Sprifermin (rhFGF18) Preserves Articular Cartilage Properties During In Vitro Culture

Alexandra JE Farran¹, Ryan A. Cocca¹, Gregory Meloni¹, Bhavana Mohanraj², Anne Gigout¹, Robert L. Mauck¹, George R. Dodge¹. ¹University of Pennsylvania, Philadelphia, PA, USA, ²Merck KGaA, Darmstadt, Germany.

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].

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 (1+6 treatment). Over six weeks, explant mechanical properties and molecular aspects were evaluated. Explants were tested in unconfined compression to determine equilibrium (10% strain, stress relaxation) and dynamic (1% strain, 1Hz) compressive properties [7]. For each explant, GAG content was determined using the DMMB assay [N=4 for Day 0, N=5 for all other time points per donor]. Media was harvested at each media change, and GAG release and MMP activity were evaluated using the DMMB assay and the SensoLyte® 520 Generic MMP assay kit (Anaspec), respectively [N=2]. Error bars in all figures are SD. Statistical analysis consisted of a 2-way ANOVA with Bonferroni post-tests (*p<0.05, **p<0.01, ***p<0.001).

Results: The equilibrium and dynamic moduli (Fig1A and 1B) of rhFGF18 treated explants did not change over the first week of culture (EY=1285 at Day0, 1313 at Wk1; G*=14511 at Day0, 13648 at Wk1), whereas the untreated explants decreased (EY=975, G*=11628 at Wk1). After the first week, both treated and untreated explants decreased in properties, with the treated samples higher than untreated samples through week 3 (Untreated: EY=563, G*=7797 at Wk3; Treated: EY=751, G*=11287 at Wk3). By 4 weeks, no statistical differences were seen between the treated and untreated groups. At week 6 however, treated samples had a lower EY but the same G* as the untreated explants (Untreated: EY=568, G*=7339 at Wk6; Treated: EY=247, G*=7382 at Wk3).

Discussion: The mechanical integrity of cartilage explants cultured in vitro decreases as early as one week after isolation, stabilizing by week 2 at ~ 40% of its original level (Fig 1A and 1B). This well-known phenomenon [8] may relate in part to loss of proteoglycan from cut surfaces [9]. When explants were cultured in serum-containing media with Sprifermin, properties were preserved during the first three weeks of culture. These differences could not solely be explained by the GAG content which was very similar in cartilage from both groups. Of note, collagen was higher in treated samples at these time points, coincident with the finding that MMP activity of treated explants was 8.7 and 5 times less than that of the untreated explants (Fig 3A). This suppression of MMP activity by rhFGF18 dissipated after the fourth week, with both groups having the same level of MMP activity.
activity. This correlates with the increase in collagen content seen in the treated explants starting from week 2 (Fig 3B), which in turn could explain the higher mechanical properties of treated explants through week 3 (Fig 1A). Taken together, our results showed that Sprifermin has an inhibitory effect on MMP activity, which in turn suppressed for 3 weeks the degradation of explant collagen. This study illustrates the potential of Sprifermin to preserve cartilage explant mechanical properties over a clinically relevant time course during which testing occurs prior to allograft implantation.

Significance: Sprifermin has the potential to preserve the mechanical integrity of cartilage explants cultured in vitro. Hence the current practice of storing allografts in the cold could be replaced by in stable maintenance of important cartilage properties in the presence of rhFGF18 during the time period required for safety screening.

Acknowledgments: This work was in part supported by Merck KGaA.
