Sprifermin (rhFGF18) Preserves Articular Cartilage Depth-Dependent Properties During in Vitro Culture

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Introduction

The current clinical practice of osteochondral allograft transplantation requires a time delay between tissue harvest and transplantation to allow for testing of bacterial and viral contamination.[1] During this time, allografts are stored in cold conditions in an attempt to preserve viability and tissue properties.[2] Although not ideal, due to the very low chondrocyte survival, this procedure is preferred to in vitro culture methods, as the latter results in the rapid loss of mechanical properties and GAG content.[3] More generally, in vitro culture of explants is widely used experimentally to study chondrocyte response in their natural environment, however the rapid turnover of matrix makes results stemming from such long-term cultures difficult to interpret. Several studies have focused on developing in vitro culture systems to preserve allograft native properties including one recent report of a serum-free media formulation containing dexamethasone that preserved mechanical properties and biochemical content in juvenile bovine explants for up to 8 weeks.[1,4] Sprifermin (recombinant human FGF18 (rhFGF18)) stimulates chondrocyte proliferation and matrix production in vitro, and reduces cartilage degeneration and increases de novo matrix formation by osteoarthritic cartilage in vivo.[5,6] We recently showed that a one day per week exposure to Sprifermin preserved explant mechanical properties in a serum-containing media, and that this preservation was due to a decrease in matrix loss and MMP activity over the first 3 weeks.[7,8] Here, we extend this analysis to query local mechanical properties to better gauge the dynamics of explant remodeling in response to Sprifermin. Our findings show that Sprifermin preserved or improve mechanical properties in a depth-dependent manner and attenuated matrix loss over six weeks of culture. These findings may provide a new method by which to preserve allografts during testing periods prior to transplantation.

Methods

Full thickness cartilage explants (4mm diameter) were harvested from the trochlear grooves of juvenile bovine knees. After overnight culture in Complete Medium (CM: DMEM with 10% FBS, 1X PSF, 1% Fungizone, 1% MEM Vitamins, 25 mM HEPES buffer, and 50 μg/ml Vitamin C), explants were trimmed to a similar thickness and cultured in CM with or without rhFGF18 (Sprifermin, 100 ng/ml) applied for 24 hours each week. Over six weeks, explant mechanical properties were evaluated using uniaxial unconfined compression. Explants were tested using a microscope mounted micrometer-driven platen and load cell assembly with a glass slide bottom for imaging (N = 3-5).[9] Cell nuclei were stained with Hoechst 33342 and images and load readings were acquired at equilibrium (0, 4, 8, 12, 16, and 20% strain). Images were processed using two-dimensional digital image correlation software (VIC-2D, Correlated Solutions) to calculate strain throughout the depth of the tissue. A custom MATLAB program (MathWorks) was then used to calculate average strains in 10 equal bins throughout the depth of the sample. The first and last bins were excluded from the analysis to avoid edge effects and the remaining data was grouped further into 20-30%, 30-60%, and 60-90% of the depth to approximate the average strains in the superficial, middle, and deep zones, respectively. Equilibrium moduli in each bin were calculated from the average strain (at an average strain of 8%) and the measured boundary stress at this step. Error bars in all figures represent the standard deviation (SD). Statistical analysis consisted of a 2-way ANOVA with Bonferroni post-tests.

Results

In the superficial zone, ranging from 10-30% of the depth, the equilibrium modulus of control cultures steadily declined through Week 4 (Day 1 E_Y = 1108 ± 343 kPa, Week 4 E_Y = 316 ± 150 kPa) followed by a slight increase in weeks 5 and 6 (Week 5 E_Y = 646 ± 150 kPa, Week 6 E_Y = 451 ± 153 kPa). Treatment with rhFGF18 increased the equilibrium modulus from Day 1 through week 2 (Day 1 E_Y = 1108 ± 343 kPa, Week 2 E_Y = 2052 ± 259 kPa) and then was maintained Day 1 levels for weeks 3 through 6 (Week 3 E_Y = 1193 ± 482 kPa). In the middle zone, 30-60% of the depth, the equilibrium modulus of controls decreased from Day 1 through Week 6 (Day 1 E_Y

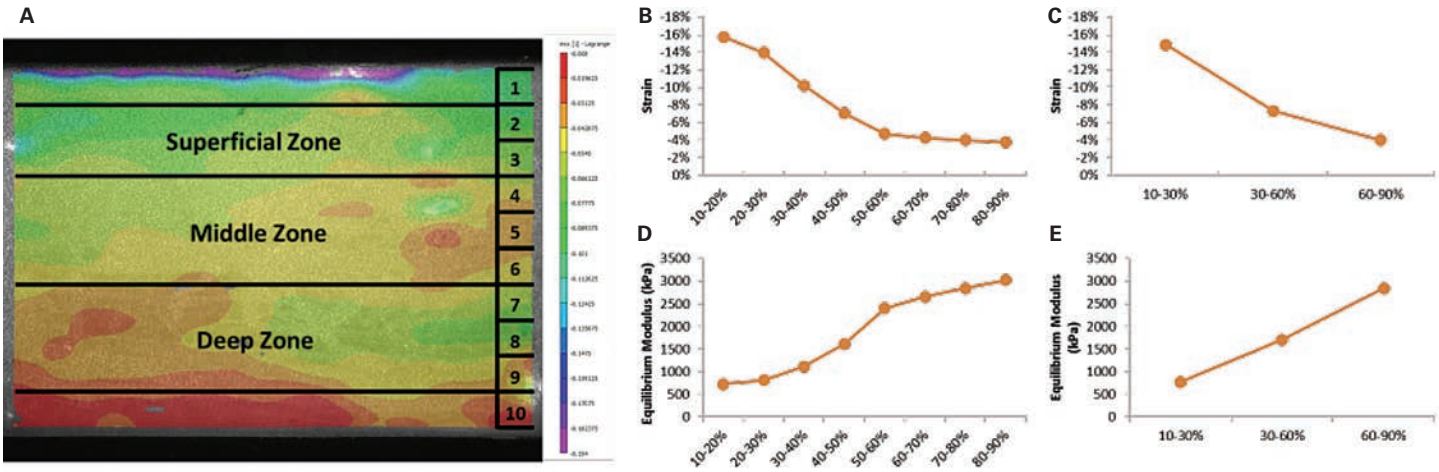

Figure 1. (A) Strain map of a day 1 juvenile bovine explant showing depth-dependent strain across the ten bins (B) and through three regions of interest (C, superficial, middle, and deep zones). Strains were highest in the superficial zone and lower in the middle and deep zones. Corresponding modulus values were calculated for each bin (D) and the three regions of interest (E) from these local strains along with the boundary stress during compression.

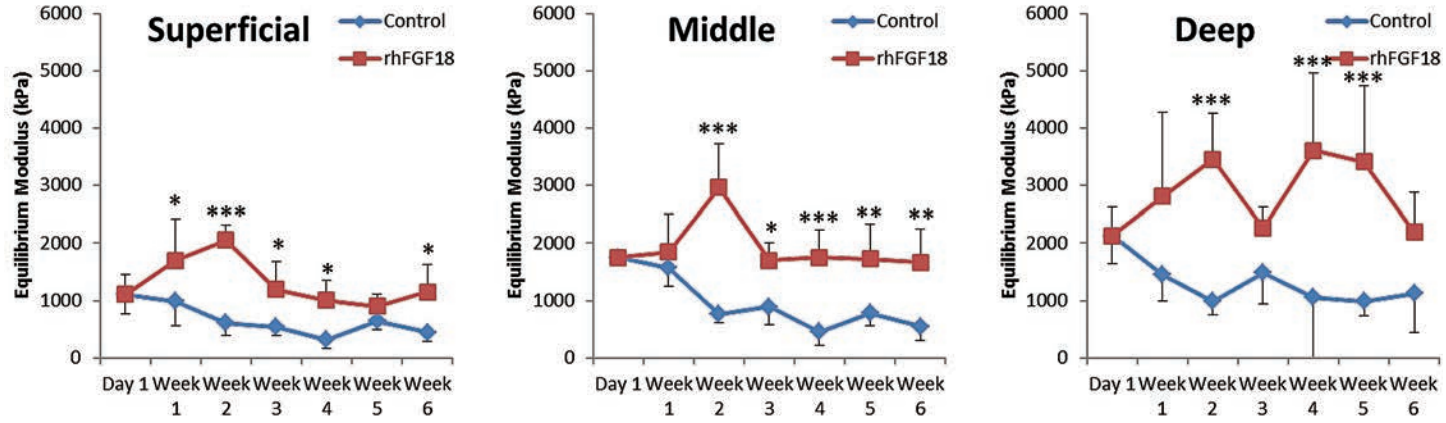

Figure 2. Equilibrium modulus in (A) the superficial zone, 10-30% of the depth, (B) the middle zone, 30-60% of the depth, and (C) the deep zone over six weeks of *in vitro* culture with and without rhFGF18 treatment (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

= 1756 ± 75 kPa, Week 6 E_Y = 554 ± 247 kPa) while the 1+6 treatment resulted in a large increase at week 2 (Week 2 E_Y = 2966 ± 766 kPa) before returning to day 1 levels for weeks 3 through 6 (Week 3 E_Y = 1700 ± 308 kPa). The deep zone showed the most variability but still showed similar trends through the first two weeks of culture, with the equilibrium modulus decreasing in the control culture (Day 1 E_Y = 2136 ± 483 kPa, Week 6 E_Y = 1129 ± 683 kPa). Treated explants increased in mechanical properties in the deep zone through Week 2 (E_Y = 3455 ± 804 kPa) with a decrease back to Day 1 levels at Week 3 (E_Y = 2259 ± 363 kPa) followed by a rebound at Week 4 (E_Y = 3609 ± 1360 kPa) and another decrease to Day 1 levels at Week 6 (E_Y = 2195 ± 696 kPa).

Discussion

Previous studies have shown that the bulk mechanical properties of articular cartilage explants decrease rapidly *in vitro* and treatment with rhFGF18 has a preservative effect on cartilage mechanics and biochemical composition.[10] Given the unique material properties of cartilage are heterogeneous and differ within discrete zones, bulk measurements only tell part of the story. Assessment of local mechanical properties enables specific analysis of depth-dependent characteristics of articular cartilage and in this study their changes over time in culture. Our findings show a distinct increase in modulus from the superficial to middle to deep zones at day 1, consistent with the reported literature. In control cultures, the mechanical properties decreased very quickly throughout the depth and stabilized between weeks 3 and 6. This resulted in preservation of depth-dependence, though at lower values than at harvest under these control conditions. Treatment with rhFGF18 increased the modulus in all three zones through week 2 of culture, before falling back to day 1 values through six weeks of culture. This indicates that rhFGF18 is able to maintain or improve the mechanical properties of cartilage in a depth-dependent manner when cultured under free swelling conditions in a serum containing media. This finding suggests that rhFGF18 is able to prevent or reverse the

loss of extracellular matrix of cartilage explants, and may be a useful additive in the preservation of cartilage properties for osteochondral allografting procedures.

Significance

Sprifermin has the potential to preserve the mechanical integrity of cartilage explants cultured in vitro and in a physiologically relevant depth-dependent manner. This finding suggests that the current practice of storing allografts in the cold could be replaced by the inclusion of rhFGF18 in standard culture conditions to stably maintain important cartilage biomechanical properties during the time period required for safety screening.

Acknowledgement

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References

1. Bian L, et al. Effects of dexamethasone on the functional properties of cartilage explants during long-term culture. *Am J Sports Med*, (2010).

2. Brighton CT, et al. Articular cartilage preservation and storage. Application of tissue culture techniques to the storage of viable articular cartilage. *Arthritis Rheum,* (1979).

3. Acosta CA, et al. Cell viability and protein composition in cryopreserved cartilage. *Clin Orthop Relat Res*, (2007).

4. Gennero L, et al. Protective effects of polydeoxyribonucleotides on cartilage degradation in experimental cultures. *Cell Biochem Func*, (2013).

5. Ellsworth JL, et al. Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. *Osteoarthritis Cartilage*, (2002).

6. Moore EE, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage*, (2005).

7. Schniagl RM, et al. Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *J Orthop Res*, (1997).

8. Wang CC, et al. An automated approach for direct measurement of two-dimensional strain distributions within articular cartilage under unconfined compression. *J Biomech Eng*, (2002).

9. Farrell MJ, Comeau, ES and Mauck RL. Mesenchymal stem cells produce functional cartilage matrix in three-dimensional culture in regions of optimal nutrient supply. *Eur Cell Mater,* (2012).

10. Farran A, et al. Enhanced integration with treatment of Sprifermin (rhFGF18) in a cartilage injury-repair model. *Orthopaedic Research Society Annual Meeting*, (2014).