of material degradation (Fig 1A and B). With pure fibrin degrading by Day 2, 75% Fibrin by day 10 and lower concentrations by Day 12. With the addition of musculoskeletal cell populations, less hydrogel degradation was seen with MSC populations (Fig 1C and D) which tend to synthesize significant ECM proteins, while the addition of SC populations let to material consolidation and lack of volume (Fig 1E and F) that is often associated with myotube formation. The addition of MSCs increased the average storage moduli of 50C:50F gels, regardless of cell concentration; indicating potentially that the cells were synthesizing ECM and degrading matrix in a density agnostic manner driven by reaching a metabolically stable density in situ. Comparatively, the addition of SC appears to have little effect on the average storage moduli even though the modulus trends upwards indicating that it is not degradation of the IPN hydrogel but potentially matrix consolidation concurrent with myotube formation (as opposed to degradation seen with volume loss in the cell free constructs).

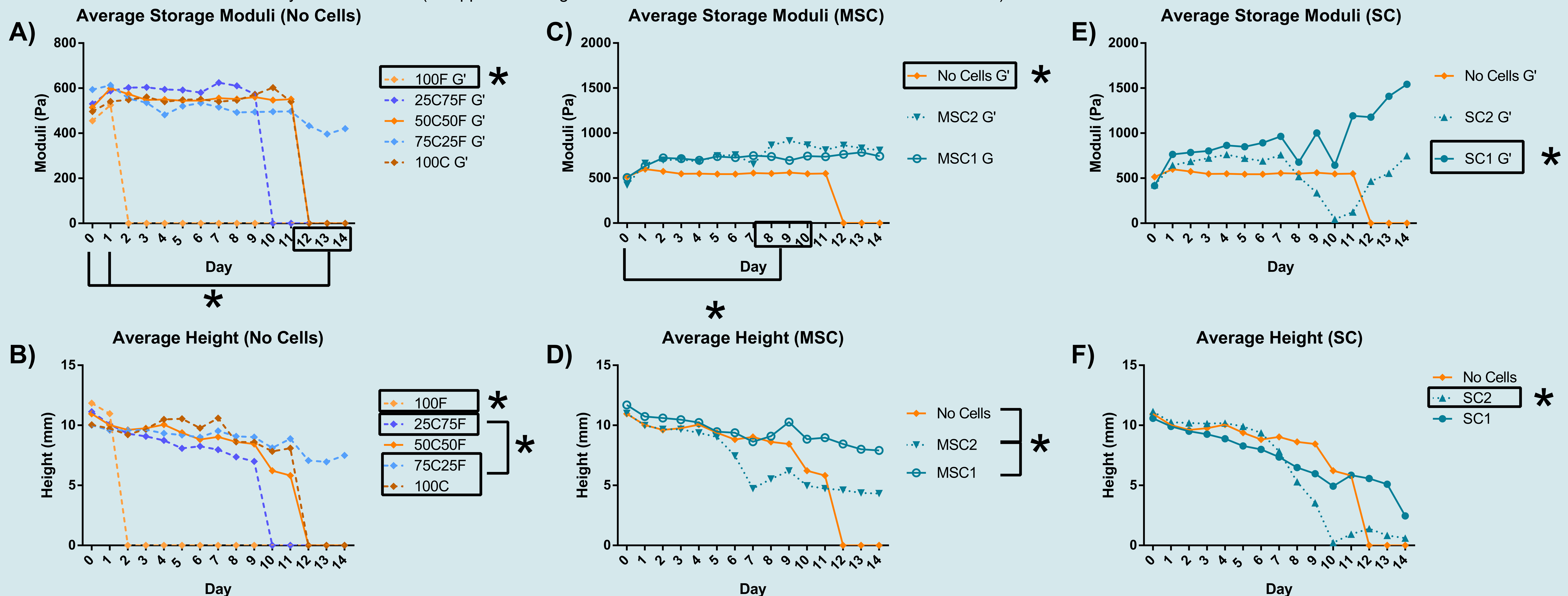


Figure1: Average Storage Moduli of (A) Col:Fib blends without cells; and 50:50 Col:Fib blend (C) with MSCs, and (E) with SCs, respectively. Average Height of corresponding (B) Col:Fib blends without cells; and 50:50 Col:Fib blend (D) with MSCs, (F) with SCs respectively.

Conclusions

Musculoskeletal cells remodel the temporary matrix at the wound site during the proliferative phase through both remodeling and matrix deposition. Depending on skeletal muscle versus bone, this sequence is impacted temporally by cell concentration and native polymers such as collagen and fibrin can be blended in interpenetrating hydrogels to support this remodeling while continually providing mechanically stable substrata.

Acknowledgements

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