Effect of the Platelet Rich Plasma and Porcine Dermal Collagen Graft Augmentation for Rotator Cuff Healing in Rabbit Model

Joo Han Oh¹, Seok Won Chung², Sae Hoon Kim³, Byung Wook Song⁴, Yeun Ho Kim⁵.
¹Seoul National University Bundang Hospital, Gyunggi-do, Korea, Republic of, ²Konkuk University Medical Center, Seoul, Korea, Republic of, ³Seoul National University Hospital, Seoul, Korea, Republic of, ⁴Nalgae Hospital, Seoul, Korea, Republic of, ⁵Seoul Jeil Hospital, Gyunggi-do, Korea, Republic of.

Disclosures:
J. Oh: None. S. Chung: None. S. Kim: None. B. Song: None. Y. Kim: None.

Introduction:
The failure of rotator cuff healing after surgical repair remains one of the most common complications. Platelet rich plasma (PRP) is blood plasma enriched with platelets, and contains numerous growth factors, which are known to be involved in tendon healing. In spite of widespread use of PRP, and much attention to enhance the healing process, the clinical results are somewhat controversial and basic experimental studies are still lacking. In addition, porcine dermal collagen patch graft which has been used as a biologic scaffold may act as a mechanical augmentation and improve the rate and quality of biological rotator cuff healing by providing a chemically and structurally beneficial environment for host cells. Therefore, the purpose of this study is to verify the effect of the PRP with/without porcine dermal collagen graft augmentation on tendon-to-bone healing in chronic rotator cuff tear model, using a rabbit supraspinatus (SST) tendon.

Methods:
Allocation We randomly allocated 80 mature New Zealand white rabbits (weight, 3.5 to 4.0 kg) into 4 groups (20 rabbits per each group, 12 for histology and 8 for mechanical test): group R (repair), group RPa (repair + patch augmentation), group RPr (repair + PRP apply), and group RPaPr (repair + patch + PRP).

Chronic rotator cuff tear model and repair For all rabbits, the chronic rotator cuff tear model was created in the right shoulder by severing SST tendon at the insertion site and wrapping the torn tendon with a silicone penrose drain to inhibit adhesion to the surrounding tissue and by leaving alone for 6 weeks. For the left shoulder, sham operation was performed (control). After 6 weeks, we repaired the torn SST tendon with 2-0 Ticron in a transosseous manner simulating double row repair after creating a bleeding bed.

PRP preparation To prepare PRP, 10 ml of autologous blood was acquired from the femoral vein of each rabbit of group RPr and RPaPr, then combined with 1.1 mL of ACD-A to prevent coagulation. After two centrifugation process (firstly at 210 g for 10 minutes and secondly at 860g for another 10 minutes), approximately 1ml of PRP was acquired, and activated by 0.1 ml of 10% calcium chloride. The mean number of platelets was \(259.06\pm88.04\times10^3/\mu l\) in whole blood and 924.77±330.86\times10^3/\mu l in PRP. In addition, the mean number of leukocytes was 6.18±2.01×10^3/μl in whole blood and was much decreased in PRP with 0.06±0.09×10^3/μl.

PRP application and/or patch augmentation The prepared activated PRP gel was immediately administered onto the repaired tendon of each rabbit of group RPr. In addition, the 1-mm thickness porcine dermal collagen graft was prepared as 1×1 cm² size, and used to augment the repair site by stitching the proximal portion of the patch to the SST tendon and the distal portion to the soft tissue at the lateral portion of proximal humerus for group RPa. For group RPaPr, the patch was augmented covering the PRP added on the repair site.

Histological evaluation All histological evaluations were performed in a randomized and blinded fashion by a 10-year trained musculoskeletal specialized pathologist, who was not involved in the study. At 4 and 8 weeks after repair, we harvested the greater tubercle with attached SST tendon of both shoulders (6 rabbits per each group at each period), and assessed vascularity and cellularity from H&E stain, collagen fiber continuity and proportion of fibers oriented parallel at the tendon-to-bone interface from Masson’s Trichrome stain, and the inflammation rate and absorption rate of the graft at the tendon-to-graft interface from H&E stain, using a microscope and an image software system. The histological findings were graded semi-quantitatively into 4 grades for the later analyses.

Mechanical evaluation In addition, 8 weeks after repair, the entire SST tendon of both shoulders along with the humeral head of each rabbit (8 per each group) was harvested. Then, we evaluated the mode of tear and the load to failure at a rate of 1mm/s with a preload of 5 N using a custom fixture clamping system and an Instron materials testing machine.

Results:
Eleven rabbits were excluded from further assessment due to deep infection in 4, death in 4, and a wide dehiscence of SST tendon repair in 3 (These events were distributed relatively evenly among groups.), therefore, 69 rabbits were used in the final analysis (4-week histology: 6, 5, 5, and 5; 8-week histology: 5, 5, 5, and 5; 8-week mechanical test: 7, 7, 7, and 7 for group R, RPa, RPr, and RPaPr, respectively).
Histologic evaluation (Figure 1 and 2) At 4 weeks, the collagen fibers are poorly organized and the fiber continuity with bone has not yet been established in all 4 groups. The vascularity and cellularity (Fig. 1A and B) were higher with granulation tissue formation in the PRP treated group (group RPr and RPaPr) than that of the PRP non-treated group (group R and RPa). At this period, most of the graft tissue was not integrated into the surrounding tissue (Fig. 2A). At 8 weeks, the tendon-to-bone interface was less cellular and more collagen fibers bridged the interface showing good tendon to bone integration, and longitudinally oriented collagen fibers were visible. The PRP treated group (group RPr and RPaPr) showed better collagen fiber continuity and more regularly arranged collagen fibers than the PRP non-treated group (group R and RPa) (Fig. 1C and D). However, no distinctive difference was found in tendon-to-bone healing between the patch augmented group and non-augmented group (group R vs. RPa and RPr vs. RPaPr). Even though the healing process was much progressed up to 8 weeks, it did not reach to the level of the control side in terms of newly formed collagen fiber regularity and continuity to the bone. Most of the patch graft was integrated into the surrounding tissue at this period (Fig. 2B).

Mechanical evaluation (Table 1) The ratio of insertional tear to mid-substance tear was 5:2 in group R, 4:3 in group RPa, 3:4 in group RPr, and 3:4 in group RPaPr. The mid-substance tear might suggest strong tendon-to-bone healing, and it was more prevalent in group RPr (57.1%) than group R (28.6%), and in group RPaPr (57.1%) than group RPa (42.8%). The load-to-failures of each group were 61.57±29.99, 76.84±16.08, 105.35±33.82, and 117.93±12.60 for groups R, RPa, RPr, and RPaPr, respectively (p=0.001). The Bonferroni’s post-hoc analysis revealed significant differences in load-to-failure between group R and RPr (p=0.018), group R and RPaPr (p=0.002), and group RPa and RPaPr (p=0.029). However, there was no differences between group R and RPa (p=0.662), and group RPr and RPaPr (p=0.779). The load-to-failures of the operated side was significantly lower than the control side in all the groups (all p<0.05).

Discussion:
This controlled animal study showed the enhancement of tendon-to-bone healing after local administration of autologous PRP assessed by histology and biomechanical test in a chronic rotator cuff tear model of rabbit. However, there was little adding effect of the porcine dermal collagen graft as a biological augmenter of PRP.

Significance:
This finding suggest that PRP might be used as a useful biological supplement to increase the cuff healing rate, which still remains unsolved even after successful cuff repair. However, due to the limitation of this animal study, our results should be interpreted with caution regarding clinical application.

Acknowledgments:
We thank Kyong-Bun Lee for the professional evaluation of histological grade of specimens.

References:

<table>
<thead>
<tr>
<th>Table 1. The result of mechanical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group R(n=7)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Load to failure (N): operated</td>
</tr>
<tr>
<td>Load to failure (N): control</td>
</tr>
<tr>
<td>P value</td>
</tr>
<tr>
<td>Insertional tear</td>
</tr>
<tr>
<td>Midsubstance tear</td>
</tr>
</tbody>
</table>