Platelet-Rich Plasma Stimulates Cell Proliferation and Enhances Matrix Gene Expression and Synthesis in Tenocytes From Human Rotator Cuff Tendons With Degenerative Tears

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Background: Platelet-rich plasma (PRP) contains various growth factors and appears to have a potential to promote tendon healing, but evidence is lacking regarding its effect on human tenocytes from rotator cuff tendons with degenerative tears.

Hypothesis: Platelet-rich plasma stimulates cell proliferation and enhances matrix gene expression and synthesis in tenocytes isolated from human rotator cuff tendons with degenerative tears.

Study Design: Controlled laboratory study.

Methods: Tenocytes were enzymatically isolated and cultured. To evaluate cell proliferation, tenocytes were cultured with 10% (vol/vol) platelet-poor plasma (PPP), PRP activated with calcium, and PRP activated with calcium and thrombin at platelet concentrations of 100, 200, 400, 800, 1000, 2000, 4000, 8000, and 16,000 X 10^3/µL for 14 days. Cell number was measured at days 7 and 14. To investigate matrix gene expression and synthesis, cells were cultured with a PPP or PRP gel (10% vol/vol) at a platelet concentration of 1000 X 10^3/µL for 14 days. Quantitative real-time reverse transcriptase polymerase chain reaction was performed to determine the expressions of type I and III collagen, decorin, tenasin-C, and scleraxis, and measurements of total collagen and glycosaminoglycan (GAG) synthesis were conducted at days 7 and 14.

Results: Platelet-rich plasma significantly increased cell proliferation at days 7 and 14 in a dose-dependent manner, and the addition of thrombin moved up the plateau of proliferation. Platelet-rich plasma significantly induced the gene expression of type I collagen at day 7 but not at day 14, while it significantly promoted that of type III both at days 7 and 14. The ratio of type III/I collagens did not change at days 7 and 14. The expressions of decorin and scleraxis significantly increased at day 14, whereas that of tenasin-C significantly increased at days 7 and 14. Platelet-rich plasma significantly increased total collagen synthesis at days 7 and 14 and GAG synthesis at day 14.

Conclusion: Platelet-rich plasma promoted cell proliferation and enhanced gene expression and the synthesis of tendon matrix in tenocytes from human rotator cuff tendons with degenerative tears.

Clinical Relevance: These findings suggest that PRP might be used as a useful biological tool for regenerative healing of rotator cuff tears by enhancing the proliferation and matrix synthesis of tenocytes from tendons with degenerative tears.

Keywords: platelet-rich plasma; rotator cuff tear; degeneration; tenocytes; matrix; gene expression

Clinical results after rotator cuff repair have been shown to be satisfactory regardless of the operative technique used, which includes open surgery,18,67 mini-open surgery,30,51 and arthroscopic surgery.17,64 Average satisfaction rates of 85% have been reported for open surgery and from 84% to 95% for arthroscopic surgery.62 However, 2 important problems, namely the quality and speed of healing, still remain to be solved despite recent advances in mechanical fixation methods.25 Furthermore, as the degenerative torn ends of rotator cuffs do not appear to contribute to healing,27,60 and as tendon healing is naturally slow,46,47 an additional biological strategy is required to improve the tissue quality of torn ends and to aid the regeneration of native tendon-to-bone insertions.26

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Platelet-rich plasma (PRP) is a platelet concentrate that typically contains more than $1000 \times 10^6$ platelets/$\mu$L, representing a 3- to 5-fold increase as compared with whole blood. Because platelets contain various growth factors in their α-granules, PRP potentially can release these growth factors at higher than physiological levels. As growth factors known to be in α-granules, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), insulin-like growth factor (IGF-I), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF), are also known to be upregulated or involved during tendon healing,

it has been suggested that the addition of PRP would aid tendon healing. However, basic experimental and clinical evidence is lacking or still controversial. A few studies have investigated the effects of PRP on human tenocytes. Anitua et al. showed that 20% PRP (vol/vol) stimulated tenocyte proliferation and increased VEGF and HGF production by tenocytes, and demonstrated that 20% PRP (vol/vol) promoted tenocyte proliferation but decreased the gene expressions of type I and type III collagen. Recently, Zargar Baboldashti et al. reported that PRP diminished the adverse effects of dexamethasone and ciprofloxacin. However, in these studies, tenocytes were isolated and cultured from the normal hamstring tendons of young patients undergoing hamstring tendon release or anterior cruciate ligament reconstruction, and has been previously mentioned, tenocytes from the degenerated torn ends of rotator cuff tendons in older patients might respond to PRP very differently from those of normal hamstring tendons in young patients. To the best of our knowledge, no study has been performed on the effects of PRP on tenocytes from human rotator cuff tendons with degenerative tears.

The purpose of this study was to determine the effects of PRP gel on the proliferation, matrix gene expression, glycosaminoglycan (GAG), and total collagen synthesis of tenocytes from human rotator cuff tendons with degenerative tears. Our hypothesis was that PRP gel would promote tenocyte proliferation and induce matrix gene expression and synthesis.

MATERIALS AND METHODS

Isolation and Expansion of Tenocytes From Human Rotator Cuff Tendons

After informed consent was obtained, tendon tissues were obtained from patients undergoing arthroscopic rotator cuff repair for the treatment of degenerative rotator cuff tears ($n = 9$). Pieces of tissue $3 \times 3$ mm in size were obtained after debriding the severely frayed portion of the lateral edge of rotator cuff tendons with a basket forceps. The study protocol was approved by the institutional review board at our institution. All 9 patients had shoulder pain with an insidious onset and no history of trauma.

Tendon tissue was harvested after the debridement of severely frayed tissue from torn free ends using a basket forceps. After removal of the overlying bursal or synovial tissue, the tendon specimens were washed twice in calcium- and magnesium-free phosphate-buffered saline (DPBS) and finely minced. Cells were released by treating with 0.3% collagenase II for 5 hours in low-glucose Dulbecco's modified Eagle medium (LG DMEM) containing antibiotic solution (100 U/mL penicillin and 100 μg/mL streptomycin) with gentle agitation. After the same volume of DPBS was added, undigested tissue was removed using a 100-μm nylon sieve, and cells were collected by centrifugation, washed twice, resuspended in LG DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotic solution (growth medium), and plated in 100-mm tissue culture dishes at a density of 2 to $5 \times 10^4$ cells/cm² at 37°C in a humified 5% CO₂ atmosphere. The medium was replaced twice weekly. When cells reached 60% to 80% confluence, they were detached by incubation for 10 minutes with 0.25% trypsin (Welgene, Daegu, Korea), washed, and then replated at a ratio of 1:3. Cells from the second passage to fifth passage were used in the study.

Preparation of PRP Gel

Platelet-rich plasma ($n = 9$) was obtained from patients undergoing arthroscopic rotator cuff repair for the treatment of degenerative rotator cuff tears using a plateletpheresis system with a leukoreduction set (COBE Spectra LRS Turbo, Caridian BCT, Lakewood, Colorado) according to a previously described standard collection program. The target concentration of platelets in the final product was 1400 $\times 10^6$ platelets/$\mu$L. The system was set and primed with saline solution and anticoagulant acid citrate dextrose as the anticoagulant according to the manufacturer's instructions. Aliquots were taken to determine complete blood counts. For the application experiments, platelet counts in PRP were first adjusted with platelet-poor plasma (PPP) to 1000 $\times 10^6$ platelets/$\mu$L and then further diluted or concentrated if necessary. To produce a gel from prepared PPP or PRP, 10% calcium gluconate with or without 166.7 IU/mL thrombin (thrombin-lyophilized powder of 5000 IU) (Rayon Pharmaceutical, Seoul, Korea) was added to PPP or PRP at 1:10 (vol/vol). The dilution and gelling procedure was performed immediately before experiments. Throughout the experiments, cells were treated with PPP, PRP activated with calcium (PRP-Ca), and PRP activated with calcium and thrombin (PRP-Ca-Thr). Cells treated with only 2% FBS were used as controls.

Assay for Tenocyte Proliferation

Cells were seeded at a density of $1 \times 10^5$ cells/cm² in the bottom of 24-well plates with cell culture inserts (SPL Lifeiences, Pochon, Korea) and were allowed to attach for 24 hours in LG DMEM supplemented with 2% FBS and antibiotic solution. Platelet-rich plasma gels (10% vol/vol) at platelet concentrations of 100, 200, 400, 800, 1000, 2000, 4000, 8000, or 16,000 $\times 10^6$ cells/$\mu$L activated with 10% calcium gluconate with or without 166.7 IU/mL bovine thrombin were then placed on the cell culture insert of
Assay for Matrix Gene Expression

Cells were seeded at a density of \(3 \times 10^5\) cells/cm\(^2\) in the bottoms of 6-well plates with cell culture inserts (SPL LifeSciences) and allowed to attach for 24 hours. Platelet-poor plasma or PRP gels (10% vol/vol) at a platelet concentration of 1000 \(\times 10^6\) cells/\(\mu\)L activated with 10% calcium gluconate with or without 166.7 IU/mL bovine thrombin were placed on the cell culture insert of each well. Media were replaced at 2, 7, and 9 days. Matrix gene expression was evaluated using real-time reverse transcription polymerase chain reaction (RT-PCR) at days 7 and 14.

Total RNA was extracted, and reverse transcription and amplification were performed as previously described. Briefly, total RNA was extracted using a Qiagen RNeasy mini kit (Qiagen, Hilden, Germany) and quantified using a NanoDrop ND-100 spectrophotometer (NanoDrop, Wilmington, Delaware). First-strand complementary DNA (cDNA) was synthesized using the Superscript III Reverse Transcription kit (Invitrogen, Carlsbad, California). Briefly, first-strand cDNA was synthesized from cellular mRNAs (1 \(\mu\)g) by heating a mixture (1 \(\mu\)g mRNA, 1 \(\mu\)L Oligo(dT)\(_{20}\) [50 \(\mu\)M], 1 \(\mu\)L dNTP [10 mM], and up to 10 \(\mu\)L DW) to 65°C for 5 minutes, cooling on ice for 1 minute, and then adding a mixture containing 2 \(\mu\)L 10\(\times\) RT buffer, 4 \(\mu\)L MgCl\(_2\) (25 mM), 2 \(\mu\)L DTT (0.1 M), 1 \(\mu\)L RNaseOut (40 U/\(\mu\)L), and 1 \(\mu\)L Superscript III Reverse Transcriptase (200 U/\(\mu\)L) (Invitrogen). The reaction mixture was held at 50°C for 60 minutes to promote cDNA synthesis, and the reaction was terminated by heating to 85°C for 5 minutes and then snap cooling at 0°C for 1 minute. Finally, RNase H (1 \(\mu\)L, 2 U/\(\mu\)L) was added and incubated at 37°C for 20 minutes to remove RNA strands from RNA-cDNA hybrids. Synthesized cDNA was either stored at −80°C or used immediately for real-time RT-PCR.

To perform real-time RT-PCR utilizing a LightCycler 480 (Roche Applied Science, Mannheim, Germany), TaqMan Gene Expression Assays (Applied Biosystems, Foster City, California) were used as a probe/primer set specified for type I collagen (assay ID: Hs00164004_ml), type III collagen (assay ID: Hs00943809_ml), decorin (assay ID: Hs00266491_ml), tenascin-C (assay ID: 115865_ml), and GAPDH (assay ID: Hs99999905_ml). The PCRs were performed in a final volume of 20 \(\mu\)L containing 10 \(\mu\)L 2X LightCyclerH 480 Probes Master (FastStart Taq DNA polymerase, reaction buffer, dNTP mix [with dUTP instead of dTTP], and 6.4 mM MgCl\(_2\)) (Roche Applied Science), 1 \(\mu\)L TaqMan Gene Expression Assay (Applied Biosystems), 5 \(\mu\)L cDNA as the template, and 4 \(\mu\)L \(\mu\)H\(_2\)O using the following program: 96°C for 10 minutes, 40 cycles at 96°C for 10 seconds, and 60°C for 1 minute, followed by 72°C for 4 seconds, and a final cooling at 40°C for 30 seconds. Experiments were performed in triplicate, and averaged values were calculated for normalized expression levels. During PCR amplification, amplified product amounts were monitored by continuous measurement of fluorescence. Gene expressions were normalized versus GAPDH as follows: the cycle number at which the transcript of each gene was detectable (threshold cycle, Ct) was normalized against the Ct of GAPDH, which is referred to as \(\Delta Ct\). Gene expressions relative to GAPDH are expressed as 2\(^{-\Delta Ct}\), where \(\Delta Ct = Ct\) gene of interest − Ct GAPDH.

Assay for Total Collagen and GAG Synthesis

To assay total collagen and GAG synthesis, cells were seeded at a density of \(3 \times 10^5\) cells/cm\(^2\) in the bottom of 6-well plates with cell culture inserts (SPL LifeSciences) and allowed to attach for 24 hours. A PRF gel (10% vol/vol) with a platelet concentration of 1000 \(\times 10^6\) cells/\(\mu\)L activated with 10% calcium gluconate with or without 166.7 IU/mL bovine thrombin was added to cell culture inserts in wells. All assays were performed in culture supernatants and in triplicate at days 7 and 14.

Total soluble collagen was measured in culture supernatants using the Sircol assay (Biocolor, Newtownabbey, Northern Ireland) according to the manufacturer's instructions. Briefly, 900 \(\mu\)L of Sirius Red reagent was added to 100 \(\mu\)L of supernatant and mixed for 30 minutes at room temperature. The collagen-dye complex was precipitated by centrifugation at 12,000 rpm for 10 minutes and washed with 750 \(\mu\)L of ice-cold Acid-Salt Wash Reagent provided with the kit (Biocolor). Wash reagent was used to remove unbound dye from the surface of pellets and the inside surface of microcentrifuge tubes. After centrifugation at 12,000 rpm for 10 minutes, supernatants were discarded, and pellets were dissolved in the acidic solution provided. Absorbance was measured at 555 nm. The calibration curve was set up on the basis of a collagen standard provided by the manufacturer.

Glycosaminoglycan amount was measured using the Blyscan 1,9-dimethylmethylened blue (DDMB) assay kit (Biocolor) according to the manufacturer's instructions. Briefly, Blyscan dye reagent (500 \(\mu\)L) was added to supernatants (500 \(\mu\)L) and the kit standard and mixed for 30 minutes at room temperature. Insoluble pellets of sulfated GAG were precipitated by centrifugation at 12,000 rpm for 10 minutes. Bound dye was released with the dissociation reagent (500 \(\mu\)L), and the absorbance was measured at 656 nm. The calibration curve was set up on the basis of a collagen standard provided by the manufacturer.

Statistical Analysis

All data values were tested for normality using the Shapiro-Wilk test and expressed as means and standard deviations. The significances of differences were determined using the independent t test and 1-way analysis of variance. For post hoc analysis, the Dunnett test was used for comparison with controls, whereas the Tukey test was used for comparisons of PPP-, PRP-Ca-, and...
Figure 1. Tenocytes from human rotator cuff tendons with degenerative tears. Cells were cultured for 14 days with a platelet-rich plasma gel (10% vol/vol) at platelet concentrations of 100, 200, 400, 800, 1000, 2000, 4000, 8000, and 16,000 \( \times 10^3 \) cells/\( \mu L \). Platelet-rich plasma was activated with 10% calcium gluconate and placed on a cell culture insert in each well. Note that cell proliferation increased with platelet concentration and plateaued at around 4000 \( \times 10^3 \) cells/\( \mu L \). No definite morphological change was observed during culture. FBS, fetal bovine serum; PPP, platelet-poor plasma.

PRP-Ca-Thr–treated cells. \( P < .05 \) was considered to be statistically significant.

RESULTS

Characteristics of Rotator Cuff Tears and PRP

The average age of the 9 patients from whom tendons were harvested was 57.8 ± 11.9 years (range, 39-69 years), and there were 4 men and 5 women. Average anteroposterior size and mediolateral retraction were 31.6 ± 18.2 mm and 15.2 ± 14.4 mm, respectively.

The average age of the 9 patients from whom PRP was prepared was 52.7 ± 19.2 years (range, 23-69 years). Platelet, red blood cell (RBC), and white blood cell (WBC) counts were determined using a fully automated analyzer (XE-2100, Sysmex, Kobe, Japan). Platelet counts increased from 199.00 ± 36.93 \( \times 10^3 \) platelets/\( \mu L \) in whole blood to 956.22 ± 55.12 in PRP, a 4.9-fold increase from baseline (\( P < .001 \)). Mean RBC and WBC counts reduced from 4.48 ± 0.31 and 6.11 ± 1.56 in whole blood to 0.15 ± 0.08 and 0.01 ± 0.01 in PRP, respectively (\( P < .001 \)). The average concentration of fibrinogen in PRP was 197.05 ± 10.55 mg/dL.

Effect of PRP on the Proliferation of Tenocytes From Tendons With Degenerative Tears

Platelet-rich plasma activated with calcium and PRP-Ca-Thr significantly induced the gene expression of type I collagen compared with the control at day 7, whereas PPP did not (Figure 3). Platelet-rich plasma activated with calcium and PRP-Ca-Thr upregulated the gene expression of type I collagen by 2.32- and 3.58-fold, respectively (\( P = .034 \) and \( P < .001 \), respectively). At day 14, although PRP-Ca and PRP-Ca-Thr increased the gene expression of type I collagen.
Figure 2. Relative cell proliferation measured using a WST colorimetric assay (EZ-CyTox assay, Daell Lab Service, Seoul, Korea). Cells were cultured for 14 days with a platelet-rich plasma (PRP) gel (10% vol/vol) at platelet concentrations of 100, 200, 400, 800, 1000, 2000, 4000, 8000, and 16,000 × 10³ cells/µL. Platelet-rich plasma was activated with calcium only (PRP-Ca) or with calcium plus thrombin (PRP-Ca-Thr). (A) At day 7, cell proliferation increased in a dose-dependent manner. The greatest proliferation was 4.98-fold at a concentration of 4000 cells × 10³/µL for PRP-Ca and 4.44-fold at a concentration of 8000 cells × 10³/µL for PRP-Ca-Thr. The addition of thrombin further enhanced cell proliferation at lower concentrations of 100, 200, and 400 cells × 10³/µL compared to cells activated with calcium only, resulting in moving up the proliferation plateau from 2000 cells × 10³/µL for PRP-Ca (solid line) to 400 cells × 10³/µL for PRP-Ca-Thr (dotted line). (B) At day 14, cell proliferation also increased in a dose-dependent manner. The greatest proliferation was 5.70-fold at a concentration of 8000 cells × 10³/µL for PRP-Ca and 5.41-fold at a concentration of 8000 cells × 10³/µL for PRP-Ca-Thr. Thrombin further enhanced cell proliferation at lower concentrations of 100, 200, and 400 cells × 10³/µL compared to cells activated with calcium only, resulting in moving up the proliferation plateau from 2000 cells × 10³/µL for PRP-Ca (solid line) to 400 cells × 10³/µL for PRP-Ca-Thr (dotted line).

collagen compared with the control, no statistically significant difference was found because of a large variation between samples.

The gene expression of type III collagen was significantly induced by PRP-Ca and PRP-Ca-Thr compared with the control at days 7 and 14, whereas PPP did not (Figure 4). At day 7, PRP-Ca and PRP-Ca-Thr upregulated the gene expression of type III collagen by 3.57- and

Figure 3. Gene expression level of type I collagen measured by real-time reverse transcriptase polymerase chain reaction. Relative quantifications were calculated by dividing the mRNA expression level in cells treated with platelet-rich plasma (PRP) by that in control cells. At day 7, PRP activated with calcium (PRP-Ca) and PRP activated with calcium and thrombin (PRP-Ca-Thr), but not platelet-poor plasma (PPP), significantly upregulated type I collagen expression by 2.32- and 3.58-fold, respectively (P = .034 and P < .001, respectively). At day 14, although PPP, PRP-Ca, and PRP-Ca-Thr also enhanced expression by 3.00-, 5.97-, and 7.40-fold, respectively, there were no statistical significances. P values were calculated as compared with the control.

Figure 4. Gene expression level of type III collagen measured by real-time reverse transcriptase polymerase chain reaction. Relative quantifications were calculated by dividing the mRNA expression level in cells treated with platelet-rich plasma (PRP) by that in control cells. At day 7, PRP activated with calcium (PRP-Ca) and PRP activated with calcium and thrombin (PRP-Ca-Thr), but not platelet-poor plasma (PPP), significantly upregulated type III collagen expression by 3.57- and 5.23-fold, respectively (all P < .001). At day 14, PRP-Ca and PRP-Ca-Thr increased the gene expression by 3.24- and 3.93-fold, respectively (P = .003 and P < .001, respectively). P values were calculated as compared with the control.
Figure 5. The ratio of the gene expression of type III to I collagen. Both at days 7 and 14, platelet-rich plasma (PRP) activated with calcium and PRP activated with calcium and thrombin did not significantly change the ratio of type III/I collagen expression level compared with the control. PPP, platelet-poor plasma.

Platelet-poor plasma, PRP-Ca, and PRP-Ca-Thr did not significantly elevate the ratio of type III/I collagen expression at days 7 and 14 compared with the control (Figure 5). Platelet-poor plasma, PRP-Ca, and PRP-Ca-Thr significantly upregulated the gene expression of decorin compared with the control at day 14 but not at day 7 (Figure 6). At day 14, PPP, PRP-Ca, and PRP-Ca-Thr upregulated the expression of decorin by 1.58-, 1.86-, and 1.73-fold, respectively ($P = .016$, $P < .001$, and $P = .003$, respectively). $P$ values were calculated as compared with the control.

For tenascin-C expression, PRP-Ca-Thr significantly enhanced by 6.20- and 5.28-fold in comparison with the controls at days 7 and 14, respectively ($P < .001$ and $P = .004$, respectively), whereas PPP and PRP-Ca did not significantly regulate the expression of tenascin-C (Figure 7). Platelet-rich plasma activated with calcium and PRP-Ca-Thr significantly increased the gene expression of scleraxis at day 14 by 2.52- and 2.51-fold with PRP-Ca and PRP-Ca-Thr, respectively ($P = .033$ and $P = .035$, respectively), whereas PPP did not (Figure 8).

Figure 6. Gene expression level of decorin measured using real-time reverse transcriptase polymerase chain reaction. Relative quantifications were calculated by dividing the mRNA expression level in cells treated with platelet-rich plasma (PRP) by that in control cells. Platelet-poor plasma (PPP), PRP activated with calcium, and PRP activated with calcium and thrombin significantly upregulated the gene expression of decorin at day 14 by 1.58-, 1.86-, and 1.73-fold, respectively ($P = .016$, $P < .001$, and $P = .003$, respectively). $P$ values were calculated as compared with the control.

Figure 7. Gene expression level of tenascin-C measured using real-time reverse transcriptase polymerase chain reaction. Relative quantifications were calculated by dividing the mRNA expression level in cells treated with platelet-rich plasma (PRP) by that in control cells. The gene expression of tenascin-C was significantly upregulated with PRP activated with calcium and thrombin only at days 7 and 14 in comparison with the control by 6.20- and 5.28-fold at days 7 and 14, respectively ($P < .001$ and $P = .004$, respectively). $P$ values were calculated as compared with the control.

Figure 8. Gene expression level of scleraxis measured using real-time reverse transcriptase polymerase chain reaction. Relative quantifications were calculated by dividing the mRNA expression level in cells treated with platelet-rich plasma (PRP) by that in control cells. At day 7, there was no significant difference between the 4 groups. Platelet-rich plasma activated with calcium (PRP-Ca) and PRP activated with calcium and thrombin (PRP-Ca-Thr), but not platelet-poor plasma (PPP), significantly increased the gene expression of scleraxis by 2.52- and 2.51-fold with PRP-Ca and PRP-Ca-Thr at day 14, respectively ($P = .033$ and $P = .035$, respectively). $P$ values were calculated as compared with the control.
The results of the present study demonstrate that PRP-activated calcium with or without thrombin significantly stimulated the proliferation of tenocytes from human rotator cuff tendons with degenerative tears in a dose-dependent manner. Platelet-rich plasma–activated calcium with or without thrombin also significantly upregulated the gene expressions of type I and III collagen but did not significantly influence the ratio of type III/I collagen expression level. Platelet-rich plasma significantly upregulated the gene expression of decorin, a representative proteoglycan of tendon, and of tenasin-C, a representative glycoprotein of tendon. In addition, PRP significantly upregulated the gene expression of scleraxis, a tendon-specific marker. Furthermore, the synthesis of total collagen and GAG were also significantly enhanced with PRP. However, gene expression levels and the amount of matrix molecules synthesized varied considerably by culture time and activation method. Meanwhile, PPP neither stimulated the proliferation of tenocytes nor induced the expression or synthesis of tendon matrix except for decorin. At day 14 and total collagen, suggesting the importance of bioactive materials released from granules in the platelet. The addition of thrombin for platelet activation significantly accelerated cell proliferation especially at lower concentrations compared with the addition of calcium only and demonstrated a tendency of lowering the platelet concentration for reaching the proliferation plateau. Meanwhile, it did not significantly affect matrix gene expression and synthesis except for tenasin-C. Considering that final cell proliferation level is more influenced by the proliferation rate in the earlier culture period than in the later period, and that matrix gene expression and synthesis were not significantly affected except for tenasin-C, these results would be because of the initial burst release of growth factors with the addition of thrombin. Taken together, the results of the present study suggest that PRP has positive effects on the proliferation, matrix gene expression, and synthesis of tenocytes from degenerated torn tendons and that the degrees of these effects are dependent on the kind of matrix molecules, culture period, and the concentration and activation status of PRP. These results suggest that PRP might be applied usefully in the treatment of rotator cuff tendons with degenerative tears.
degenerative tendinopathy, whereas its application strategy, such as the concentration, activation method, timing, and application numbers of PRP should be further investigated.

In this study, we investigated the effects of PRP on tenocytes from human rotator cuff tendons with degenerative tears. The in vitro effects of PRP on tenocytes have been reported previously by a few authors.4,12,48,56,60,68 Three of these studies described the effects of PRP on tenocyte proliferation and/or matrix synthesis.3,4,12 Anitua et al14 reported that 30% PRP releasate (vol/vol) increased cell proliferation and the production of VEGF and HGF, and de Mos et al12 demonstrated that PRP releasate increased cell number and total collagen levels but decreased the gene expressions of type I and III collagen without affecting the III/I ratio. However, both studies were performed using tenocytes isolated from the hamstring tendons of healthy young donors3 or children aged 13 to 15 years.12 It has been reported that tendons from different sites have different structures, compositions, cell phenotypes, and metabolic characteristics.1,14 Furthermore, the behaviors of tenocytes are known to depend on donor age, anatomic site or origin, and status; that is, whether they are obtained from intact, injured, or degenerated tendons.6,12,20,68,69 A number of authors have demonstrated that tendons or fibroblasts from intact and torn tendons48 or ligaments58 also behave differently and they have suggested that intact tendon tissue is not appropriate for studying cellular tendon degeneration.18,68 Taken together, it appears that the previous studies based on the use of tenocytes from the hamstrings of young, healthy donors might not reflect the biology of tenocytes from rotator cuff tendons with degenerative tears in older patients or the responses of tenocytes to PRP. We consider that the effects of PRP on any specific disease or injury should be investigated using tissue or cells from appropriate diseased or injured sites, and thus, we suggest that the results of this study provide useful information about potential effects of PRP on the repair of torn rotator cuffs.

Previous studies on the effects of PRP on tenocytes suffer from a lack of standardization or from inadequate characterization of PRP preparations, which result in interstudy differences in platelet concentrations, activations, and WBC contamination levels. Furthermore, these factors could affect the concentrations and the release kinetics of growth factors, which make it difficult to compare study results and could explain contradictory results. Accordingly, in the present study, we used a plateletpheresis system to prepare PRP,26 which, we believe, provides the most consistent and reproducible PRP with minimal WBC contamination. Furthermore, we agree with Zimmermann et al69 that this type of preparation provides the only practical means of elucidating the platelet-dependent and leucocyte-independent mechanisms underlying the clinical effects of PRP.

The results of this study show that PRP stimulated tenocyte proliferation in a dose-dependent manner. Moreover, PRP was found to have no inhibitory effect on cell proliferation at levels of more than 50-fold over the physiological level. A number of studies have addressed the effect of PRP concentration on the proliferation of osteoblasts, periodontal cells, or mesenchymal stem cells.21,23,24,34,35,36 Some authors have reported that PRP at higher concentrations did not further promote or even suppressed proliferation.10,20,24,28 On the other hand, only a few studies have investigated the effect of different concentrations of PRP on the proliferation of tendon fibroblasts.6,12 We agree with Anitua et al6 that the biological effects of PRGF may depend on platelet concentration and on the anatomic origins of cells. In the current study, we tested a variety of platelet concentrations from 100 to 16,000 cells \( \times 10^9/\mu L \) and examined 2 different activation methods, that is, calcium and calcium plus thrombin. Considering that physiological platelet concentrations range from 150 to 300 cells \( \times 10^9/\mu L \), the concentrations used in the study correspond to approximately 0.5- to more than 50-fold over the physiological levels, which would seem to be sufficient for simulating clinical situations. In addition, considering that the in vivo half-life of platelets is about 7 days,12 some authors who reported negative effects for PRP on cell proliferation may have investigated too short a period, 3 to 7 days, to examine the effects of PRP on cell proliferation.10,20,24,28 Our results support this suggestion, as cell proliferation plateaued at a lower concentration, 400 cells \( \times 10^9/\mu L \) at day 7, whereas at day 14, cell proliferation was highest at a concentration of 2000 cells \( \times 10^9/\mu L \).

Several studies have examined matrix molecular changes in rotator cuff tendons with chronic tendinopathy.31,41,48-45,65 In one study of ruptured supraspinatus tendons, a significant reduction in total collagen content and an increase in the expression of type I and II collagen with an increase in type III/I ratio were found.45 However, relatively little is known about changes in proteoglycans and glycoprotein.31,48,44,55 In the case of proteoglycan, a generalized increase in the amounts of hyaluronan and sulfated GAG has been reported44 but little is known of other proteoglycans.41 Lo et al31 reported a significant increase in aggrecan and a significant decrease of decorin in ruptured rotator cuff tendons. Glycoproteins such as tenasin-C and fibronectin are known to be increased in ruptured tendons.43,55 On the other hand, the results of the present study show that PRP has some reversing or recovering effects, at least in part, to the above changes in chronic tendinopathy and tendon rupture on tenocytes from human rotator cuff tendons with degenerative tears; that is, in the present study, PRP significantly increased the gene expression of type I collagen but maintained the type III/I ratio, stimulated total collagen synthesis, and enhanced the gene expression of decorin. These results, along with the observed increased cell proliferation and the increased expression of decorin, a tendon-specific marker,56 suggest that PRP could reverse matrix molecular changes caused by tendon degeneration.

Type III collagen is present in normal tendons (less than 10% of total collagen) and is weaker than type I collagen. It is characterized by a small fibril in a woven pattern that is deficient in cross-linking.58 Type III collagen is synthesized as a repair response to tissue injury, especially in adhesive
or scar tissue, in a larger amount up to 20% to 30%.

In conclusion, this study demonstrates that PRP promotes the proliferation of tenocytes from human rotator cuff tendons with degenerative tears and that it enhances the gene expression and the synthesis of tendon matrix. The results of this study indicate that PRP might offer a useful biological strategy for promoting the regeneration of rotator cuff tears.

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Axial Load-Bearing Capacity of an Osteochondral Autograft Stabilized With a Resorbable Osteoconductive Bone Cement Compared With a Press-Fit Graft in a Bovine Model

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Background: Osteochondral autografts in mosaicplasty are inserted in a press-fit fashion, and hence, patients are kept non-weightbearing for up to 2 months after surgery to allow bone healing and prevent complications. Very little has been published regarding alternative fixation techniques of those grafts.

Hypothesis: Osteochondral autografts stabilized with a resorbable osteoconductive bone cement would have a greater load-bearing capacity than standard press-fit grafts.

Study Design: Controlled laboratory study.

Methods: Biomechanical testing was conducted on 8 pairs of cadaveric bovine distal femurs. For the first 4 pairs, 6 single osteochondral autografts were inserted in a press-fit fashion on one femur. On the contralateral femur, 6 grafts were stabilized with a calcium triglyceride osteoconductive bone cement. For the 4 remaining pairs of femurs, 4 groups of 3 adjacent press-fit grafts were inserted on one femur, whereas on the contralateral femur, grafts were cemented. After a maturation period of 48 hours, axial loading was applied on all single grafts and on the middle graft of each 3-in-a-row series.

Results: For the single-graft configuration, median loads required to sink the press-fit and cemented grafts by 2 and 3 mm were 281.87 N versus 345.56 N (P = .015) and 336.29 N versus 454.08 N (P = .018), respectively. For the 3-in-a-row configuration, median loads required to sink the press-fit and cemented grafts by 2 and 3 mm were 260.31 N versus 353.47 N (P = .035) and 384.83 N versus 455.68 N (P = .029), respectively.

Conclusion: Fixation of osteochondral grafts using bone cement appears to improve immediate stability over the original mosaicplasty technique for both single- and multiple-graft configurations.

Clinical Relevance: Achieving greater primary stability of osteochondral grafts could potentially accelerate postoperative recovery, allowing early weightbearing and physical therapy.

Keywords: articular cartilage; knee; autografting; transplantation; bone cement