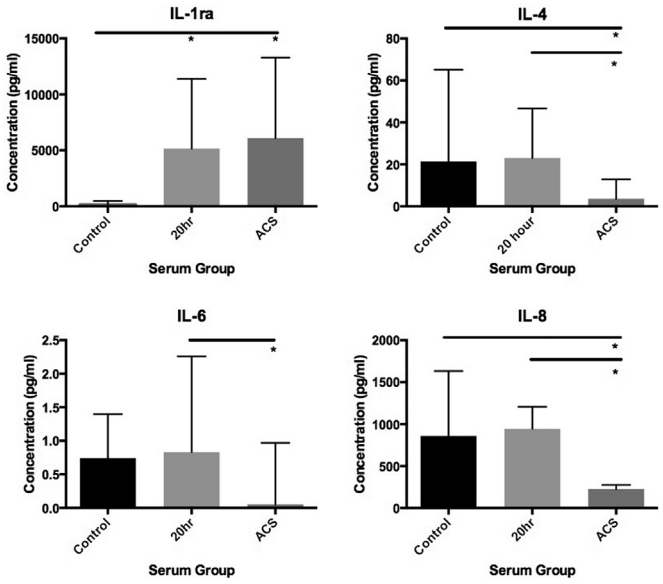

Figure 1Median concentrations (with range) of IL-1ra, IL-4, IL-6, and IL-8 cytokines between: (1) unconditioned, unincubated control serum (control), (2) unconditioned, incubated control serum (mean 20hr), (3) autologous conditioned serum. (*) denotes statistical significance ($p < 0.05$).

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AN EXAMINATION OF GROWTH FACTOR LEVELS IN PLATELET-RICH PLASMA AND CORRELATIONS WITH AGE, GENDER, PLATELET COUNT, AND DHEAS

A. Osawa †, Y. Maruyama †, K. Kaneko ‡. † *Juntendo Univ. Urayasu Hosp., Urayasu, Japan;* ‡ *Juntendo Univ., Tokyo, Japan*

Purpose: Platelet-Rich Plasma (PRP) is used as an injective treatment or as a surgical augmentation procedure for the repair of tissue with low healing potential. PRP contains a number of different growth factors such as TGFb1, VEGF, EGF, and PDGF. It is clear that PRP has a positive role in tissue repair. However, up to now, there have been only a few reports examining the correlation between growth factor and individual differences. The aim of this study is to analyze the correlation between varying factors: age, gender, and platelet count with growth factor level for the purpose of healing potential. This study also introduces a fourth difference, DHEAS, which is a steroid hormone and is used as an aging marker to show its correlation with growth factor level.
Methods: This study was approved by the Institinal Review Board and the local Ethics Commmittee. Thirty-two healthy volunteers were enrolled to provide a blood sample. These subjects included 12 males and 20 females within an age range of 19 to 59. The collected blood was then drawn from the collection tube into an Arthrex ACP (PRP) double syringe system (Arthrex systems, Naples, Florida) and spun at 1500 rpm for 5 minutes in a centrifuge (Hettich Rotofix 32, Tuttlingen, Germany) as described by the standard manufacturer protocol. Aliquots of whole blood were measured for platelet concentrations and DHEAS. Four growth factors were analyzed in this study using enzyme-linked immunosorbant assay kits specific for each growth factor (R & D systems). TGFb1, VEGF, EGF and PDGF were measured through the manufacturer's recommendations. All standards and samples were run in duplicate. A Mann-Whitney rank-sum test was conducted to examine the relationship with each growth factor. Pearson's correlations coefficients were used to test the correlation between age, platelet count, DHEAS and each of the four growth factors.
Results: In this study, the tested samples of male and female subjects showed a significant difference in terms of TGFb1 ($p<0.05$). In terms of age, younger subjects displayed an increased TGFB and PDGF in comparison to elder subjects. DHEAS was correlated with PDGF and PLT was correlated with VEGF.
Conclusions: This study shows correlations between varying growth factors and individual differences. However, as the sample group was relatively small, more research needs to be done to confirm these findings. Furthermore, there remains a need to examine the healing potential in vivo.

Therapy: Intraarticular

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ADMINISTRATION OF A SINGLE INTRAARTICULAR DOSE OF DEXAMETHASONE MITIGATES THE IMPACT OF PTOA-LIKE CHANGES: A HISTOLOGIC STUDY IN A RABBIT MODEL

B.J. Heard †·‡, K.I. Barton †·‡, M. Chung †·‡, Y. Achari †·‡, C.B. Frank †·‡, N.G. Shrive †·‡, D.A. Hart †·‡. † *Univ. of Calgary, Calgary, AB, Canada;* ‡ *The McCaig Inst. for Bone and Joint Hlth., Calgary, AB, Canada*

Purpose: Despite surgical restoration of joint stability through the reconstruction of an injured anterior cruciate ligament, many patients still develop post traumatic osteoarthritis (PTOA). It has been hypothesized that the surgery itself may be a secondary injury to the joint that increases the risk for development of PTOA. In a rabbit model created to investigate the impact of surgery on the joint, while not directly impacting any of the stability providing structures, it was found that this drill hole surgery alone was enough to induce PT-OA in a short time period (6 weeks). The aim of the present study was to investigate the efficacy of a single dose of Dexamethasone (DEX), a potent glucocorticoid currently used to treat many inflammatory disorders, administered at the time of surgery on the integrity of the infrapatellar fat pad (IFP), synovium (SYN), and cartilage within the joint. As evidence is mounting that the acute inflammatory phase after traumatic injury to the joint may be in part responsible for the future development of PTOA, we hypothesize that the mitigation of the acute phase through the use of a potent anti-inflammatory drug will have a positive effect on these key joint tissues.
Methods: Thirty, one year old female albino New Zealand white rabbits were randomly allocated to five groups (each group n=6) to investigate two post surgical time points (48 hours and 9 weeks): Non operated control (CTRL), 48 hour drill (D48h), 48 hour drill + DEX (D+D48h), 9 week drill (D9wk), and 9 week drill + DEX (D+D9wk). The drill injury was inflicted using a rabbit intraarticular (IA) bone injury model created and described by Huebner et al. (2013). Briefly, surgical joints (of the hind right limbs) were opened laterally and drilled twice (drill hole 1.1mm) into the marrow cavity in a non-cartilaginous area of the femoral notch, allowing for bleeding into the joint space. The joint capsule and skin were both closed using standard suturing procedures. The D+D48h and D+D9wk joints received one IA administration of DEX (0.5mg/kg) as soon as the joint capsule had been closed. Upon sacrifice, IFP, SYN from the patella-femoral area, and four areas of cartilage (medial and lateral femoral condyles and tibial plateau) were harvested, embedded in OCT, cut and stained (H&E for SYN and IFP, Saf-O + Fast green for cartilage), and then graded for inflammation / early PTOA like changes using previously described protocols. For each tissue, and at each time point, data were investigated in two separate analyses: change from normal (CTRL, Drill, and Drill+DEX: ANOVA with Bonferroni Post Hoc to account for multiple comparisons) and a direct comparison of Drill and Drill + DEX (Student's T-Test, two tailed). For all statistics significance was accepted at $P <= 0.05$.
Results: At 48 hours post surgery, significant inflammation was quantified histologically within the IFP and SYN of D48h joints. Grades for IFP and SYN of D+D48h joints were significantly lower than D48h in direct comparisons (Fig 1). SYN remained lower at 9 weeks post surgery, however D9wk and D+D9wk IFP histological scores were not different (Fig 1). It was found that the grades for cartilage of D9wk animals were greater than those of CTRL, and grades for cartilage of the lateral femoral condyle and tibial plateau were significantly lower in the D+D9wk joints than those of D9wk in direct comparisons (Fig 2).
Conclusions: While some inflammation is required for healing to occur, it appears that mitigating the acute inflammatory response with a single IA administration of DEX at the time of surgery (injury) has a protective capacity within key joint tissues. At 9 weeks post surgery, DEX treated joints exhibited significantly better histological grades for synovium and cartilage (lateral compartments) suggesting that mitigation of the acute phase inflammation could lead to better future joint health after traumatic joint injury.

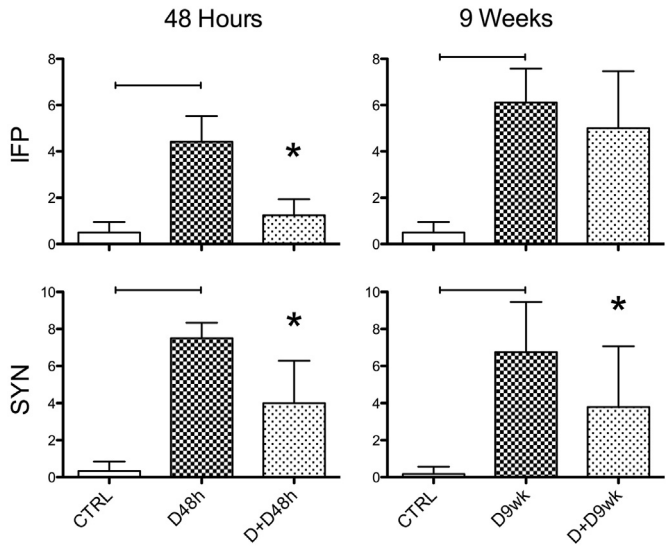

Fig 1. Histological Grading of the IFP and SYN (Mean +/- SD). Bar = statistical difference from group comparison. * = Single IA administration of DEX significantly lowered the histological grade in direct comparison between Drill and Drill+DEX.

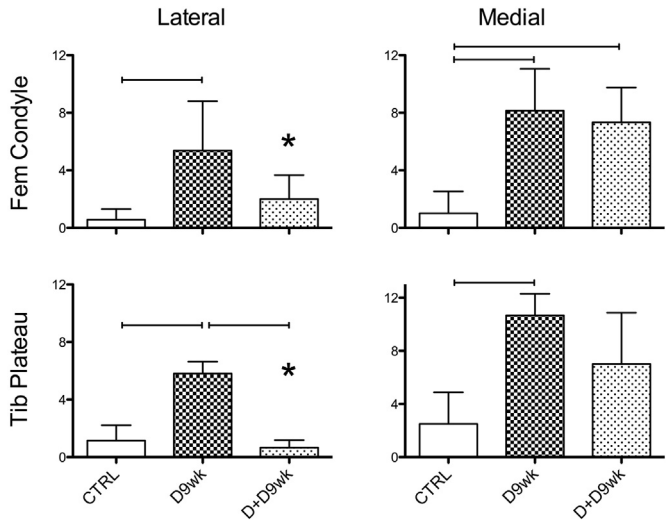

Fig 2. Cartilage Histology (Mean +/- SD). Bar = statistical difference from group comparison. * = Single IA administration of DEX significantly lowered the histological grade in direct comparison between Drill and Drill+DEX.

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INJECTION OF EXTRACELLULAR MATRIX GEL FOLLOWING ANTERIOR CRUCIATE LIGAMENT INJURY MITIGATES OSTEOARTHRITIC CHANGES IN A RAT MODEL

B.L. Proffen †, J.T. Sieker †, M.M. Murray †, K.E. Chin ‡, T.K. Patel ‡, E. Robbins ‡, M.R. Akelman ‡, J.T. Machan §, B.C. Fleming ‡. † *Boston Children's Hosp., Harvard Med. Sch., Boston, MA, USA;* ‡ *Warren Alpert Med. Sch. of Brown Univ./Rhode Island Hosp., Providence, RI, USA;* § *Rhode Island Hosp., Providence, RI, USA*

Purpose: To investigate whether the development of post-traumatic osteoarthritis (PTOA) is prevented or modulated in a rat model of anterior cruciate ligament (ACL) injury following a single intraarticular injection of an extracellular matrix (ECM) gel/autologous whole blood composite at day 0 or 14 after ACL injury.
Methods: ECM gels were prepared according to good-laboratory-practice guidelines from bovine source ECM. Thirty-two Lewis rats underwent unilateral ACL transection and either subsequent intra-articular injection of ECM gel/autologous whole blood composite on day 0 (n=11 rats, INJ0 group) or day 14 (n=11 rats, INJ14 group) or no further treatment (n=10, ACLT group), while ten additional animals received capsulotomy but no ACL transection (n=10, SHAM group). Before and 35 days after surgery gait analysis was performed using a pressure sensing walkway, followed by euthanasia and the assessment of PTOA-related phenotypes in cartilage, subchondral bone and synovial tissue. Specifically, histological scoring was performed according to the recommendations of the OARSI histopathology initiative, while the subchondral bone-mineral density was assessed using micro-computed tomography (micro-CT). The ratio of intact contralateral/treated hind limb maximum applied forces, was calculated for the pre-surgery and 5 weeks post-surgery timepoints. Then the difference of the pre and post-surgery ratios were compared between the groups. Further the differences of intact contralateral and treated tibial subchondral bone-mineral density (mg hydroxyappatit/cm3), as well as the tibial OARSI histological scores of the treated knees were compared between the groups using a one-way analysis of variance with post-hoc Bonferroni test for normally distributed and Kruskal Wallis test with Dunn post-hoc test for non-normally distributed data. Adjusted p values < 0.05 were considered statistically significant.
Results: Weight bearing on the operated limb was decreased by 15% in the ACLT group five weeks after surgery (P<0.05), while rats in INJ0 and INJ14 groups had only non-significant 3% and 10% decrease from their preoperative baseline values, respectively (P=0.44 and 0.18). All groups that have received ACLT regardless of the consecutive treatment demonstrated a loss of bone mineral density, with a loss of 99.7 mg/cm3 (95% CI 81.4, 117.9; P<0.001) in the ACLT, 115.1 mg/cm3 (95% CI 85.1, 145.0; P<0.001) in the INJ0, and 93.8 (95% CI 72.3, 115.3; P<0.001) in the INJ14 group, which was significant in the comparison to the SHAM group, that did not demonstrate a loss of bone-mineral density (-4.6mg/cm3; 95% CI -21.4, 12.3). In the histological assessment, the cartilage degeneration sum score was statistically significantly worse in the ACLT group (7.5, 95% CI 7.0, 8.0) than in the SHAM (5.0, 95% CI 4.0, 6.0, P< 0.001), INJ0 (6.1, 95% CI 5.3; 6.9, P=0.031), and INJ14 group (6.0, 95% CI 5.3, 6.7; P=0.022), while the difference of INJ0 and INJ14 from SHAM was not considered statistically significant (P= 0.161 and 0.276, respectively). In the histological assessment of the synovium, no significant differences between the ACLT (2.6, 95% CI 2.0, 3.2) and the INJ0 (2.5, 95% CI 1.7, 3.4; P=1.0) or the INJ14 (3.1, 95% CI 2.7, 3.5; P=0.957) groups were detected in the histological synovial inflammation score, which were all significantly worse (all P<0.001) than SHAM (0.9, 95% CI 0.4, 1.4).
Conclusions: Injection of ECM gel/autologous whole blood composite reduced damage of cartilage surface after ACL transection, and resulted in fewer changes in gait asymmetry. However, the treatment did not prevent subchondral bone-mineral-loss or synovial inflammation. Early or delayed injection of ECM gel/autologous whole blood composite modulates the development of PTOA after ACL transection in a rat model, particularly in regards to the cartilage surface and joint function.

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EARLY INTRAARTICULAR TRIAMCINOLONE ACETONIDE ADMINISTRATION ALTERS ANTERIOR CRUCIATE LIGAMENT INJURY-INDUCED CHANGES IN SYNOVIAL MEMBRANE GENE EXPRESSION

J.T. Sieker †, U.M. Ayturk †, B.L. Proffen †, B.C. Fleming ‡, M.M. Murray †. † *Boston Children's Hosp., Harvard Med. Sch., Boston, MA, USA;* ‡ *Warren Alpert Med. Sch., Brown Univ. and Rhode Island Hosp., Providence, RI, USA*

Purpose: First, to define early alterations in gene expression (genes and pathways) within synovial membranes of a porcine post-traumatic osteoarthritis [PTOA] model. Second, to assess the efficacy of intra-articular triamcinolone acetonide to attenuate the synovial membrane's response to the surgical anterior cruciate ligament [ACL] injury.
Methods: Eighteen minipigs (mean weight of 33.4 kg) were allocated to unilateral ACL transection [ACLT] alone, unilateral ACLT and immediate injection of 20mg triamcinolone acetonide, or no surgery (n = 6 each group). At 14 days after ACLT, synovial membranes were harvested and used for RNA-sequencing (RNA-seq) to quantify transcriptome wide gene expression changes. Raw reads were mapped to the pig genome (Susscr3, released Aug. 2011) with the RNA-seq unified mapper (RUM) pipeline. Reads uniquely aligned to the exons of each gene were counted with a custom R script that utilizes Rsamtools and Genomics-Features packages, and used for differential expression analysis with the edgeR package. P-values were then corrected using a Benjamini & Hochberg approach to adjust for multiple hypothesis testing to adjust for the transcriptome-wide analysis. A filter was applied to exclude very