The Anti-inflammatory and Matrix Restorative Mechanisms of Platelet Rich Plasma in Osteoarthritis.

[1]Sundman EA; [2]Cole BJ, [2]Karas V; [2]Della Valle CJ, +[1]Fortier LA
+[1]Cornell University, Ithaca NY, [2]Rush University Medical Center, Chicago IL
Laf4@cornell.edu

Introduction: Pain and dysfunction associated with osteoarthritis (OA) is associated with major loss of performance and expensive treatments that are often ineffective. *The broad objective* of the studies in this study were to investigate the ability of platelet rich plasma (PRP) to modulate an OA environment. PRP is a relatively inexpensive, simple, efficient, and minimally invasive method of obtaining a natural concentration of autologous growth factors and other bioactive molecules that enhance tissue repair, diminish pain, and restore joint function. Cartilage and synovium were obtained from patients undergoing total knee arthroplasty and cultured with hyaluronic acid or PRP. We aimed to In this study, For the studies outlined in this proposal, we determine if either of the treatment choices decreased joint pain and/or improve the articular environment through decreased catabolism and increased anabolism in synoviocytes and articular cartilage.

Methods: Human knee bone, cartilage, and joint capsule (n=21) were procured by Rush University Medical Center from patients undergoing total knee arthroplasty, de-identified, and shipped overnight to Cornell University. Cartilage was diced into 3x5x5 mm explants, and incubated overnight in DMEM + 10% FBS. Synovium was dissected from the joint capsule, digested in collagenase, filtered, and cells were plated with DMEM + 10% FBS and allowed to adhere overnight. Synoviocyte / cartilage co-cultures were then established with hanging well inserts. Treatments groups were established with hyaluronan (HA; 2.5mls SYNVISC (Genzyme, Cambridge, MA) with 1.5mls media), ACP (2.5mls ACP with 1.5mls media), and control (4mls media) (Figure 1).

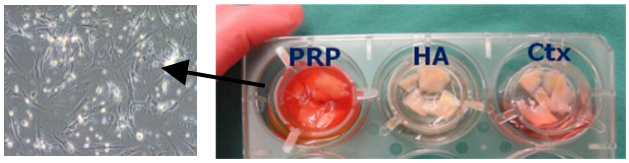

Figure 1. Co-cultures of synoviocytes (left) on the bottom of the well and cartilage (right) suspensions with culture medium of platelet rich plasma (PRP), hyaluronic acid (HA), or control medium (Ctx).

A cartilage sample was also retained for time zero analysis and for routine histologic processing with Mankin scoring by two individuals. To generate PRP, venous blood was obtained from 21 healthy volunteers and processed using the Double Syringe Autologous Conditioned Plasma System (Arthrex Inc, Naples, FL). Co-cultures were incubated for 96 hours. Media were snap frozen at -80. Synoviocyte and cartilage RNA were isolated using the PerfectPure Fibrous Tissue Kit (5Prime, Gaithersburg, MD and used for quantitative PCR. Cartilage RNA was assayed for Collagen Type 1 α1 (COL1A1), COL2A1, aggrecan, lubricin, matrix metalloproteinase-3 (MMP-3), interleukin 1β (IL-1β), MMP-13, and 18S. Synoviocyte RNA was assayed for hyaluronan synthase 1 (HAS-1), HAS-2, MMP-1, MMP-3, tumor necrosis factor α (TNF-α), MMP-13, and 18s. ΔCT results were normalize to 18S. Statistical analysis was performed using a one-way ANOVA and Tukey's post-hoc test with Statistix 9 (Analytical Software, Tallahassee, FL).

Results: The average Kellgren score for OA was 3.8. The average Mankin score on cartilage sections was 2.5. In synoviocytes, HAS2 was significantly increased in PRP treated co-cultures compared to control and HA groups and there was no difference between control and HA (Table 1). MMP-13 was significantly decreased in PRP treated co-cultures compared to control and HA groups (Table 1). There was no significance difference between control and HA. There were no significant differences between the effects of control, PRP, or HA treatment in expression of HAS1, MMP-1, MMP-3, TNF-α ($p>0.05$).

Table 1. Synoviocyte gene expression in co-cultures of synoviocytes and cartilage treated with platelet rich plasma (PRP) or hyaluronic acid (HA). Data are expressed as the mean of 2^{-ddCT} ± sem. Letters denote groups with significant differences ($p<0.05$).

Gene of interest	Treatment Group		
	control	PRP	HA
HAS1	1 ± 0	1.4 ±0.25	1.40±0.19
HAS2	*1 ± 0[a]*	*26.4±8.8[b]*	*2.01±0.40[a]*
MMP-1	1 ± 0	1.31±0.42	1.10±0.51
MMP-3	1 ± 0	2.67±1.05	0.86±0.27
MMP-13	*1 ± 0[a]*	*0.29±0.13[b]*	*0.77±0.13[a]*
TNFα	1 ± 0	2.19±1.17	3.48±1.25

In the cartilage, COL2A1 expression was decreased in PRP-treated groups compared to control, but the effect was not significantly different from that of HA (Table 2). In contrast, COL1A1 was significantly decreased by HA compared to control and PRP treated groups. There was no significant difference in expression of aggrecan, IL-β, MMP-3, or MMP-13 in cartilage samples.

Table 2. Cartilage gene expression in co-cultures of synoviocytes and cartilage treated with platelet rich plasma (PRP) or hyaluronic acid (HA). Data are expressed as the mean of 2^{-ddCT} ± sem. Letters denote groups with significant differences ($p<0.05$).

Gene of interest	Treatment Group		
	control	PRP	HA
Aggrecan	1 ± 0	0.83±0.14	1.18±0.15
COL2A1	1 ± 0[a]	0.26±0.14[b]	0.94±0.42[ab]
COL1A1	1 ± 0[a]	1.35±0.59[a,b]	0.35±0.13[b]
IL-1β	1 ± 0	4.121±3.08	3.52±1.63
MMP-3	1 ± 0	4.06±2.65	3.04±1.92
MMP-13	1 ± 0	4.06±2.65	7.40±4.97

Discussion: In OA cartilage, aggrecan gene expression is decreased while COL2A1 and COL1A1 are increased.[1] Presuming this is the disease state, a return of matrix gene expression to baseline might be considered desirable in the treatment of OA. Our results indicate that PRP dampens the upregulation of COL2A1 and HA has a similar effect on COL1A1 expression. In OA synoviocytes, MMP-13 is known to be significantly upregulated[2] and HAS1 & 2 are downregulated[3]. Our data indicate that PRP significantly decreases MMP-13 expression, but HA has no effect. In addition, it appears that PRP, but not HA, stimulates hyaluron synthesis as indicated by an upregulation of HAS2. These data indicate that PRP and HA have some effect on returning cartilage matrix gene expression to normal. However, only PRP is capable of modulating synoviocyte expression to decrease expression of catabolic MMP-13 and to stimulate HA synthesis. This would suggest that PRP is superior to HA in restoring the OA environment to a more normal state with less inflammation/pain and increased lubrication.

Significance: This study indicates that intra-articular PRP administration might be more beneficial than HA in patients affected with OA.

References: [1]Brew CJ, et al. Ann Rheum Dis. 2010;69:234-40. [2]Davidson RK, et al. Arthritis Res Ther 2006;8:R124. [3]Yoshida M, et al. Arthritis Res Ther 2004,6:R514-R520.

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