Distinct Effects Of Platelet-rich Plasma And Bmp13 On Rotator Cuff Tendon Healing In A Rat Model

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Introduction: Tissue engineering may enhance the biological environment of rotator cuff healing and reduce the incidence of re-tears after surgical repair. Platelet-rich plasma (PRP) is an autologous concentration of platelets and growth factors including TGF-ß, VEGF, PDGF, FGF-2 and IGF-1. PRP is used for orthopaedic indications including augmentation of rotator cuff repair. However, definitive functional improvement has not been proven. Bone morphogenetic proteins (BMPs) 12, 13 and 14 have each demonstrated the ability to promote formation of tendon in rats and may improve tendon healing. Of these, BMP13 has been shown as the most tenogenic and least osteogenic. We examine the molecular effects of exogenous delivery of BMP13 compared to the growth factors found in highest concentrations in PRP (TGF-ß1, VEGF and PDGF) in tenocytes. We also compare the differential effects of PRP and BMP13 on the biomechanical properties of injured rat supraspinatus tendons.

Methods: Tenocytes were isolated from the Achilles tendon of 4-wk CD-1 mice, minced and digested in PBS with 1% penicillin/streptomycin solution with 3 mg/ml collagenase type I and 4 mg/ml dispase for 1h. Cells were plated at equal confluency and treated AdBMP13, AdTGF-ß1, AdVEGF-A, AdPDGF-BB or AdGFP in equal titers determined by dose titration with visualization under fluorescence. RNA was isolated from tenocytes at 5d after infection with the indicated adenovirus using TRIZOL reagents. RT-PCR was performed and cDNA products generated for sqPCR analysis. Gene expression levels were normalized with GADPH and amplified with mouse gene-specific primers. sqPCR data was analyzed by densitometry of gel bands using ImageJ.

Two donor Sprague-Dawley rats were used to demonstrate continuing BMP13 or GFP expression in vitro. Harvested tendons were cultured in vitro in complete DMEM with 10% FBS following direct injection with 10 µl (10^8 pfu) of AdBMP13 or AdGFP. PRP was harvested via intracardiac puncture. Concentrated platelets achieved a final concentration of 10^9/mL and were irradiated and activated with thrombin. 32 Sprague-Dawley rats were divided into 4 groups of 8 rats: AdGFP control, PRP, AdBMP13, and PRP+AdBMP13. Under standard anesthesia, the supraspinatus tendon was exposed through a deltoïd-splitting approach followed by division of the acromioclavicular joint. A 2x2 mm defect was created using a sterile punch at the insertion of the supraspinatus tendon. Under direct visualization, the supraspinatus tendon at the defect was injected with either 10 µl of AdGFP (108 pfu), 10 µl of PRP (10^9 platelets/mL), 10 µl of AdBMP13 (10^8 pfu) or 10 µl of PRP (10^9 platelets/mL) + 10 µl of AdBMP13 (10^8 pfu). All rats were sacrificed at 2 weeks and supraspinatus tendons harvested for biomechanical testing. Maximum stress-to-failure data was and stiffness for each specimen was recorded. A power analysis demonstrated that a strength increase of 20% between groups would be considered clinically significant using 8 specimens per group with α= 0.05.

Nonparametric statistical methods (Mann-Whitney U tests) were used for the analysis of the biomechanical data. Microsoft Excel was used to calculate standard deviations and statistically significant differences of band densitometric analysis of tendon matrix- and gene-associated genes. All in vitro experiments were performed in triplicate.

Results: We analyzed the molecular effects of AdBMP13 and each of the growth factors found in high concentrations in PRP (TGF-ß1, VEGF-A or PDGF-BB) on the expression of 4 major genes involved in tendon healing. cDNA levels were standardized to GAPDH demonstrating no difference between treatment groups and control (p>0.20). Only treatment with AdBMP13 up-regulated type III collagen (COL3A1) expression compared to AdGFP control (p<0.01).

Furthermore, COL3A1 was up-regulated when treated with AdBMP13 compared to each of the PRP growth factors tested (p<0.01). Treatment with AdBMP13, AdTGF-ß1, AdVEGF-A or AdPDGF-BB up-regulated fibronectin expression compared to AdGFP control (p<0.01). Furthermore, fibronectin was more up-regulated when treated with AdBMP13 compared to each of the growth factors of PRP tested (p<0.05). Treatment with AdBMP13, AdTGF-ß1 or AdVEGF-A significantly up-regulated both tenascin C and scleraxis expression compared to AdPDGF-BB or AdGFP (p<0.05).

Tendons harvested for in vitro direct injection of BMP13 were visualized under fluorescent microscopy at 7 and 14 days post-harvest and demonstrated GFP signal consistent with continuing gene expression at each time point. All tendons were found to fail at the bone-tendon interface, and none failed by intra-substance tear. The mean stress-to-failure was higher in the intact, untreated tendons (23.5 ± 1.3 MPa) compared to GFP control (10.1 ± 3.1 MPa, p<0.01), PRP (13.9 ± 1.76 MPa, p<0.01) and BMP13 (18.1 ± 2.8 MPa, p<0.05) but not PRP+BMP13 (20.0 ± 4.7 MPa, p=0.38). Compared to the GFP control, the mean stress-to-failure was higher in PRP (p<0.05), BMP13 (p<0.01) and PRP+BMP13 (p<0.01). Compared to PRP alone, the mean stress-to-failure was higher in BMP13 (p<0.01) and PRP+BMP13 (p<0.05).

The mean stiffness was higher in the intact, untreated tendon (27.6 ± 5.2 MPa) compared to GFP control (10.7 ± 3.5 MPa, 0.7 ± 3.5 MPa).
p<0.01), PRP (14.4 ± 2.9 MPa, p<0.01) and PRP +BMP13 (19.3 ± 7.2 MPa, p<0.05) but not BMP13 (22.7 ± 7.7 MPa, p=0.30) (Table 4B). The mean stiffness was higher in the PRP, BMP13 and PRP+BMP13 groups compared to GFP control (p<0.05). There was no significant difference in mean stiffness between PRP, BMP13 or PRP+BMP13.

**Discussion:** First, we showed that BMP13 expression continues for up to 2 weeks following direct injection into the supraspinatus tendon, providing visual confirmation and validation of sustained adenoviral delivery of BMP13. Our tenocyte gene expression analysis suggests that compared to the major growth factors in PRP, BMP13 up-regulates expression of tendon cell- and matrix-associated genes known to be critical in the early tendon healing process. Our biomechanical analysis demonstrated that treatment with PRP and BMP13, in combination or alone, increases the stress-to-failure and stiffness of the supraspinatus tendon during the first two weeks following injury compared to GFP control. Furthermore, BMP13 increased stress-to-failure significantly more than PRP alone. While we found no significant differences in stress-to-failure or stiffness between BMP13 and BMP13+PRP treatment groups, we did find significant differences in stress-to-failure when comparing BMP13 or BMP13+PRP to PRP alone. Thus, the enhanced tensile strength appears to be attributable to BMP13 and not PRP.

**Significance:** Adenoviral delivery of BMP13 significantly increases supraspinatus tendon stress-to-failure during the early stages of healing following injury. BMP13 also significantly increases expression of genes with critical roles in the healing process following tendon injury. While we demonstrate beneficial effects of PRP on tendon healing, BMP13 was superior to PRP. Our biomechanical and molecular results provide strong evidence that BMP13 may be a better therapeutic alternative for intraoperative surgical augmentation than PRP.

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3. Carpenter JE, Thomopoulos S, Flanagan CL, DeBano CM, Soslowsky LJ. Rotator cuff defect healing: a biomechanical and
histologic analysis in an animal model. Journal of shoulder and elbow surgery / American Shoulder and Elbow Surgeons [et al.
Stress-to-Failure

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<th>PRP</th>
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Stress-to-Failure (U-test)

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*p<0.05; **p<0.01