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# Recombinant Human FGF18 Preserves Depth Dependent Mechanical Inhomogeneity in Articular Cartilage

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## Abstract

Cartilage is a specialized tissue that has a relatively homogenous endogenous cell population but a diverse extracellular matrix (ECM) with depth-dependent mechanical properties. Cartilage repair remains an elusive clinical goal, with biological interventions preferred to arthroplasty in younger patients. Osteochondral transplantation (OCT) has emerged for the treatment of cartilage defects and osteoarthritis. Fresh allografts stored at 4 °C for periods of time have been utilized, though matrix and cell viability loss remained an issue. To address this, several studies have developed media formulations to maintain cartilage explants *in vitr*o. One promising factor for these applications is sprifermin, a human-recombinant fibroblast growth factor-18 which stimulates chondrocyte proliferation and matrix synthesis and is in clinical trials for osteoarthritis treatment. We hypothesized that addition of sprifermin during storage would maintain the unique depth-dependent mechanical profile of cartilage explants, a feature of cartilage not often evaluated. In this study, cartilage explants were maintained for up to 6 weeks with or without a weekly 24-hour exposure to sprifermin (100 ng/ml) and the compressive modulus was assessed. Results showed that sprifermin treated samples maintained their depth-dependent mechanical profile through 3 weeks, whereas untreated samples lost their mechanical integrity over 1 week of culture. Sprifermin also affected ECM balance by maintaining levels of extracellular collagen and suppression of matrix metalloproteinase production. These findings support the use of sprifermin as a medium additive for OCT allografts during *in vitro* storage, and present a potential

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mechanism where sprifermin may impact a functional characteristic of cartilage in repair strategies.

**Keywords**

cartilage; fibroblast growth factor-18; mechanical properties

## Introduction

The normal function of cartilage is to resist compressive loads generated with normal activity. In order to accomplish this task, over a lifetime of use, the tissue has evolved to possess complex mechanical properties, including biphasic interactions between the solid and fluid phases of the tissue (Ateshian *et al.*, 1994; Mow *et al.*, 1980), tension-compression non-linearity, imbued by the high concentration and direction-dependent organization of collagen (Huang *et al.*, 2003; Mow *et al.*, 1992; Park *et al.*, 2003; Soltz and Ateshian, 2000; Sophia Fox *et al.*, 2009), and depth-dependent compressive properties, largely dictated by the high concentration and distribution of proteoglycans through the depth (Chen *et al.*, 2001; Korver *et al.*, 1990). In degenerative conditions, this complex distribution of ECM is disrupted, and mechanical function is compromised, becoming more isotropic and less specialized.

Despite its complex function over a lifetime of normal use, cartilage function can be compromised by acute and localized injury. For instance, a recent review revealed that treatment of focal cartilage lesions has increased 5 % annually, with an incidence rate of 90 per 10,000 patients (McCormick *et al.*, 2014). When symptomatic, these focal injuries can be treated with chondroplasty, cell-based strategies, such as ACI, MACI, and microfracture, or may be treated with osteochondral transfer (OCT), wherein an entire osteochondral unit is transferred from one location to another (Bartlett *et al.*, 2005; Basad *et al.*, 2010; Brittberg, 2008; Brittberg, 2010; Horas *et al.*, 2003; Knutsen *et al.*, 2007; Knutsen *et al.*, 2004; Mithoefer *et al.*, 2005; Peterson *et al.*, 2010; Steadman *et al.*, 2001). OCT can be performed in an autologous fashion (where living donor tissue is moved from one location to another in an individual) or in an allogeneic fashion (wherein fresh cadaveric tissue is the source the donor segment) (Aubin *et al.*, 2001; Bugbee and Convery, 1999; Chow *et al.*, 2004; Chu *et al.*, 1999; Hangody *et al.*, 2010; Hangody and Fules, 2003; Hangody *et al.*, 2008; Marcacci *et al.*, 2007; Williams *et al.*, 2007). While early versions of this procedure used fresh-frozen and devitalized cadaveric tissue, recent studies have suggested that outcomes for allogeneic OCT are better when a living implant is used (Malinin *et al.*, 2006; McCarty *et al.*, 2010).

While promising as a therapeutic, donor osteochondral units must first be screened for communicable disease prior to implantation, necessitating storage of tissues for several weeks. Several studies have examined long-term storage of allografts at freezing (−70 °C), refrigerated (4 °C), and body (37 °C) temperatures, with the most common primary outcome being chondrocyte viability (Allen *et al.*, 2005; Ball *et al.*, 2004; Ohlendorf *et al.*, 1996; Pallante-Kichura *et al.*, 2013; Pallante *et al.*, 2009; Pallante *et al.*, 2012; Williams *et al.*, 2004; Williams *et al.*, 2003). Storage of allografts in hypothermic conditions may maintain

the integrity of extracellular matrix, but does so at the expense of chondrocyte viability. While maintaining viability is readily achieved over such a time period when storing grafts at body temperature, this storage and screening process is detrimental to the mechanical properties of the osteochondral unit, potentially limiting its efficacy upon implantation.

Indeed, *in vitro* culture or storage of living cartilage results in the rapid loss of mechanical function (Bian *et al.*, 2008). This occurs as a consequence of the rapid loss and or degradation of the dense extracellular matrix (ECM) (Hascall *et al.*, 1983b). Once removed from the load-bearing synovial environment, both mechanical and biochemical cues that would normally promote tissue homeostasis are lost, and so the tissue begins to degrade. There have been numerous attempts to preserve or promote the cartilage phenotype during *in vitro* culture of living cartilage, with varying degrees of success. Early studies in this field, employing mechanical loading systems and supplementation of media with growth factors, demonstrated an anabolic effect and have explicated many of the key factors that regulate cartilage homeostasis (Fitzgerald *et al.*, 2004; Guilak *et al.*, 1994; Hall *et al.*, 1991; Hascall *et al.*, 1983a; Luyten *et al.*, 1988; Sah *et al.*, 1994; Sah *et al.*, 1989). Towards the practical application of preserving implant properties prior to OCT procedures, these media formulations have been increasingly well defined, with some making their way to clinical and commercial application (Mickevicius *et al.*, 2015; Teng *et al.*, 2008). For instance, based on a chemically defined media formulation containing transforming growth factor-beta3, the Missouri Osteochondral Preservation System enables the transfer of very large and highly viable osteochondral segments for total joint restoration (Garrity *et al.*, 2012; Kuroki *et al.*, 2017; Stoker *et al.*, 2017; Stoker *et al.*, 2018).

Despite this progress in the field, it is not yet clear whether such preservation systems fully retain the graded and refined mechanical properties of native tissue. It is also not clear whether the current media formulations represent the optimal, and whether other molecules may have a preservative effect. To that end, we and others have recently shown that fibroblast growth factor 18 (FGF18) stimulates chondrocyte proliferation and matrix production *in vitro*, and reduces cartilage degeneration and increases *de novo* matrix formation by osteoarthritic cartilage *in vivo* (Ellsworth *et al.*, 2002; Moore *et al.*, 2005). A recombinant version of this protein, known as sprifermin, is currently a non-approved drug candidate and in clinical development for osteoarthritis, also has positive effects *in vitro*, *in vivo*, and in several recent pre-clinical and clinical trials in humans (Dahlberg *et al.*, 2016; Gigout *et al.*, 2017; Lohmander *et al.*, 2014; Mori *et al.*, 2014; Power *et al.*, 2014; Reker *et al.*, 2017). Sprifermin has decreases collagen type I expression in monolayer culture, and decreases collagen type II expression at dosing concentrations >100 ng/mL. With regards to cell morphology, Sprifermin results in chondrocytes taking on a more rounded morphology, with a loss of elongated shape and stress fibres (Gigout *et al.*, 2017). In this study, we tested whether the addition of sprifermin (recombinant human FGF18) to standard media formulations could maintain the refined mechanical properties of articular cartilage during long-term in vitro culture, with a particular focus on the characteristic depth-dependent compressive properties of the native tissue.

## Materials and Methods

### Cartilage Explant Harvest and Culture

Full thickness articular cartilage explants were harvested from the trochlear groove of juvenile (3–6 month old) bovine stifle joints (Research 87, Boylston, MA) using aseptic technique and a sterile 4 mm diameter biopsy punch. Explants were washed three times in sterile phosphate buffered saline (PBS) supplemented with 200 units/mL penicillin, 200 μg/mL streptomycin and 0.5 μg/mL Amphotericin B (Gibco by Life Technologies, Carlsbad, CA). Following washing, explants were sharply dissected to remove non-cartilaginous tissues (subchondral bone) and cultured overnight in complete medium consisting of Dulbecco's Modified Eagle Medium (Gibco by Life Technologies, Carlsbad, CA) with 10 % by volume fetal bovine serum, 100 units/mL of penicillin, 100 μg/mL of streptomycin, 2.5 μg/mL of Amphotericin B (Gibco by Life Technologies, Carlsbad, CA), 1x MEM Vitamins (Corning Cellgro, Manassas, VA), 25 mM HEPES Buffer (Gibco by Life Technologies, Carlsbad, CA), and 50 μg/ml L-Ascorbic Acid 2-Phosphate (Sigma-Aldrich, St. Louis, MO). Following overnight culture, samples were randomly assigned into treatment and time groups per the study design. Samples were maintained fully submerged in 1 mL complete medium per sample and cultured at 37 °C and 5 % $CO_2$. Media were changed three times per week, with 1 ml collected at each media change frozen at −80 °C for later analysis. The initial study was planned for a duration of 3 weeks with follow up studies planned for end points between 3 and 6 weeks. The data presented was gleaned from all samples at the same time points over multiple experiments. Replicate numbers are provided with each figure in addition to the summarized data in Table 1. Note that the reduction in sample numbers for MMP and media GAG assessments was the result of sequential tissue removal at each weekly time point.

### Recombinant Human FGF18 (rhFGF18) Treatment

Recombinant human fibroblast growth factor 18 (rhFGF18, alias sprifermin, Merck KGaA, Darmstadt, Germany) was added to complete medium at a concentration of 100 ng/ml. The concentration was chosen as low to medium based on previous in vitro assays demonstrating a dose dependency in a wide range of concentrations (Gigout *et al.*, 2017). As established by previous studies, a treatment duration of 24 hours each week was chosen to enhance the 'hit and run' effect that Sprifermin has been shown to operate by, where a short term exposure and then remove elicits the greatest anabolic response. After treatment, media was then replaced with fresh complete medium (Gigout *et al.*, 2017). Controls were treated similarly, with exchange of complete medium after the identical 24 hours. Thereafter, all cultures were changed into fresh medium every 3 days.

### Mechanical Testing

For unconfined compression testing, samples were tested using a custom-built device (Figure 1) (Mauck *et al.*, 2000; Mauck *et al.*, 2006). Briefly, a 0.02 N creep load was applied at a velocity of 10 μm/s and held for 300 seconds, followed by 10 % compressive strain applied over 200 seconds followed by a 1000 second hold. Equilibrium modulus was calculated by dividing the measured load at the end of the 1000 second hold by the cross-sectional area and applied strain. To assay the depth-dependent mechanical properties of the

cartilage explants, samples were tested in unconfined compression using a custom micrometer-driven inverted microscope mounted apparatus (Farrell *et al.*, 2012). The deep zone of each sample was trimmed on a freezing stage microtome to ensure parallel surfaces for mechanical testing, while still preserving the superficial zone. The sample was then measured with digital calipers and cut diametrically to produce a semi-cylinder. While this may cause some fiber discontinuity in the sample, all specimens were tested in the same fashion. One half was fixed in 4 % paraformaldehyde for histology and the other half was stained with Hoechst 33342 (1:100 dilution) to identify cell nuclei that would later be used as fiducial markers. The tissue was then placed cut side down into the device and the loading platens were brought into contact with the sample. Fluorescent images were taken on an inverted microscope (Nikon Eclipse TE2000-U) and equilibrium load readings were recorded at initial contact, and following each of five 4 % strain increments, up to 20 % strain. Samples were loaded manually using a micrometer-driven linear stage at approximately 1% strain per second and held for 800 seconds. Image correlation software (Vic2d, Correlated Solutions) was then used to compute a 2D strain field with a resolution of 16.25 microns throughout the depth of the tissue using the nuclei as fiducial markers (Farrell *et al.*, 2012). The data was further analyzed using a custom MATLAB program to calculate the average strain the direction of loading in 50 μm bins across the depth of the sample, starting at the cartilage surface and continuing through the depth of the sample. Strain data from the first and last 100 microns of each sample was discarded due to edge effects. The equilibrium modulus for each bin was calculated by dividing the equilibrium load by the measured cross-sectional area and bin strain. This results in a depth-dependent profile of the compressive equilibrium modulus. To compare these local data to the tissue-scale modulus data acquired using the test described above, the average strain across the whole tissue was calculated. This data was then normalized by dividing the measured modulus at each time point by average modulus at the start of each experiment. This was done to account for differences in the testing modalities and biological variability between studies. This data was then included in the analysis of the tissue-scale mechanics.

## Biochemical Assays

Sulfated glycosaminoglycan (GAG) content was quantified via the colorimetric 1,9-dimethylmethylene blue assay (Farndale *et al.*, 1986). Collagen content was quantified using the orthohydroxyproline assay and a conversion factor 7.6 (Stegemann and Stalder, 1967). Matrix metalloproteinase (MMP) activity levels in the media were quantified with the SensoLyte Fluroimetric assay (AnaSpec) using a fluorometric assay reading at ex/em 490 nm/520 nm. This Generic MMP Assay Kit is designed to detect MMP-1, 2, 3, 7, 8, 9, 12, 13, and 14, making it ideal for high throughput screen and detecting generic MMP activity. Wet and dry weights were recorded before samples were digested in a Proteinase K digestion buffer for 18 hours at 60 °C with frequent mixing (50 units/ml in 100 mM Tris-HCl, Worthington, Lakewood NJ). GAG and collagen content were all quantified on digested samples. GAG and MMP levels in the medium were quantified on a weekly basis. Solid volume fraction was calculated as the quotient of the dry weight divided by the wet weight of each sample. From this, the change in water content was expressed as a percentage by calculating the difference in solid volume fraction at each week relative to the mean week 0 value.

### Data Analysis and Statistics

For mechanical testing data analysis, studies were normalized to mean time zero values and combined. GAG and collagen content were normalized to wet weight to account for variability in the size of each individual tissue segment. Error bars in figures represent the standard error of the mean. Statistical significance was determined using a 1-way or 2-way ANOVA with Bonferroni's post-hoc test, as appropriate.

## Results

### Cartilage Explant Tissue-Scale Mechanics with FGF18 Treatment

The equilibrium modulus of cartilage explants decreased with in vitro culture over six weeks, regardless of sprifermin treatment (Figure 2). Samples cultured in control medium (Figure 2, white bars) had significantly lower equilibrium moduli compared to week 0 values at every week following one week of culture ($n=12–23$, $p<0.05$). Conversely, the equilibrium modulus of samples treated with rhFGF18 (black bars) did not become significantly lower until week 6 ($n=17–29$, $p<0.05$). Similarly, the tissue-scale equilibrium modulus of the explants treated with rhFGF18 was higher than control explants at time points of 2, 3, and 4 weeks ($n=12–29$, $p<0.05$).

### Cartilage Explant Local Mechanics with Sprifermin Treatment

To better understand these differences in tissue-scale compressive mechanics, each sample was tested using a custom device to assay tissue modulus in 50 μm increments from the superficial zone through the deep zone. Baseline, or week 0, depth-dependent mechanics showed an increase in modulus as function of distance from the cartilage surface (Figure 3, grey), consistent with previous findings (Schinagl *et al.*, 1996; Wang *et al.*, 2003; Wang *et al.*, 2002). Control samples (dashed lines, n=12–17) had moduli that were below baseline levels throughout the depth of the cartilage explants. These differences became more pronounced with culture duration. Additionally, control samples quickly lost the depth-dependence characteristics of native cartilage, seen here as a flattening of the modulus profile. In contrast, sprifermin treated samples (solid colored lines, n=12–17) retained a depth dependent profile matching that of the baseline week 0 control through five weeks of *in vitro* culture, with modulus increasing as a function of distance from the cartilage surface. Further investigation of this data were assessed in the 100–1000 micron region of the tissue, in order to determine differences in the superficial and lower/mid zone of the cartilage samples, rather than the full depth presented in Figure 3. This layer spanning 1mm thickness represents the layer of cartilage that will remain in the adult. These trends were quantified as average moduli in regions near the surface (100–150 microns), in the middle (450–500 microns), and in the deep (950–1000 microns) zones of the tissue as a function of culture duration in Figure 4. At these distances from the cartilage surface, the control samples (dashed lines) showed a decreased modulus by week 3 near the surface and in the middle zone, and by week 4 in the deep zone. The rhFGF18 treated samples (solid lines) showed no differences in the deep zone, a decrease at week 4 in the middle zone, and decreases at weeks 4 and 5 near the surface. Overall, control samples showed a decrease in modulus in 11 of 18 samples (61 %) whereas sprifermin samples had differences in only 3 of 18 samples (17 %).

### Explant Biochemical Content with Sprifermin Treatment

Following mechanical testing, the biochemical composition of the tested samples was determined. The biochemical composition was determined on the half that was mechanically tested. The adjacent hemi-cylinder was fixed in 4 % para-formaldehyde. Total collagen content, as a percentage of wet weight, decreased significantly in control samples after one week of culture (Figure 5A, white bars) compared to week 0 levels ($n=19–30$, $p<0.05$). In comparison, sprifermin treated explants (solid bars) had significantly lower collagen content after only 4 and 6 weeks of *in vitro* culture ($n=19–30$, $p<0.05$). Additionally, sprifermin treated explants had higher collagen content compared to controls at all time points after week 2. GAG content of explants was also measured as a percentage of wet weight with time (Figure 5B). There was a significant decrease in GAG content for control samples starting at week 2 (white bars), while for sprifermin treated explants these decreases were not apparent until week 3. Only at week 2 was there any detectable difference in GAG content between groups.

### Explant Macroscopic Appearance and Water Content with Sprifermin Treatment

Through six weeks, explants in control conditions appeared to visibly swell and distort from their original cylindrical shape, whereas sprifermin treated explants maintained their size and shape. Both control and sprifermin treatment groups showed a significant difference in solid volume fraction after one week of culture (Figure 6A). However, the solid volume fraction with sprifermin treatment was higher than controls at each week following. This can be expressed as a change in water content (Figure 6B), where there was a significant increase in water content in control samples at all time points. For treated explants, while there was an increase at after week 1, this change was attenuated compared that seen in control conditions.

### Matrix Loss from Explants with Sprifermin Treatment

Assays on the medium were also performed to determine GAG and MMP release from the tissue. Although there were some minor differences in weekly GAG released into the medium, none of these differences reached the level of significance (Figure 7A). Similarly, cumulative GAG release into the medium did not differ between treatment groups (Figure 7B). Control samples also showed a steady level of MMP released to the medium each week. Conversely, sprifermin treated samples showed a marked reduction in MMP in the medium through the first three weeks of culture (Figure 8).

## Discussion

In this study, we demonstrated the efficacy of sprifermin as storage media additive to preserve articular cartilage during extended *in vitro* storage. At the tissue level, sprifermin had a significant effect on the equilibrium modulus. Control samples quickly decreased modulus starting after week 2, while sprifermin treated samples showed no change in modulus through five weeks of *in vitro* culture. Additionally, the sprifermin treatment group had a higher modulus when compared to control samples at weeks 2 through 4 of culture. These findings were replicated using two different measurement techniques, while not directly comparable the differences between treated and untreated were reproducibly similar.

To visualize the order of magnitude of these changes, the results from one study are shown in Figure 2. In this replicate study, sprifermin maintained or increased the tissue-scale modulus from week 0 through week 2 of culture, whereas control explants decreased in modulus at all time points.

To further examine and identify changes, we utilized a technique to track strain (and so calculate modulus) throughout the depth of the cartilage tissue, allowing us to determine the depth-dependent mechanical properties of each sample. This is an important feature, given that the native surrounding tissue shows such depth dependence, and so functional integration of the implanted osteochondral unit might be compromised if the implant does not match native tissue values. This high-resolution method resulted in a plot of modulus in 50 micron increments. As early as one week of *in vitro* culture, there was a divergence in mechanical properties between the control (Figure 3, dashed lines) and sprifermin samples (Figure 3, solid lines), where the former decreased from 2000 to 3000 microns from the cartilage surface and the latter increased over initial values at the same depth. This divergence point moved closer to the cartilage surface as the duration of culture increased, indicating that control samples were losing their mechanical properties in the deep zones faster than in the superficial zones. This pattern results in control samples that have lost their intrinsic inhomogeneity, whereas samples from the sprifermin treatment group maintained a depth-dependent modulus profile through five weeks of culture.

The largest differences in local mechanical properties were observed in the deepest zones of our samples, furthest from the cartilage surface. The samples used in this study are from immature bovine cartilage, where the cartilage thickness will be much less when the animals fully mature. Thus, it was important to examine changes in this tissue closer to the cartilage surface, in regions that will likely remain as mature, permanent cartilage and not transition into bone. In these more superficial regions, sprifermin also preserved the mechanical properties of the cartilage for at least three weeks, as shown in Figure 4. This finding demonstrates that sprifermin is able to preserve the depth-dependent mechanical characteristics of articular cartilage in an *ex vivo* environment for an extended duration. Given that this three-week time window is sufficient for screening of implants for communicable diseases, this finding may improve preservation methods for allogeneic OCT procedures.

The mechanical testing carried out in this study to determine both the tissue-scale and local properties of the tissue consisted of uniaxial compression. This is a sensible first assay, given that the native tissue functions in compression and testing of the tissue in this way is necessary to understand the change in mechanical properties (Korhonen *et al.*, 2003). There are two principle extracellular matrix components that regulate the mechanical properties of cartilage, proteoglycans (PGs) and collagen (Julkunen *et al.*, 2007). Although the PG content is known to resist compressive loads through charge-charge repulsion (via the fixed negative charges on the PGs) and water retention and interstitial fluid pressurization (Khoshgoftar *et al.*, 2013) (via the Donnon osmotic effect and the frictional drag of fluid flowing through the small pores), it appears that PGs are not the central player in the action of sprifermin. Rather, the preservation of cartilage mechanical integrity with weekly sprifermin treatment appears to be due to maintenance of the collagen network. While collagen acts to resist

tensile loads, it is also a key contributor to the mechanical response in compression by resisting the outward expansion of the cartilage tissue (the Poisson effect). The equilibrium Poisson ratio of native cartilage is quite low (on the order of 0.1–0.2), due in large part to the high tensile properties in the plane of the tissue from the high collagen content (Wang *et al.*, 2003). Explants treated with rhFGF18 had higher collagen levels when compared to control samples at all time points from week 3 to 6. Additionally, treated samples did not differ from baseline levels until week 4. These significant differences were not seen in either the GAG levels in the tissue itself, or the GAG measured in the medium. Given that the mechanical response of the tissue is largely governed by these two components, we conclude that sprifermin maintained native cartilage mechanical properties through preservation of the collagen network.

Further evidence of the importance of collagen and the ability of sprifermin to maintain this crucial network is in the solid volume fraction or swelling of the tissues during *in vitro* culture. Solid volume fraction and collagen content are directly related (Bank *et al.*, 2000). The change in solid volume fraction can also be expressed as the influx of water. Control samples were unable to resist the imbibition of water into the tissue, and so the tissue swelled substantially. We hypothesize that since control samples are unable to resist this expansion, they may also lose their ability to undergo interstitial fluid pressurization during loading, resulting in reduced mechanical functionality. Cartilage samples from the sprifermin treatment group were better able to resist swelling as the collagen network was better preserved. The changes in the collagen content are likely due to differential expression and activity of MMPs. We measured the MMP activity in the culture medium as an indicator of MMP expression and activation during *in vitro* culture. Sprifermin treatment significantly suppressed MMP levels for three weeks, while control samples had a higher, steady concentration of MMPs in the media. This is the same period over which we determined that sprifermin treatment maintained tissue-scale mechanical properties, local depth-dependent mechanical properties, and collagen content. The mechanism of action of FGF18 is to induce cell proliferation, which is in the articular joint context the specific proliferation of chondrocytes because of the FGF-receptor subtype distribution. The chondrocytes need space for proliferation and therefore this process is started with the expression of matrix dissolving proteases, e.g. MMP13. Chondrocytes that recently proliferated are producing more matrix (more, because the cartilage is populated by more chondrocytes and more, because of an increased matrix production rate per cell) by activation of the ERK pathway (Gigout *et al.*, 2017). In 3D culture, FGF18 increases the number of matrix-producing chondrocytes, improves the type II:I collagen ratio, and enables chondrocytes to produce a hyaline extracellular matrix. Furthermore, FGF18 displays a ‘hit and run’ mode of action, which is a common phenomenon for growth factors (Gigout *et al.*, 2017). The chondrocyte survival time in cartilage explants seems to be increased in in-vitro experiments, however, cell viability was not evaluated in this current study.

The findings of this study suggest that sprifermin may be used as a medium supplement during storage or extended culture of viable cartilage tissue has significant clinical application. Currently, donor tissue for allograft procedures is refrigerated while it is screened prior to transplant. This practice maintains some of the mechanical properties of the native tissue, but also results in low chondrocyte viability. Using sprifermin under

normal culture and storage conditions, we showed that cartilage mechanical inhomogeneity can be maintained over a similar time course. In this study, we used cartilage from multiple bovine sources of approximately the same age. The advantage to this was in reducing the number of variables, namely age of the cartilage. However, in future work and for clinical applications, the maturity of the cartilage will be an important factor to consider as it has a great effect on the tissue homeostasis and activity. Studies to address this issue and to replicate these findings in human sourced materials are now underway.

## Conclusions

Sprifermin preserved the intrinsic depth-dependent mechanical properties of articular cartilage during long-term *in vitro* culture/storage conditions through maintenance of extracellular collagen and suppression of matrix metalloproteinase production. This methodology may be applied in a clinical scenario to improve on current transplantation practice, where prior to surgery the tissue is stored during screening, compromising its mechanical properties. Furthermore, this study also presents potential mechanisms for sprifermin mode of action in protecting the integrity of cartilage and ultimately impact a functional characteristic of articular cartilage in restorative cartilage strategies.

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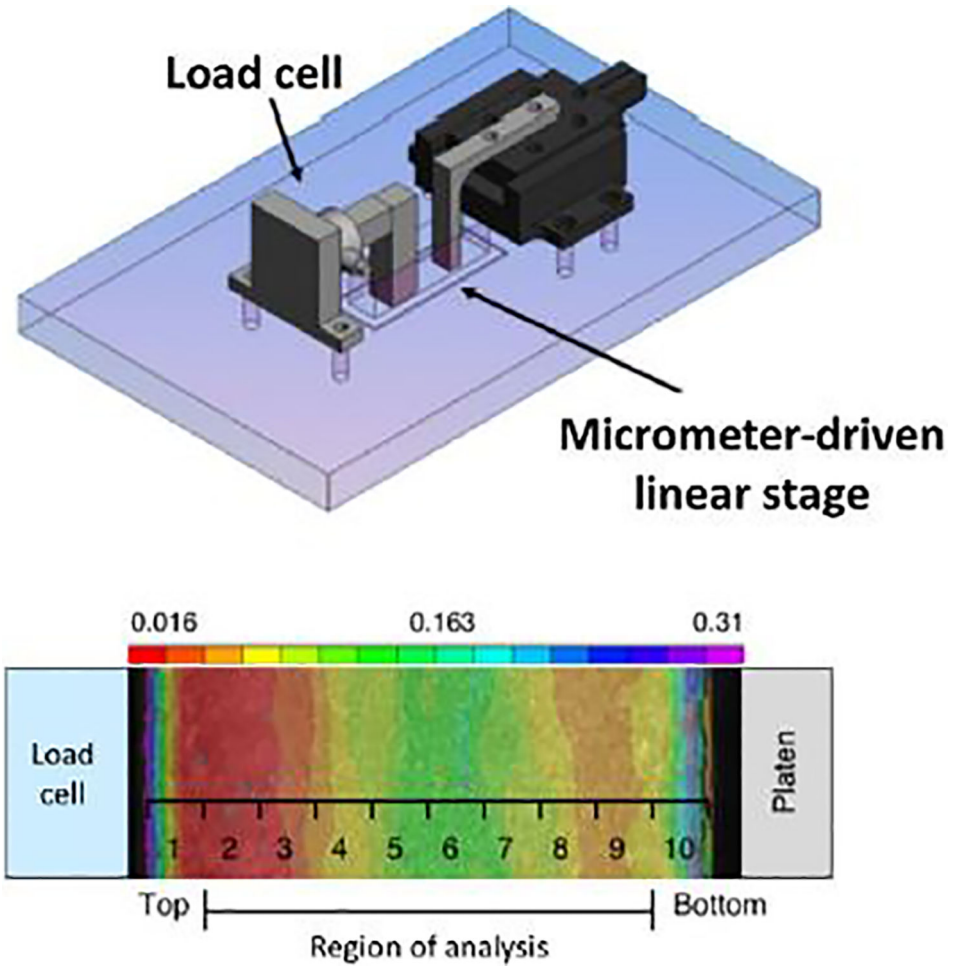


**Fig. 1.**

Schematic of custom compression device mounted on an inverted fluorescent microscope and heat map of strain mapped to ten distinct regions of analysis from the superficial to deep zone. Adapted from Farrell *et al.* (2012), used with permission.

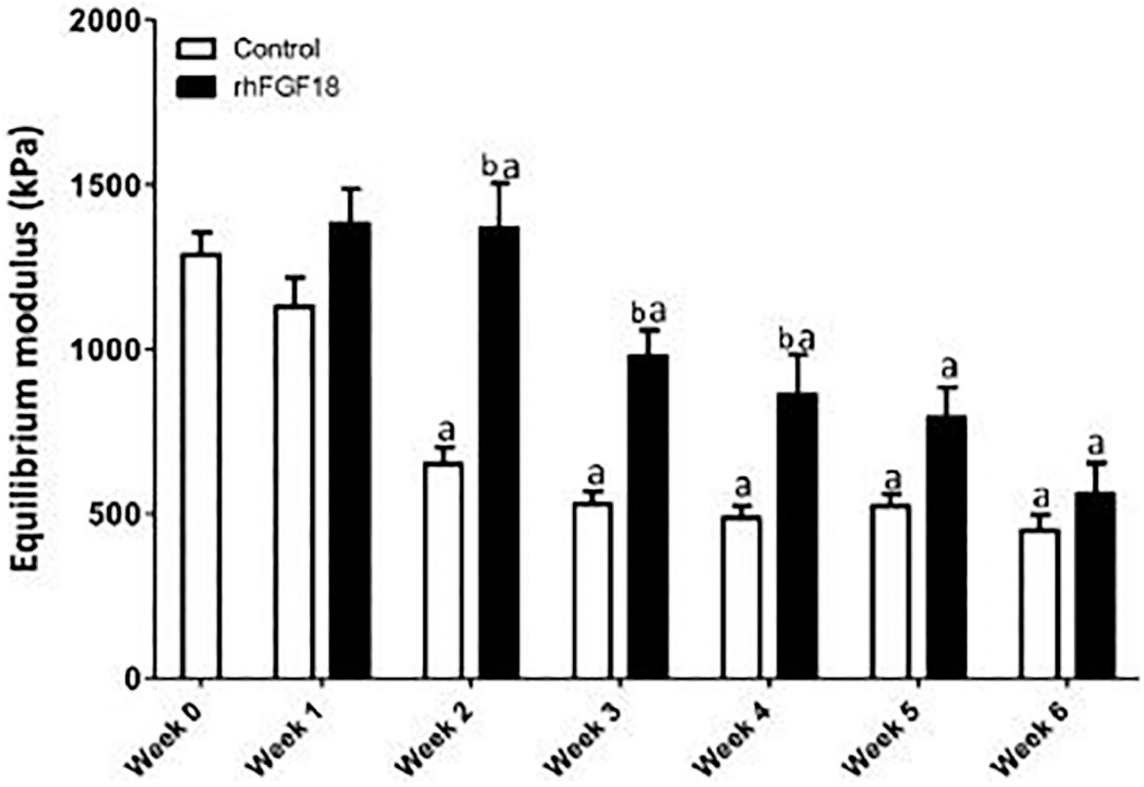


**Fig. 2.**

Equilibrium modulus of AC on the tissue scale. Samples were cultured *in vitro* for up to 6 weeks ± 100 ng/mL rhFGF18. Treated explants did not differ from baseline modulus throughout 5 weeks. Additionally, the modulus of the treated explants was significantly higher than that of the control at weeks 2, 3 and 4 ([a] $p < 0.05$ *vs.* week 0, [b] $p < 0.05$ *vs.* control; $n = 17–25$).

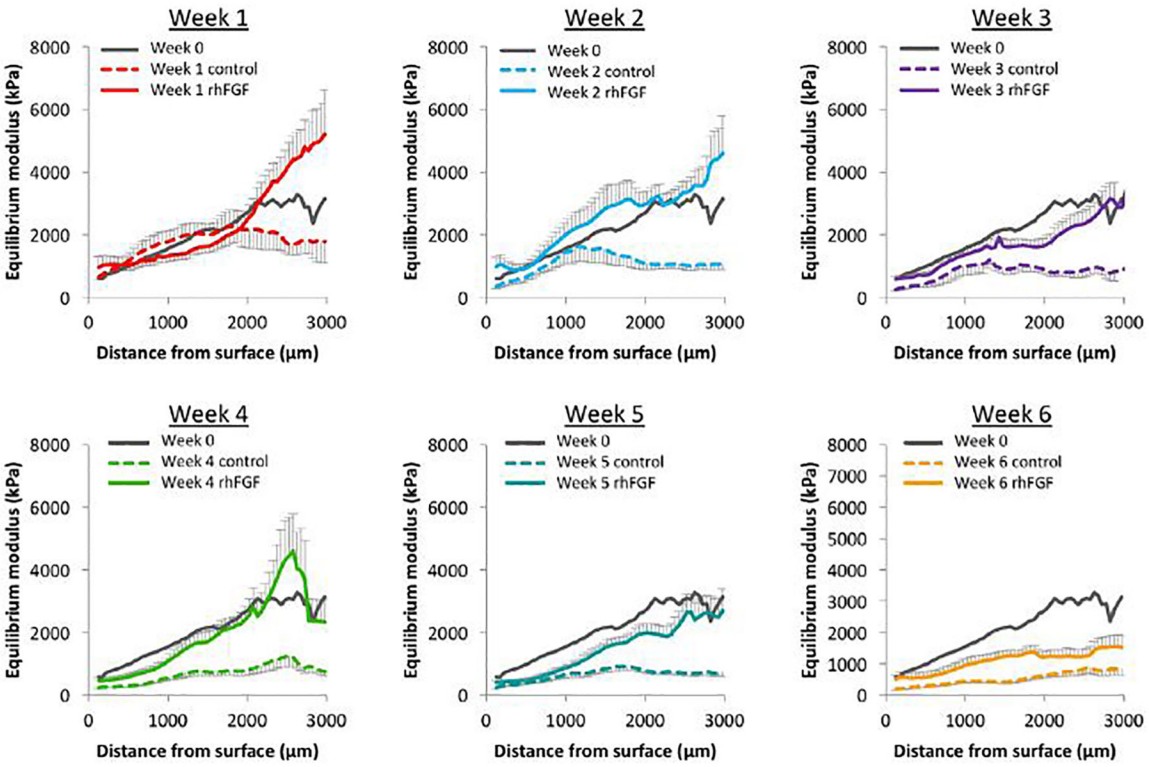


**Fig. 3.**

Depth-dependent equilibrium modulus of AC explants cultured *in intro* for 0–6 weeks. Baseline modulus through the depth is shown in grey *(n* = 12–17).

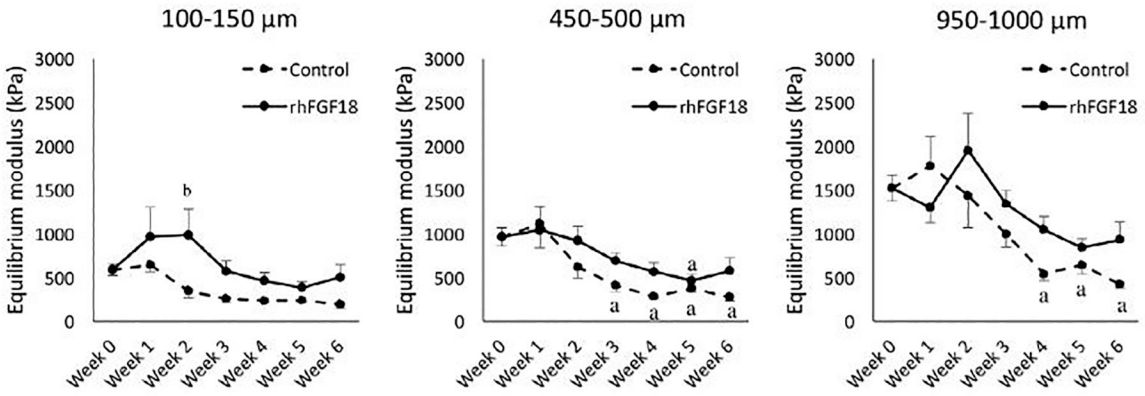


**Fig. 4.**

Mean equilibrium modulus of AC explants at distances of 100–150,450–500 and 950–1,000 pm from the AC surface over the course of 6 weeks of *in vitro* culture. [a] $p < 0.05$ *vs.* week 0, [b] $p < 0.05$ *vs.* control.

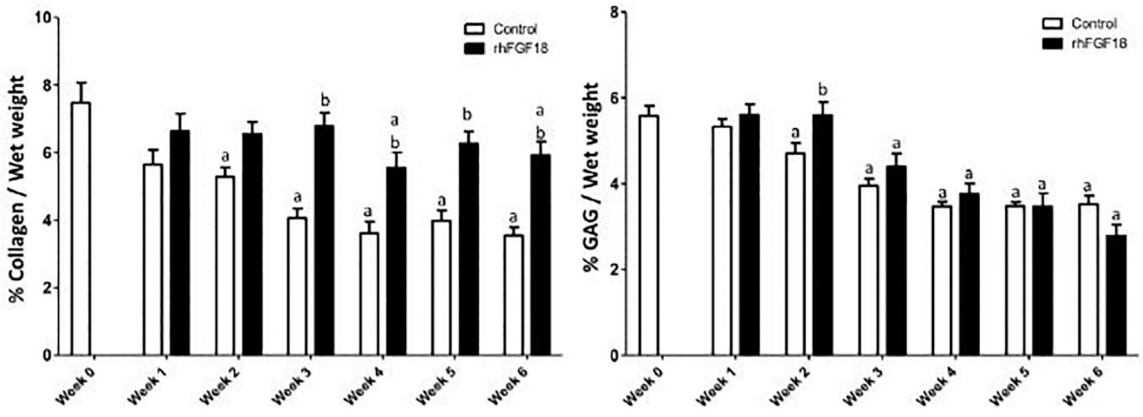


**Fig. 5.**

Collagen content as measured by the orthohydroxyproline assay and sulphated-GAG contented measured by the 1,9-dimethylmethylene blue assay for AC explants cultured *in vitro* for 0–6 weeks. Data were normalised to wet weight. [a] $p < 0.05$ *vs.* week 0 control, [b] $p < 0.05$ *vs.* control at same time point ($n = 19–31$).

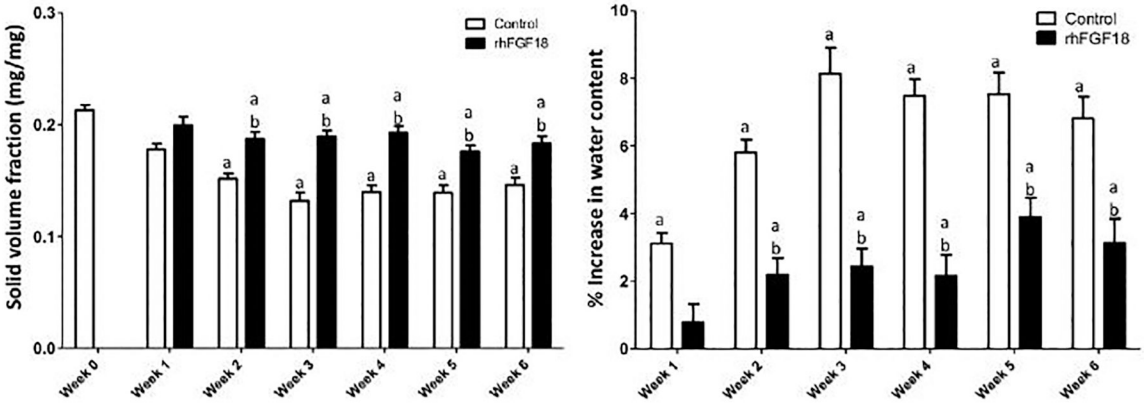


**Fig. 6.**

Increase in water content is attenuated with sprifermin treatment. [a] $p < 0.05$ *vs.* week 0 control, [b] $p < 0.05$ *vs.* control at same time point ($n = 19–31$).

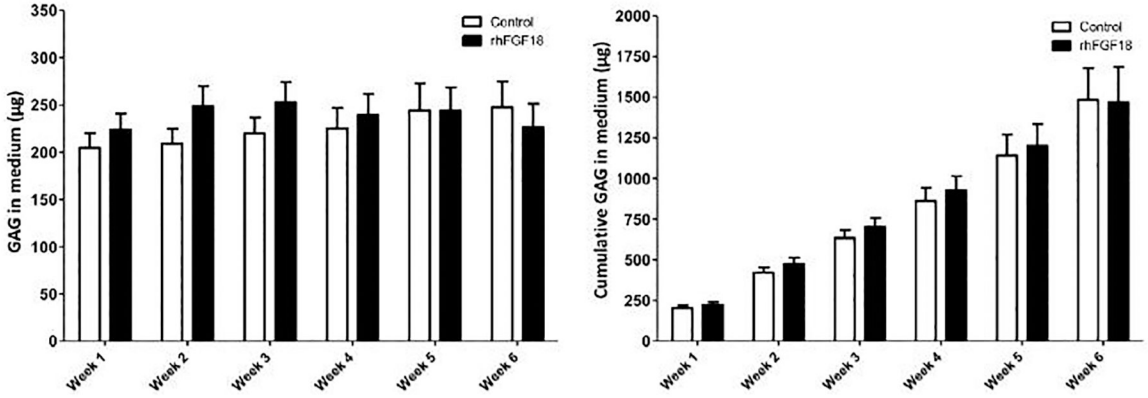


**Fig. 7.**

GAG released into the culture medium per explant and cumulative GAG in the medium over the course of 6 weeks of *in vitro* culture ($n$ = 7–25).

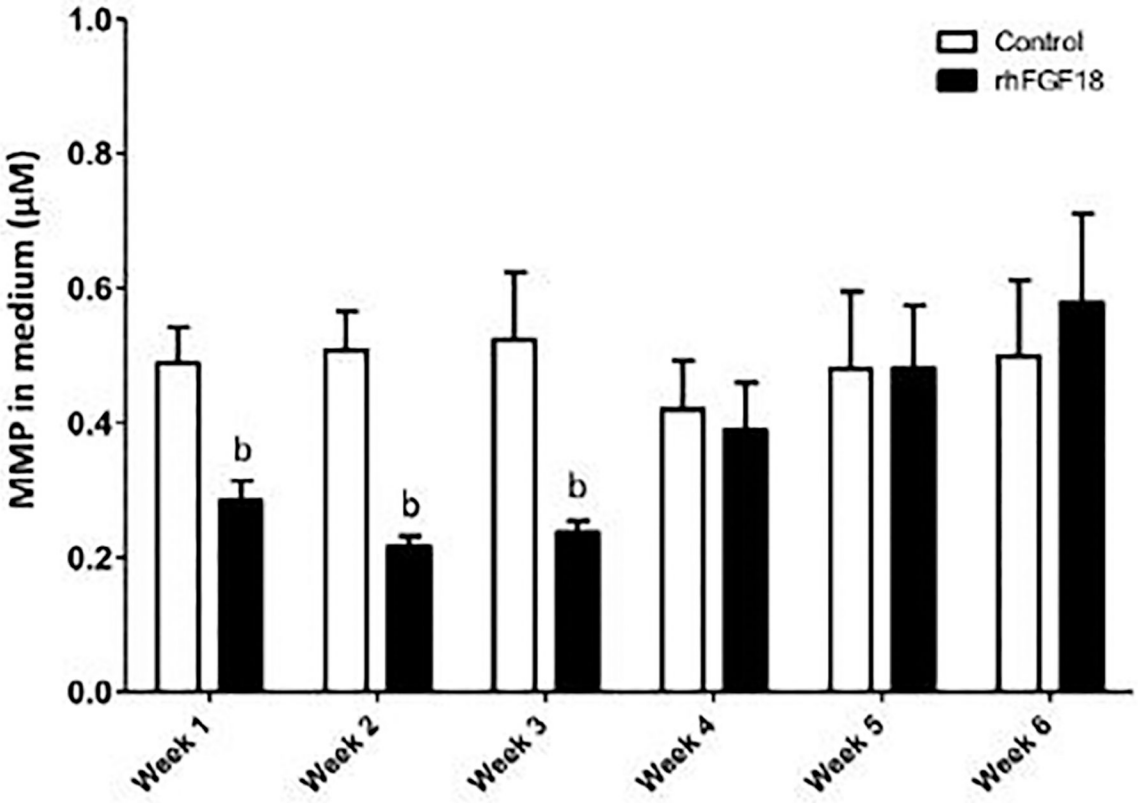


**Fig. 8.**

MMP concentration in the medium. MMP release into the medium was significantly suppressed with weekly sprifermin treatments through the first 3 weeks of exposure ($^{b}p < 0.05$ *vs.* control at same time point, $n = 7–25$).

**Table 1:**

N for various assays

| | | Collagen | GAG | Solid Vol | MMP | Media GAG | Normalized Bulk Modulus | Local Mechanics |
|---|---|---|---|---|---|---|---|---|
| Week 0 | Control | 23 | 23 | 19 | | | 23 | 15 |
| Week 1 | Control | 26 | 26 | 17 | 25 | 24 | 21 | 16 |
| | Sprifermin | 24 | 25 | 15 | 25 | 25 | 19 | 14 |
| Week 2 | Control | 26 | 26 | 15 | 21 | 21 | 20 | 15 |
| | Sprifermin | 27 | 27 | 17 | 21 | 21 | 21 | 16 |
| Week 3 | Control | 30 | 30 | 20 | 17 | 17 | 25 | 16 |
| | Sprifermin | 31 | 31 | 21 | 17 | 17 | 24 | 17 |
| Week 4 | Control | 19 | 19 | 19 | 13 | 13 | 19 | 15 |
| | Sprifermin | 19 | 19 | 19 | 13 | 13 | 17 | 13 |
| Week 5 | Control | 19 | 19 | 19 | 10 | 10 | 18 | 14 |
| | Sprifermin | 19 | 19 | 19 | 10 | 10 | 17 | 13 |
| Week 6 | Control | 23 | 23 | 18 | 7 | 7 | 21 | 12 |
| | Sprifermin | 24 | 24 | 19 | 7 | 7 | 24 | 15 |