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

Seok Won Chung,* MD, Byung Wook Song,† MD, Yeun Ho Kim,‡ MD, Kyoung Un Park,§ MD, PhD, and Joo Han Oh,|| MD, PhD

Investigation performed at Seoul National University Bundang Hospital, Seongnam, Korea

Background: The rate of healing failure after surgical repair of chronic rotator cuff tears is considerably high.

Purpose: To verify the effect of platelet-rich plasma (PRP) with and without porcine dermal collagen graft augmentation on tendon-to-bone healing, using the rabbit supraspinatus tendon.

Study Design: Controlled laboratory study.

Methods: A total of 80 rabbits were randomly allocated into 4 groups (20 rabbits per group: 12 for histological and 8 for mechanical testing): repair (R), repair + patch augmentation (RPa), repair + PRP (RPr), and repair + patch + PRP (RPaPr). The right shoulder was used for experimental interventions, and the left served as a control. Six weeks after the detachment of the supraspinatus, the torn tendon was repaired in a transosseous manner, simulating double-row repair in all groups. Platelet-rich plasma was prepared and applied onto the repair site in the RPr and RPaPr groups, and the patch was used to augment the repair in the RPa and RPaPr groups. The mechanical tensile strength test was performed at 8 weeks after repair and the histological evaluation at 4 and 8 weeks.

Results: At 4 weeks, the collagen fibers were poorly organized, and fiber continuity was not established in all groups. However, vascularity and cellularity were higher with granulation tissue formation in the PRP-treated groups (RPr and RPaPr) than the nontreated groups (R and RPa). At 8 weeks, tendon-to-bone integration was much improved with more collagen fibers, and longitudinally oriented collagen fibers were visible in all groups. The PRP-treated groups showed better collagen fiber continuity and orientation than the nontreated groups; however, no distinctive difference was found between the patch-augmented groups (RPa and RPaPr) and nonaugmented groups (R and RPr). The mean load-to-failure results were 61.57 ± 29.99 N, 76.84 ± 16.08 N, 105.35 ± 33.82 N, and 117.93 ± 12.60 N for the R, RPa, RPr, and RPaPr groups, respectively, and they were significantly different between the R and RPr (P = .018), R and RPaPr (P = .002), and RPa and RPaPr (P = .029) groups.

Conclusion: This animal study showed the enhancement of tendon-to-bone healing after local administration of autologous PRP assessed by histological and biomechanical testing in a rabbit model of chronic rotator cuff tears. However, there was little additive effect of the patch graft.

Clinical Relevance: The use of PRP might be a biological supplement to increase the rotator cuff healing rate, which still remains low even after successful cuff repair, but this result should be interpreted with caution regarding clinical applications.

Keywords: shoulder; rotator cuff; rabbit model; growth factors/healing enhancement; platelet-rich plasma; porcine dermal collagen graft

A rotator cuff tear is a common condition that causes pain and dysfunction.21,25 In spite of the rapid development of the techniques and instruments used for surgical repair of torn rotator cuffs, failure of rotator cuff healing after surgical repair remains one of the most common and well-known complications. Recent studies have reported that approximately 50% of surgically repaired rotator cuffs do not heal properly, independent of the surgical procedures utilized.8,12,14,38 Consequently, many efforts to support the repair construct mechanically and enhance tendon-to-bone healing biologically have been undertaken to decrease the failure rate after rotator cuff repair.

Platelet-rich plasma (PRP) is blood plasma that is enriched with platelets by centrifugation, and it contains numerous growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor–β 1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), which are also known to be involved in tendon healing.27,29 Recently, PRP has gained widespread use clinically in the
orthopaedic field, and much attention has been paid to enhance the healing process by providing endogenous growth factors as a biological approach. However, the clinical results are somewhat controversial, and basic experimental studies are still lacking, especially on tendon-to-bone healing after rotator cuff repair.2,13,41 In addition, many attempts have been made to augment the repair with a patch graft as a mechanical and biological scaffold to facilitate healing. A porcine dermal collagen patch graft is a tissue-engineered xenograft that has been used as a biological scaffold to provide a stronger repair.5 It is commercially made of a chemically cross-linked, acellular collagen/elastin material derived from the porcine dermis; thus, it is resistant to enzymatic degradation and minimizes potential cell-mediated responses.17 The use of patch grafts for rotator cuff repair may act as a mechanical augmentation and improve the rate and quality of biological healing by providing a chemically and structurally beneficial environment for host cells.6 Therefore, the purpose of this study was to verify the effect of PRP with or without porcine dermal collagen graft augmentation on tendon-to-bone healing in a chronic rotator cuff tear model, using the rabbit supraspinatus tendon. We hypothesized that locally administered PRP and porcine dermal collagen graft augmentation could enhance tendon-to-bone healing.

MATERIALS AND METHODS

All experimental procedures were approved by the Experimental Animal Committee of the Clinical Research Institute of the authors’ institution (IACUC No. BA1104-081/027-01).

Allocation of Rabbits

Power analysis indicated that a required sample size would be 8 for biomechanical tests to detect a significant difference in ultimate load to failure (mean difference, 90 ± 40 N; α error = .05; β error = .2; dropout rate = 25%), based on previous studies.32,46 For the histological evaluation, we assigned 6 rabbits per group at 4 weeks and 8 weeks (Figure 1). Thus, 80 mature New Zealand White male rabbits (weight, 3.5-4.0 kg) were randomly allocated into 4 groups (20 rabbits per group: 12 for histological and 8 for mechanical testing): the repair group (R), repair + patch augmentation group (RPa), repair + PRP group (RPr), and repair + patch + PRP group (RPaPr). All experimental procedures were performed in the right shoulder, and sham operations were performed in the left shoulder (control).

Chronic Rotator Cuff Tear Model

Under anesthesia and sterile conditions, a 3-cm skin incision was made between just proximal to the acromion process and greater tuberosity, and then the supraspinatus tendon was exposed after incising and retracting the deltoid muscle. For all rabbits, a chronic tear was created in the right shoulder by completely severing the supraspinatus tendon at the insertion site to the greater tuberosity and wrapping the torn tendon with a 10 mm–long silicone Penrose drain (8 mm in outer diameter, Yushin Corp, Bucheon, Korea) to inhibit adhesion to the surrounding soft tissue, and it was left alone for 6 weeks (Figure 2). For the left shoulder, a sham operation was performed that omitted the critical steps of tear or repair, including PRP application and patch augmentation.
Repair of Rotator Cuff Tendon

After 6 weeks, we repaired the torn supraspinatus tendon with No. 2-0 Ticon (Tyco, Waltham, Massachusetts) in a transosseous manner after creating a bleeding bed at the footprint of the greater tuberosity. Repair procedures were as follows: under the same anesthesia and with the same approach, we removed the Penrose drain wrapped around the supraspinatus tendon and then gently roughened the exposed greater tuberosity with a scalpel blade. Four bone tunnels were created at the anterior and posterior aspects of the footprint and mediolaterally at the articular surface and 5 mm from it to simulate a double-row repair. The suture ends grasping the tendon at the medial and lateral aspects of the tendon were then passed through the bone tunnels (Figure 3) and tied over the lateral humeral cortex, reattaching the supraspinatus tendon to the footprint.

PRP Application and/or Patch Augmentation

The prepared activated PRP (type 4B according to the classification by Mishra et al35), which was changed into a gelatinous substance by adding calcium chloride (PRP gel), was immediately administered on the repaired tendon of each specimen in the RPr group. That is, the PRP gel with adhesive properties was applied onto the site of the repaired tendon-to-bone approximation, as Beck et al5 did (Figure 4). The 1 mm–thick porcine dermal collagen graft (Permacol, Tissue Science Laboratories Plc, Aldershot, United Kingdom) was prepared as a 1 × 1–cm² patch. The collagen patch was not soaked in saline before usage, as no hydration or other preparatory procedures were required according to manufacturer instructions.

The collagen patch was used to augment the repair site by stitching the proximal portion to the supraspinatus tendon and the distal portion to the soft tissue at the lateral portion of the proximal humerus for the RPa group (Figure 5). We did not mix or soak the patch with PRP to minimize the possible loss of PRP. For the RPaPr group, the patch was applied over PRP after it had been added to the repair site.

Histological Evaluation

At 4 and 8 weeks after repair, the rabbits (6 per group for each period) were fully anesthetized and euthanized with carbon dioxide, and the greater tubercle of the humeral head with the attached supraspinatus tendon of both shoulders of each rabbit was harvested. Specimens were fixed in neutral buffered 10% formalin (pH 7.4) and decalcified, and paraffin blocks were made in the repair site including the supraspinatus tendon and greater tuberosity. Sections that were 5 μm thick were cut in the coronal plane and stained with hematoxylin and eosin (H&E) and Masson trichrome. We assessed vascularity, cellularity, collagen fiber continuity, and proportion of fibers oriented parallel at the tendon-to-bone interface, and we also evaluated the inflammation rate and the absorption rate of the graft at the tendon-to-graft interface. For each of these
items, the histological findings were graded semiquantitatively into 4 stages (grades 0, 1, 2, and 3). Vascularity, cellularity, inflammation rate around the graft, and absorption rate around the graft were graded as absent or minimally present (grade 0), mildly present (grade 1), moderately present (grade 2), and severe or markedly present (grade 3). In addition, for the items of collagen fiber continuity and collagen fibers oriented parallel, we divided their stages by percentage: present with <25% of proportion (grade 0), 25%-50% of proportion (grade 1), 50%-75% of proportion (grade 2), and >75% of proportion (grade 3). All examinations were performed in a randomized and blinded fashion to eliminate observer bias by a musculoskeletal specialized pathologist with 10 years of training, who was not involved in the study. The cross-sections were examined under a microscope (DP 70, Olympus, Tokyo, Japan) and analyzed using an image software system (Image-Pro Plus, Media Cybernetics Inc, Bethesda, Maryland).

**Mechanical Evaluation**

In addition, 8 weeks after repair, the entire supraspinatus tendon of both shoulders along with the humeral head of each rabbit was harvested after being euthanized (8 per group). We then evaluated the mode of tear and the load to failure at a rate of 1 mm/s with a preload of 5 N after 5 consecutive preconditioning loads (5-50 N at a loading rate of 15 N/s), using a custom fixture clamping system and an Instron materials testing machine (5565A, Instron, Norwood, Massachusetts) (Figure 6). We designed and
statistical analysis was performed using SPSS software (version 15.0, SPSS Inc, Chicago, Illinois). Comparisons between the groups were performed using a 1-way analysis of variance, followed by Bonferroni post hoc analysis with the significance level set at $P < .05$. Before the analysis of variance test, the Kolmogorov-Smirnov test for normality and the Levene test for homogeneity of variances were performed, and they fulfilled the assumption of normality and homogeneity of variances with all $P$ values >.05.

RESULTS

Four specimens (1 RPa, 1 RPr, and 2 RPaPr) showed a deep wound infection at the time of harvest, and another 4 rabbits died either during the operation (2 rabbits: 1 RPr and 1 RPaPr) or within a few days after the operation (2 rabbits: 1 R and 1 RPa) of unknown causes. These events were distributed relatively evenly among the groups, and we do not believe that the added procedures of blood collection for PRP or patch augmentation affected these events. In addition, a wide dehiscence of the supraspinatus tendon repair was noted in 3 rabbits (1 R, 1 RPa, and 1 RPr) at harvest. These 11 rabbits were excluded from further assessment; therefore, 69 rabbits were used in the final analysis (4-week histological testing: $n = 6, 5, 5,$ and 5; 8-week histological testing: $n = 5, 5, 5,$ and 5; 8-week mechanical testing: $n = 7, 7, 7,$ and 7 for R, RPa, RPr, and RPaPr groups, respectively), as shown in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Complications and Number of Rabbits Included in Each Analysis</th>
<th>R Group</th>
<th>RPa Group</th>
<th>RPr Group</th>
<th>RPaPr Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep infection</td>
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<td>1</td>
<td>2</td>
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<tr>
<td>No recovery from anesthesia</td>
<td>0</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Death from unknown causes</td>
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<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wide dehiscence of repaired supraspinatus tendon</td>
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<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4-week histological evaluation</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8-week histological evaluation</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8-week mechanical evaluation</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

*R, repair; RPa, repair + patch augmentation; RPaPr, repair + patch + platelet-rich plasma; RPr, repair + platelet-rich plasma.

### Histological Evaluation

The results of histological grading are described in Table 2. At 4 weeks, the collagen fibers were poorly organized, and fiber continuity with bone had not yet been established in all 4 groups. Evidence of vascularization was noted by the presence of blood vessels, and cells were present in the irregularly arranged fibrovascular interface tissues in all 4 groups. However, vascularity and cellularity (Figure 7, A and B) were higher with granulation tissue formation in the PRP-treated groups (RPr and RPaPr) than in the nontreated groups (R and RPa). In all 4 groups, the histological findings of the 4-week specimens were clearly different from those of the control side, which showed dense regular collagen fibers with complete continuity to the bone. At this period, most of the graft tissue was not integrated into the surrounding tissue, mostly keeping its shape, which was covered with fibrous tissue and had many inflammatory cells around it (Figure 8A).

At 8 weeks, the tendon-to-bone interface was composed of less cellular and less vascular fibrous tissues in all 4 groups. Instead, the number of collagen fibers in the interface was greatly increased, and collagen organization was obviously improved. In addition, more collagen fibers bridged the interface, showing good tendon-to-bone integration, and longitudinally oriented collagen fibers were visible in all 4 groups. However, the PRP-treated groups (RPr and RPaPr) showed better collagen fiber continuity and more regularly arranged collagen fibers than the nontreated groups (R and RPa) (Figure 7, C and D). The collagen fibers in the interface were more perpendicular from the bone in the PRP-treated groups. However, no distinctive difference was found in tendon-to-bone healing between the patch-augmented groups and nonaugmented groups (RPa vs R and RPaPr vs RPr). Even though the healing process was much progressed up to 8 weeks, it did not reach the level of the control side in terms of the regularity of newly formed collagen fibers and continuity to the bone. Most of the patch graft was integrated into the surrounding tissue at this period (Figure 8B).
Mechanical Evaluation

In the failure mode analysis, the ratio of the insertional tear to the midsubstance tear was 5:2 in the R, 4:3 in the RPa, 3:4 in the RPr, and 3:4 in the RPaPr groups (Table 3); the midsubstance tear suggests strong tendon-to-bone healing, while the insertional tear suggests relatively weak tendon-to-bone healing.45 In this study, midsubstance tears were more prevalent in the RPr (57.1%) than the R (28.6%) group and in the RPaPr (57.1%) than the RPa (42.8%) group. The load-to-failure values of the operated side (right side) were significantly lower than those of the control side (left side) in all groups (\( P < .05 \) for all cases). Mean load-to-failure values for the R, RPa, RPr, and RPaPr groups were 61.57 \( \pm \) 29.99 N, 76.84 \( \pm \) 16.08 N, 105.35 \( \pm \) 33.82 N, and 117.93 \( \pm \) 12.60 N, respectively (\( P < .001 \)). The Bonferroni post hoc analysis (Table 4) revealed significant differences in load to failure between the R and RPr (\( P = .018 \)), R and RPaPr (\( P = .002 \)), and RPa and RPaPr (\( P = .029 \)) groups. However, there was no difference between the R and RPa (\( P = .662 \)) and RPr and RPaPr (\( P = .779 \)) groups.

**DISCUSSION**

This study demonstrated that the local administration of PRP on the repaired supraspinatus tendon facilitated biological tendon-to-bone healing and increased the load to failure of the repaired rotator cuff; however, these biological and mechanical properties were not enhanced by porcine dermal collagen graft augmentation.

Platelet-rich plasma is an autologous platelet concentrate that contains various growth factors that can stimulate tendon healing. The platelets collected in PRP by the centrifugation process are activated by the addition of thrombin or calcium chloride, which induces the release of these growth factors from \( \beta \) granules.9 Platelet-rich plasma usually represents a 3- to 5-fold increase in platelet count compared with whole blood. Even though there is

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**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>R Group</th>
<th>RPa Group</th>
<th>RPr Group</th>
<th>RPaPr Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0 G1 G2 G3</td>
<td>G0 G1 G2 G3</td>
<td>G0 G1 G2 G3</td>
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<td>0 0 4 1</td>
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<td>Collagen fiber continuity 8-week evaluation, n</td>
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<td>Absorption rate around graft 4-week evaluation, n</td>
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<td>5 0 0 0</td>
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<td>Absorption rate around graft 8-week evaluation, n</td>
<td>5 0 0 0</td>
<td>5 0 0 0</td>
<td>5 0 0 0</td>
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</tr>
</tbody>
</table>

*Grades were as follows: G0 = absent or minimal or <25% of proportion; G1 = mild degree or 25%-50%; G2 = moderate degree or 50%-75%; and G3 = severe (marked) degree or >75%. R, repair; RPa, repair + patch augmentation; RPaPr, repair + patch + platelet-rich plasma; RPr, repair + platelet-rich plasma.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>R Group (n = 7)</th>
<th>RPa Group (n = 7)</th>
<th>RPr Group (n = 7)</th>
<th>RPaPr Group (n = 7)</th>
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<tr>
<td>Load to failure–operated side, N</td>
<td>61.57 ( \pm ) 29.99</td>
<td>76.84 ( \pm ) 16.08</td>
<td>105.35 ( \pm ) 33.82</td>
<td>117.93 ( \pm ) 12.60</td>
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<td>&lt;.001</td>
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<td>Midsubstance tear–operated side, n</td>
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</table>

*Values are expressed as mean \( \pm \) standard deviation unless otherwise indicated. R, repair; RPa, repair + patch augmentation; RPaPr, repair + patch + platelet-rich plasma; RPr, repair + platelet-rich plasma.
some controversy, the 3- to 5-fold concentrate seems to be suggested as a therapeutic PRP concentrate for tissue healing and regeneration.\textsuperscript{33} In the current study, we demonstrated a consistent result in platelet count in PRP, which increased approximately 4-fold compared with whole blood (mean, 924.77 ± 330.86 × 10\(^3\)/\(\mu\)L vs 259.06 ± 88.04 × 10\(^3\)/\(\mu\)L, respectively). Thus, we believe that PRP preparation process was well performed and that the level of platelets was within the therapeutic range. In addition, the reduction of leukocytes in PRP may enhance healing by decreasing the recruitment of leukocyte-induced catabolic signaling molecules such as interleukin-1 \(\beta\), tumor necrosis factor \(\alpha\), and matrix metalloproteinase-9.\textsuperscript{34,44} As it is known that the presence of leukocytes can result in proinflammatory cellular signaling and local tissue catabolism, a higher concentration of leukocytes is likely undesirable for musculoskeletal applications associated with tendon healing.\textsuperscript{11,28} We believe that even though prepared PRP contains a mixture of anabolic and catabolic mediators, the net effect of PRP was anabolic, thus facilitating tendon-to-bone healing, as we used leukocyte-reduced PRP in this study. In addition, we used calcium chloride alone to activate PRP and to produce an autologous PRP gel. Calcium chloride without thrombin may allow platelets to be functionally viable over a 7-day time and also a slower release of growth factors enhancing cell migration and healing.\textsuperscript{18} Moreover, the formation of fibrin glue (PRP gel) may provide an adhesive support, which can confine the secretion of growth factors to a chosen site, thus resulting in enhanced activity.\textsuperscript{1}

By applying this PRP gel onto the repair site of torn rabbit supraspinatus tendons, we were able to verify the facilitation of biological tendon-to-bone healing and improvement in the mechanical load to failure. This positive effect of PRP on rotator cuff healing may be caused by the growth factors secreted from the \(\alpha\) granules of platelets. Growth factors released from platelets include PDGF, TGF-\(\beta\), IGF-1, VEGF, EGF, platelet-derived angiogenesis factor, and bFGF, which are also known to be upregulated or involved during tendon healing.\textsuperscript{26} These growth factors contribute to stem cell recruitment, mitogenesis, angiogenesis, collagen synthesis, extracellular matrix production, and remodeling.\textsuperscript{3,24} Several in vitro studies have demonstrated that PRP may possibly contain and release these growth factors abundantly and stimulate the proliferation of fibroblasts, tendon cells, osteoblasts, and mesenchymal stem cells.\textsuperscript{15,30} In addition, hepatocyte growth factor, which is a plasmatocyte factor, released from PRP could induce cell proliferation and promote the synthesis of angiogenic factors during the healing process.\textsuperscript{3,47} The actions of these growth factors released from the platelets in PRP may have helped tendon-to-bone healing of the rabbit supraspinatus tendon in this study. However, this should be interpreted with caution because the dose-response relationship between platelet count and growth factor level in PRP was previously demonstrated only in TGF-\(\beta\),\textsuperscript{46} and we did
not evaluate whether a high amount of the growth factors was actually released and worked on tendon-to-bone healing on a molecular level.

There have been several studies that investigated the effect of PRP on tendon healing, and most of the basic research using animal models has suggested that PRP may improve tendon healing. However, all these studies investigated the effect of PRP on tendon-to-tendon healing and not on tendon-to-bone healing. To the best of our knowledge, there has only been 1 animal study, by Beck et al, which investigated the effect of PRP on tendon-to-bone healing of the rotator cuff. Beck et al showed that the application of PRP during rotator cuff repair in rats rather decreased tissue strain because of the cellular infiltration, increased areas of fibrinoid necrosis, and the lack of collagen orientation at the 7-day point and did not affect the load to failure of the repair. However, in their study, the initial histological changes at the 7-day time point were normalized at the 14-day point, and, compared with the control group, the collagen fibers of the PRP group were more organized in a more linear fashion, directed toward the native tendon footprint. Moreover, they only evaluated the effect of PRP at an early time point up to 21 days. We believe that there can be a positive effect of PRP at a later time point when the maturation of collagen is more progressed. Recently, several authors performed prospective randomized clinical trials and tested the ability of PRP to enhance tendon-to-bone healing of the rotator cuff. Randelli et al and, more recently, Gumina et al reported that the use of PRP in the treatment of rotator cuff tears improved repair integrity. Conversely, Castricini et al and Rodeo et al revealed that PRP application at the time of rotator cuff repair had no effect on healing or functional scores. We believe that these conflicting results may come from the variability of growth factor content between the donors and methods of PRP preparation. The methods and length of time involved in blood collection, centrifugation, platelet activation, and delivery to the target site also affect the final concentration and composition of growth factors and may alter the effectiveness of PRP. In addition, the presence or absence of leukocytes in preparations could affect the effectiveness of PRP, as a higher concentration of leukocytes is likely undesirable for tendon healing because of their inflammatory and catabolic effect on local tissue. Moreover, the effect of PRP may not reach the level at which the retear rate shows a significant difference or at which patients could detect a subjective difference of function in clinical studies, considering that the clinical studies dealt not with massive tears but with mostly small-to large-sized tears, which are already expected to have good anatomic and functional results. In this study, we collected the blood quickly and safely from the femoral vein under anesthesia to prevent blood clotting and destruction of platelets, and all procedures for PRP extraction were consistently performed by 1 senior specialist in laboratory medicine (K.U.P.). In addition, the administration of PRP on the repaired rotator cuff was performed immediately, within minutes after extracting and activating PRP. We believe that these efforts to reduce the variability resulting from the PRP preparation may contribute in enhancing the effect of PRP and further rotator cuff healing in this study.

Regarding the delivery method, there could have been different results if we had infiltrated PRP into the tendon or bone. We applied the PRP gel onto the repaired tendon because the infiltration of whole PRP into a chronically torn small rabbit tendon will cause a lot of resistance, accompanied by much leakage outside the tendon to an unwanted space. This loss of PRP may influence the results. The placement of PRP between the tendon and bone may be another possible method. However, this method would also cause much loss of PRP by the squeezing effect during the repair process. On the other hand, the infiltration of PRP into bone may stimulate mesenchymal stem cells, thus enhancing tissue healing. Moreover, much of the leukocytes were separated from PRP by the centrifugation protocol of this study, and the final product contained a very small amount of leukocytes (0.06 ± 0.09 × 10³/μL in PRP). This reduction of leukocytes in PRP may be another factor to enhance the effect of PRP on tissue healing by decreasing the recruitment of leukocyte-induced catabolic signaling molecules.

As a secondary objective, we evaluated the effect of the patch graft on rabbit rotator cuff healing by using a porcine dermal collagen graft. However, the result was less satisfactory, showing no significant effect on rotator cuff healing both mechanically and biologically, even with the application of PRP. Several authors have investigated the effect of the patch graft on rotator cuff healing in animal models and showed improved biological and mechanical properties by the use of a porcine small intestinal submucosa patch. However, all these studies used the patch as an interpositional graft, which was different from the present study, where it was used as an augmentation graft in rotator cuff repair. In one animal study, Schlegel et al used the patch to augment sheep infraspinatus tendon repair and showed that the load to failure and histological findings did not indicate a significant difference between the augmented and nonaugmented groups at 12 weeks. They demonstrated that patch augmentation was insufficient to prevent the anatomic failure of rotator cuff repair. Their result was quite similar to that of the present study.

We do not know the exact reason why there was no effect of patch augmentation in this study; however, we believe that the evaluation time point may be a reason. Even though Zheng et al suggested that the grafted patch was totally replaced by dense irregular collagen fibers at 8 weeks in a rabbit supraspinatus tendon model, on the other hand, Funakoshi et al showed that the scaffold materials still remained in the regenerated tissues at 12 weeks and that the tensile strength in the regenerated tissue after patch grafts was significantly improved from 4 to 12 weeks postoperatively in a rabbit rotator cuff model. Thus, 8 weeks may not be enough for the full integration of patch grafts with the surrounding tendon tissue and for mature tendon-to-bone healing. Further study with a long-term evaluation may be necessary. However, an 8-week period is sufficient for the evaluation of early to midterm tendon healing. Thus, we believe that the results of this study, which inform us that patch augmentation was
not effective on tendon-to-bone healing at an early to midterm period, are still meaningful, even though we do not know the changes at a late period. In addition, if the tension for the repaired supraspinatus tendon to be pulled back to the footprint was high, such as the clinical situation of chronic retracted rotator cuff tears, the result could be different because of the load distribution effect of patch augmentation at an early to midterm period. However, all supraspinatus tendons of each rabbit were repaired onto the greater tuberosity with almost no tension in this study. That is, if the possibility of good healing already exists in a situation of very low tension at the repair site, patch augmentation at the repair site may not have the ability to show improvement. Moreover, the different kinds of patch grafts used may affect the result. The porcine dermal collagen patch graft used in this study has a long residence time after implantation, which may result in late integration with the surrounding tendon tissue and a late effect on tendon-to-bone healing, which we were not able to check in this study.

To our knowledge, this is the first study evaluating the effect of PRP on rotator cuff repair combined with patch augmentation in a rabbit model using a relatively large number of animals. In this animal model, we used only 1 shoulder for the repair procedure with and without PRP or patch augmentation and the other shoulder as a control. The use of bilateral shoulders for the experimental procedures in each rabbit could reduce the number of animals needed. However, we believe that it may cause too much harm or sometimes be lethal to the animals because of the long operation time and much blood loss, especially for the animals for which blood was collected for the PRP preparation. In addition, to use only 1 shoulder for the experimental intervention may enable the reduced use of that shoulder during the initial phase of healing, which may decrease the initial detachment and possibly aid in enhancing tendon-to-bone healing. Another consideration is that the healing pathway is fundamentally different between an acute injury and chronic injury. Different from acute injuries, chronic injuries presumably have a terminated inflammatory phase, with a paucity of platelets and a decrease in healing potential because of poor-quality tissues. In this situation, the increased growth factors produced by PRP may stimulate the tissue and restart the inflammatory and stalled healing process, thereby facilitating healing. Considering that rotator cuff tears usually develop over a long period of time as a result of intrinsic or extrinsic factors, and that most rotator cuff surgeries are performed on chronic tears, the use of a chronic tear model, like in this study, is essential for this research; still, most animal models evaluate acute injuries and repair conditions.

Nevertheless, the current study has several limitations. First, this study was an animal study, which limits the generalization of the results. The differences in the anatomy of the shoulder, the intrinsic healing potential, and the platelet function between rabbits and humans prevent the clinical application of the findings of this preliminary study without further investigation. Second, we studied the status of tendon-to-bone healing only at an early to midterm period until 8 weeks after repair with and without PRP and patch augmentation and had no information on further changes at a late postoperative period. Even though we selected 8 weeks based on previous studies, there certainly could be more mechanical and biological changes after 8 weeks. Further study with a longer period may be needed. Third, we did not measure the repair tension or tendon quality. The different tension and tendon qualities may affect the result of tendon-to-bone healing profoundly. Finally, we could not confirm that PRP stayed in the desired site after closure nor determine how the growth factors secreted from PRP acted in the enhancement of tendon-to-bone healing. However, as the PRP-treated groups actually showed a significant effect on tendon-to-bone healing in this study, we believe that PRP was present at the repair site in sufficient quantities to create an effect, and the effect might come from the growth factors or cytokines released from PRP. Further studies, such as an immunohistochemical assay, to detect the expression of PRP-related growth factors or cytokines, using different application methods of direct infiltration of PRP into the tendon or bone, may be needed as a next step and to confirm the results of this study.

In conclusion, this controlled animal study showed the enhancement of tendon-to-bone healing after local administration of autologous PRP assessed by histological and biomechanical testing in a chronic rotator cuff tear model in rabbits. However, there was little additive effect of the porcine dermal collagen graft as a biological augmenter of PRP. This finding suggests that PRP might be used as a biological supplement to increase the rotator cuff healing rate, which still remains low even after successful rotator cuff repair. However, because of the limitations of this animal study, our results should be interpreted with caution regarding clinical application.

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REFERENCES


