

# Enhanced Integration with Treatment of Sprifermin (rhFGF18) in a Cartilage Injury-Repair Model

<sup>1</sup>Alexandra JE Farran

<sup>1</sup>Ryan A. Cocca

<sup>1</sup>Gregory Meloni

<sup>1</sup>Bhavana Mohanraj

<sup>2</sup>Anne Gigout

<sup>1</sup>Robert L. Mauck

<sup>1</sup>George R. Dodge

<sup>1</sup>University of Pennsylvania, Philadelphia, PA, USA

<sup>2</sup>Merck KGaA, Darmstadt, Germany

## Introduction

Osteochondral autograft transplantation (OAT) is a common procedure for the treatment of focal articular defects. Multiple factors likely influence the effectiveness of this procedure, including the source of donor cartilage, health of cartilage surrounding the defect site, and ultimately the degree to which integration occurs at the interface with native tissue. In animal models and in vitro experiments it has been shown that sprifermin (recombinant human FGF18, rhFGF18) promotes chondrocyte proliferation and extra-cellular matrix (ECM) biosynthesis,<sup>1</sup> and stimulates cartilage repair.<sup>2</sup> Since in many instances OAT procedures result in poor integration (due to low cellularity and fibrous tissue formation at the interface), we tested the hypothesis that addition of Sprifermin would increase cartilage-to-cartilage integration by increasing cell proliferation and ECM accumulation at the interface. We tested this hypothesis in an in vitro cartilage explant injury model and evaluated outcomes using mechanical, histological, and micro-computed tomography ( $\mu$ CT) assays of the interface.

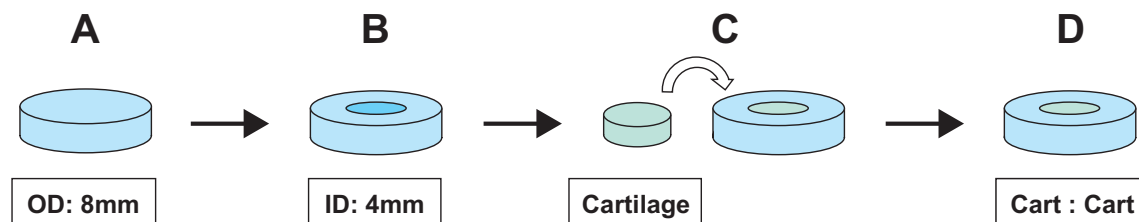
## Methods

Fresh hyaline cartilage was harvested from the trochlear groove of juvenile bovine knees (3-6 months old). Cylindrical explants (8mm, Figure 1A) were removed with a biopsy punch and cultured overnight in complete medium (DMEM 4.5g/L D-Glucose and L-Glutamine, 10% FBS, 1% PSF, 1% Fungizone, 1% MEM Vitamins, 25mM HEPES and 50 $\mu$ g/ml Vitamin C). Samples were trimmed of bone and defects (4mm diameter) were created to form a core and annulus repair construct (Figure 1B). Both the inner core and outer annulus were cultured

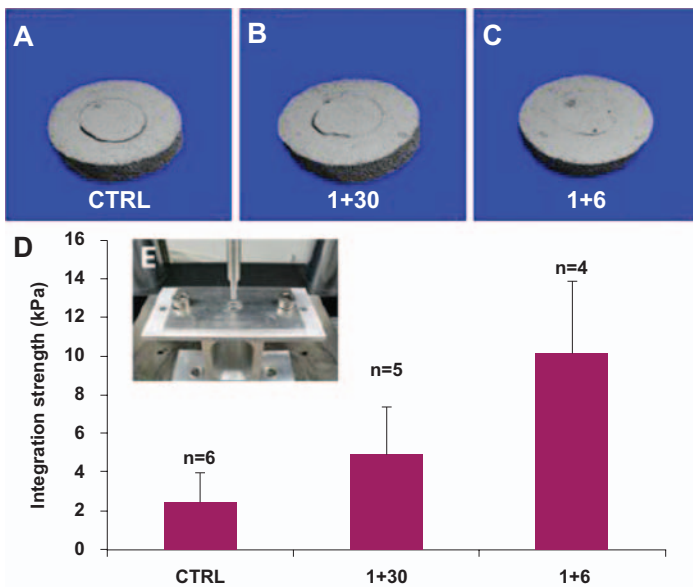
separately for 24 hours before the defect was filled with the original core. Samples were then cultured in complete medium, or treated with Sprifermin (rhFGF18, 100 ng/ml). Treatments consisted of one dose of rhFGF18 for 24 hours, applied once a week (and repeated weekly) (1+6) or one 24 hour treatment followed by 1 month of culture in complete medium (1+30 days). Samples were harvested after 4 weeks of culture. Push-out mechanical testing (n=4-6) was performed (Instron 5848, Instron, Norwood, MA) using a custom testing rig (Figure 2E<sup>3</sup>). Integration strength was calculated by dividing the peak force by the integration area. For 3D visualization, samples (n=6) were soaked in a modified Lugol's solution (2.5% I<sub>2</sub> and 5% KI in dH<sub>2</sub>O) for 24 hours<sup>4</sup> and scanned by  $\mu$ CT at an energy level of 55kV and intensity of 145 $\mu$ A with a voxel size of 6 $\mu$ m and 10.5 $\mu$ m ( $\mu$ CT 35 and vivaCT 40, SCANCO Medical, Wayne, PA). Scans were analyzed and reconstructed using the manufacturers software, and cross sections were used to evaluate defect integration. Additional samples (n=3) were fixed overnight in 4% PFA and analyzed histologically for cell and matrix deposition at the interface.

## Results

The integration strength (Figure 2D) of control samples was the lowest ( $2.5 \pm 1.4$  kPa), with progressively increasing properties with the 1+30 ( $5.0 \pm 2.4$  kPa) and 1+6 ( $10.2 \pm 3.7$  kPa) treatments. While the results are striking when comparing controls and treated groups, with the replicate numbers possible in this study, statistical significance was not achieved.  $\mu$ CT analysis of control constructs (Figure 3, top left) showed a distinct dark circle, indicating separation between the outer annulus and inner



**Figure 1.** Preparation of cartilage defect-repair model: (A) 8mm cartilage plug, (B) central 4mm defect creation, (C) insertion of cartilage into defect, and (D) long-term culture of repair construct.

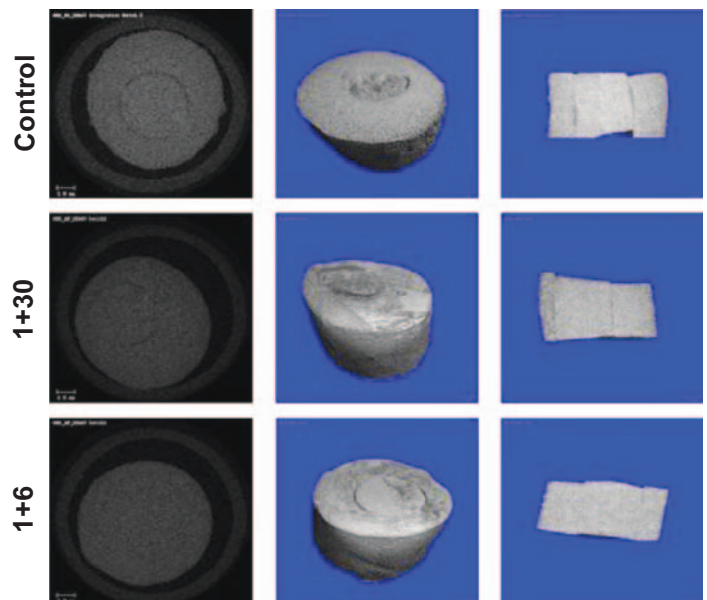


**Figure 2.** (A-C) Transverse cross sections of 3D  $\mu$ CT reconstruction with different treatments. (D) Integration strength of the repaired defect showing increasing strength from the control to the 1+30 treatment to the 1+6 treatment. (E) Experimental setup of the push-out testing rig. Error bars are SEM.

core, and thus poor integration. The 1+30 treatment (Figure 3, middle left) showed a less distinct circle, suggesting a smaller gap and greater integration, and the 1+6 treatment (Figure 3, bottom left) showed very homogenous  $\mu$ CT signal across the interface, indicative of the greatest degree of integration. Evidence of this increased integration was apparent on both vertical and transverse cross sections throughout the samples.

## Discussion

A successful cartilage repair requires that the repair material (engineered or native) be well-integrated into the surrounding cartilage to ensure continuous load transfer (and lack of stress concentrations) across the interface. In this study we investigated the potential of Sprifermin to enhance integration of cartilage in a well-defined ex vivo (explant) cartilage repair model. Sprifermin has an established proliferative effect on chondrocytes,<sup>1</sup> where transient (24 hour) exposure to this biologic agent elicits the most striking response. Using this dosing regimen, our findings clearly demonstrate that Sprifermin improves integration strength and matrix deposition at the interface (as evidenced by contrast-enhanced  $\mu$ CT showing a more uniform attenuation by increase in GAG-containing proteoglycans). In this study, one 24 hour administration weekly for 4 weeks leads to an overall better outcome than one 24 hour treatment over one month. This study represents for the first time a biologic (and



**Figure 3.**  $\mu$ CT scans of cartilage-to-cartilage repair constructs. Left: single  $\mu$ CT scan slice representative of the sample. Center: 3D reconstruction. Right: cross-section of the reconstruction. The  $\mu$ CT scans demonstrate increasing integration from control to 1+30 to 1+6 treatments.

in particular an FGF) has improved the integration of cartilage surfaces in a clinically relevant repair model.

## Significance

This study demonstrates that the biologic Sprifermin improved the integration of cartilage surfaces in a model of cartilage repair. The findings implicate its potential usefulness in surgical procedures such as OATS and in tissue engineering approaches where cartilage like biomaterials will be required to successfully integrate with native cartilage in order to achieve clinical success.

## Acknowledgments

This work was supported by Merck KGaA.

## References

1. Ellsworth JL, et al. Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. *Osteoarthr. Cartil. OARS Osteoarthr. Res. Soc.* 10, 308–320 (2002).
2. Moore EE, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthr. Cartil. OARS Osteoarthr. Res. Soc.* 13, 623–631 (2005).
3. Erickson IE, et al. Improved cartilage repair via in vitro pre-maturation of MSC-seeded hyaluronic acid hydrogels. *Biomed. Mater. Bristol Engl.* 7, 024110 (2012).
4. Palmer AW, Goldberg RE, Levenston ME. Analysis of cartilage matrix fixed charge density and three-dimensional morphology via contrast-enhanced microcomputed tomography. *Proc. Natl. Acad. Sci. U. S. A.* 103, 19255–19260 (2006).