Autologous Platelet-rich Clot Releasate Stimulates Proliferation and Inhibits Differentiation of Adult Rat Tendon Stem Cells Towards Nontenocyte Lineages

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OBJECTIVE: To explore the effects of autologous platelet-rich clot releasate (PRCR) on proliferation and differentiation of adult rat tendon stem cells (TSCs) in vitro, following intense mechanical stretching. METHODS: TSCs were subjected to 8% mechanical stretching and subsequently incubated in control medium or medium supplemented with 2% or 10% PRCR. Collagen types I and III, peroxisome proliferator-activated receptor-γ (PPARγ), sex determining region Y-box 9 (SOX-9) and runt-related transcription factor 2 (RUNX2) concentrations were assessed via Western blotting and flow cytometry. Transforming growth factor (TGF)-β1 and vascular endothelial growth factor concentrations were measured using enzyme-linked immunosorbent assay. Treated TSCs were also cultured in adipogenic, chondrogenic or osteogenic culture media. RESULTS: PRCR increased the number of TSCs, and the concentrations of collagen types I and III and TGF-β1. In contrast, PRCR significantly reduced PPARγ, SOX-9 and RUNX2-positive cell numbers, and significantly reduced the numbers of TSC-derived adipocytes, chondrocytes and osteocytes. CONCLUSION: PRCR induced tenocyte differentiation while suppressing the adipocyte, chondrocyte and osteocyte lineages believed to impede tendon healing.

KEY WORDS: DIFFERENTIATION; PLATELET-RICH CLOT RELEASATE; TENDINOPATHY; TENDON STEM CELLS; TRANSFORMING GROWTH FACTOR-β1; VASCULAR ENDOTHELIAL GROWTH FACTOR

Introduction
Tendons are subjected to frequent and intense mechanical loading, and excessive mechanical stress often leads to tendon lesions, which are collectively termed tendinopathies. Typical histopathological features of tendinopathy are accumulation of adipocytes, mucoid degeneration and tissue calcification.1 Tendon stem cells (TSCs) have been identified in adult humans, mice, rats and rabbits.2 These cells have the potential to differentiate into several cell types (including tenocytes, adipocytes, chondrocytes and osteocytes) and...
differentiation towards these various cell fates may impact tendon healing.

Mechanical loading of TSCs in vitro has been used to assess intrinsic cellular stress responses and extracellular signalling events that may contribute to the pathophysiology of tendinopathy.\(^3,\)\(^4\) Low-level mechanical stretching (4%) increase TSCs proliferation and promote the differentiation of TSCs into tenocytes, but more intense stretching (8%) has been shown to induce some TSCs to differentiate into cells of the adipocyte, chondrocyte or osteocyte lineages.\(^4\) Potentially damaging mechanical stress may, therefore, direct TSCs towards nontenocyte lineages, resulting in lipid accumulation, mucoid formation and tissue calcification, which are the typical features of late-stage tendinopathy.\(^4\)

The platelet-rich plasma (PRP) fraction of blood contains a myriad of growth factors with the potential for tissue healing. Concentrations of many of these, including platelet derived growth factor (PDGF), epidermal growth factor, transforming growth factor (TGF)-\(\beta_1\) and insulin-like growth factor (IGF)-1, are increased in tendons during healing.\(^5,\)\(^6\) Autologous PRP has been used to promote the healing of tendons or ligaments.\(^7,\)\(^8\) Active releasate of PRP (platelet-rich clot releasate [PRCR]) can stimulate TSC proliferation and collagen production in vitro, in addition to increasing the deposition of collagen-rich extracellular matrix.\(^9,\)\(^10\) Studies have demonstrated that PRCR treatment can increase tenocyte numbers and the in vivo production of collagen types I and III, which are the main components of tendons.\(^9\)\(^–\)\(^11\) Treatment with PRCR following intense mechanical loading may also regulate the differentiation of TSCs towards nontenocyte lineages known to impede healing, but this has not been directly tested. The present study explored the effects of autologous PRCR on adult rat TSCs in vitro following intense mechanical stretching, in order to determine whether PRCR can improve tendon healing by suppressing TSC differentiation into adipocytes, chondrocytes and osteocytes.

Materials and methods

ANIMALS

This study took place at the Department of Orthopaedics, Southwest Hospital, Third Military Medical University, Chongqing, China. Male Sprague–Dawley rats \((n = 9; 4 – 6\)-weeks-old; 200 – 250 g) were obtained from the Animal Experiment Centre, Third Military Medical University. The animal experiments in the study followed European Community guidelines for the care and use of experimental animals,\(^12\) with free access to food and water, and housing in a 12-h light/12-h dark cycle. After an overnight fast, rats were fully sedated with 10 mg/kg ketamine administered by intramuscular injection and were sacrificed. The study was approved by the Ethics Review Committee of the Third Military Medical University.

RAT TSC ISOLATION AND CULTURE

The TSC isolation procedure was carried out as previously described.\(^2\) Briefly, the patellar and Achilles tendons were dissected after the animals were sacrificed. The middle section of the tendon was minced into fine pieces (1 mm\(^3\)) and 100 mg of tissue was digested with 3 mg type I collagenase (Worthington Biochemical Corp., Lakewood, NJ, USA) and 4 mg dispase (Stem Cell Technologies, Vancouver, Canada) in 1 ml phosphate buffered saline (PBS; 0.01 M, pH 7.4) at 37 °C for 1 h. The resulting cell suspension was centrifuged at 1500 \(g\) for 15 min, the supernatant was discarded and the cells were resuspended in Dulbecco’s Modified Eagle’s Medium (Gibco Life Technologies,
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Gaithersburg, MD, USA) supplemented with 20% fetal bovine serum (Gibco Life Technologies) (growth medium). Cells were diluted to 10 cells/µl in the growth medium and cultured in 25 cm² flasks (5 ml/flask). The TSCs formed adherent colonies after 6 – 9 days in culture. The colonies were then subcultured in new flasks for one or two passages, to obtain sufficient numbers of TSCs for further experiments. The cells were stained with 0.5% crystal violet (Sigma-Aldrich, St Louis, MO, USA) and observed under a light microscope.

PREPARATION OF PRCR

Blood (approximately 5 ml) was extracted from the heart of each rat after sacrifice using a 22-gauge needle. The blood was mixed with 1 ml sodium citrate (0.1 M), centrifuged at 400 g for 15 min, and the supernatant containing concentrated platelets (PRP) was collected. The PRP fraction comprised approximately 37% of the total blood volume. To activate the platelets, the PRP fraction was treated with 22 mM calcium chloride for 1 h at 37 °C, followed by centrifugation at 2000 g for 10 min. The resulting PRCR was collected by aspiration and stored at 4°C until use.

CELL STRETCHING

The TSCs were subjected to cyclic uniaxial mechanical stretching using an in vitro system comprising a control unit, driving motor and silicone dishes that were elastic, transparent and nontoxic. The culture surface measured 3 × 6 cm and was engineered with micro-grooves (10 × 3 µm) oriented along the stretching axis. The silicone dishes were precoated with 10 µg/ml ProNectin® F (Sigma-Aldrich) to promote cell attachment, and TSCs were plated at 5 × 10⁴ cells/dish in growth medium. After incubation for 12 h, cyclic 8% stretching at 0.5 Hz was applied for 12 h. After stretching, the cells were cultured in growth medium alone or medium supplemented with 2% or 10% PRCR for 3 days. Parallel cultures that had not undergone stretching were used as controls. Cell morphology was examined by phase contrast light microscopy after stretching. Each treatment condition was performed using 20 silicone dishes and a minimum of six dishes were analysed. Experiments were performed in triplicate.

ADIPOGENESIS

After stretching, the TSCs were cultured in adipogenic medium (Iscove’s modified Dulbecco’s medium [IMDM] supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 1 µM hydrocortisone, 0.1 mM indomethacin and 10% rabbit serum [Sigma-Aldrich]) or adipogenic medium containing 2% or 10% PRCR for 21 days. Oil red O staining was performed after adipogenesis. Briefly, the medium was removed and cells were washed three times with PBS for 5 min each wash, fixed in 4% paraformaldehyde in PBS for 40 min at room temperature, then washed three times with PBS and twice with water (5 min per wash). The fixed adipocytes were incubated with 0.36% oil red O solution (Millipore, Billerica, MA, USA) for 50 min and washed three times with deionized water. Lipid droplets within adipocytes appeared red under light microscopy. Images were recorded, using a charge-coupled device camera, and 10 random fields (× 200 magnification) were analysed with SPOT™ software, version 5.0 (Diagnostic Instruments, Sterling Heights, MI, USA).

CHONDROGENESIS

After stretching, the TSCs were cultured in chondrogenic medium (high-glucose DMEM [Biofluids, Rockville, MD, USA] supplemented with 0.1 µM dexamethasone, 50 µg/ml ascorbic acid, 100 µg/ml sodium pyruvate, 40 µg/ml proline, 10 ng/ml TGF-β1, and 50
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mg/ml ITS+ premix [Becton Dickinson, Franklin Lakes, NJ, USA]) or chondrogenic medium containing 2% or 10% PRCR for 21 days. Safranin O staining was performed after chondrogenesis. Cells were fixed in ice-cold ethanol for 1 h, washed twice with distilled water (5 min per wash), stained at room temperature for 30 min with toluidine blue O solution (Sigma-Aldrich), then rinsed five times with distilled water. Cells were examined as above. Stained glycosaminoglycan-rich matrix produced by chondrocytes appeared blue under light microscopy.

OSTEOGENESIS

After stretching, the TSCs were cultured in osteogenic medium (IMDM supplemented with 0.1 μM dexamethasone, 10 mM β-glycerol phosphate and 0.2 mM ascorbic acid) or osteogenic medium containing 2% or 10% PRCR for 21 days. Alizarin red S staining was performed after osteogenesis. Cells were fixed in chilled 70% ethanol for 1 h, washed twice in distilled water (5 min per wash) stained with Alizarin red S (Millipore) at room temperature for 30 min, and washed five times in distilled water (3 min per wash). Cells were examined as above. Mineral deposits within osteocytes appeared orange-red under light microscopy.

DETERMINATION OF CELL PROLIFERATION

Manual cell counting and lysate protein concentration were used as indicators of TSC proliferation. Cell counts were performed 3 days after stretching, using a haemocytometer under a light microscope. The number of cells per field (equivalent to 0.1 μl of cell suspension) was counted, and the mean of four fields from each sample was recorded as cells/μl.

For estimation of cell numbers by lysate protein concentration, each culture plate was washed twice with PBS. Cells were harvested and resuspended in 100 μl radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) containing 1% phenylmethanesulphonyl fluoride protease inhibitor (Sigma-Aldrich) for 20 min at 4°C. The resulting lysate was centrifuged at 12000 g for 30 min and the protein concentration in the supernatant was measured using a bicinchoninic acid (BCA) protein assay kit (Pierce Protein Research Products, Rockford, IL, USA).

WESTERN BLOTTING

Total protein was extracted from TSCs (5 × 10⁶ cells) in RIPA buffer (Beyotime) and protein concentration was determined using a BCA protein assay kit (Pierce Protein Research Products). Protein (50 μg) was separated by 8% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and electrophoretically transferred onto nitrocellulose membranes (Amersham Bioscience, Piscataway, NJ) at 80 V for 150 min. The membranes were blocked with 5% nonfat milk power in 0.1 M Tris buffered saline containing 0.1% Tween-20 (TBST; pH 7.4) for 30 min at room temperature, then incubated overnight at 4°C with one of the following primary antibodies: rabbit antirat collagen type I; rabbit antirat collagen type III; rabbit antirat peroxisome proliferator-activated receptor (PPAR)-γ; rabbit antirat sex determining region Y-box 9 (SOX-9); rabbit antirat runt-related transcription factor 2 (RUNX2); mouse antirat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1 : 1000 dilution). Membranes were washed three times with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies (antirabbit or antimouse immunoglobulin (Ig)G-peroxidase conjugate, 1 : 2000 dilution; Jackson ImmunoResearch, West Grove, PA,
USA) at room temperature for 1 h. Specific protein bands were visualized with enhanced chemiluminescence detection reagents (Amersham Biosciences) and autoradiography. Any differences in protein loading were normalized to the GAPDH control.

FLOW CYTOMETRY
Flow cytometry analysis was used to determine the cell fractions (as a percentage of the total cell number) positive for PPAR\(\gamma\), SOX-9 and RUNX2. The TSCs were detached with trypsin, pelleted and resuspended at a final concentration of \(1 \times 10^6\) cells/ml in staining buffer (BD Biosciences Pharmingen, San Diego, CA, USA). A 95-µl sample of cell suspension was incubated with 5 µl of rabbit antirat PPAR\(\gamma\), SOX-9 or RUNX2 antibody (all Santa Cruz Biotechnology; 1 : 1000 dilution) for 30 min in a covered ice bath. Antibody-labelled cell suspensions were washed three times with PBS (15 min per wash) and then incubated with fluorescein isothiocyanate-conjugated goat antirabbit IgG (1 : 2000 dilution; Santa Cruz Biotechnology) for 30 min in a covered ice bath. Cells were washed three times with PBS (15 min per wash) then analysed on a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA). Data acquisition and analysis were performed using BD CellQuest™ Pro software, version 5.1 (BD Biosciences). The presence or absence of antibody staining was determined by comparison to the appropriate isotype control.

VEGF AND TGF-\(\beta_1\) ELISA
Vascular endothelial growth factor (VEGF) and TGF-\(\beta_1\) in cell suspensions were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R& D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. All assays were performed in duplicate.

STATISTICAL ANALYSES
Data were expressed as mean ± SD. Pairwise comparisons were made using one-way analysis of variance followed by Fisher’s protected least significant difference post hoc test. All statistical analyses were performed using SPSS® version 14.0 (SPSS Inc., Chicago, IL, USA) for Windows®. A P-value < 0.05 was considered to be statistically significant.

Results
Cultured TSCs were observed under a light microscope before (Fig. 1A) and after (Fig. 1B)

![FIGURE 1: Representative light photomicrographs of rat tendon stem cells (TSCs) before and after mechanical stretching. (A) Phase contrast photomicrograph showing morphology of cultured TSCs at day 7 after isolation. (B) TSCs stained with methyl violet. (C) Phase contrast photomicrograph of TSC morphology following 8% mechanical stretching.](image-url)
staining with methyl violet. In response to 8% mechanical stretching, the TSCs became highly elongated and aligned along microgrooves in the axis of stretching (Fig. 1C).

Cell numbers were significantly higher in cultures treated with 2% or 10% PRCR after stretching, compared with cultures subjected to stretching alone (P < 0.05 and P < 0.001, respectively; Fig. 2A). In addition, protein concentrations in cell lysates from cultures treated with 2% or 10% PRCR following mechanical stretching were significantly higher than in control cell lysates (stretching alone) (P < 0.01 and P < 0.001, respectively; Fig. 2B).

Treatment with 10% PRCR significantly increased levels of collagen types I and III and reduced levels of the adipocyte marker PPARγ, the chondrocyte marker SOX-9, and the osteocyte marker RUNX2 compared with control cultures (P < 0.05 for all comparisons; Fig. 3A). PRCR treatment (2% or 10%) significantly reduced the proportions of PPARγ, SOX-9, and RUNX2-positive TSCs compared with control cultures (P < 0.05 for all comparisons; Fig. 3B).

In addition, PRCR treatment significantly reduced the numbers of oil red O-positive TSC-derived adipocytes (Fig. 4A), Safranin O-positive TSC-derived chondrocytes (Fig. 4B) and Alizarin red S-positive TSC-derived osteocytes (Fig. 4C) (P < 0.05 for all comparisons).

Treatment with 2% or 10% PRCR significantly increased VEGF levels compared with control cultures (P < 0.01 and P < 0.001, respectively; Fig. 5A), but this increase was no longer significant when normalized to cell numbers (Fig. 5B). PRCR treatment (2% or 10%) also significantly increased TGF-β1 levels compared with control cultures (P = 0.01 and P < 0.001, respectively; Fig. 5C). When normalized to cell numbers, TGF-β1 was still significantly enhanced by 2% and

![Figure 2: Effect of incubation with 2% or 10% autologous platelet-rich clot releasate (PRCR) for 3 days on proliferation of rat tendon stem cells (TSCs), previously subjected to 8% mechanical stretching for 12 h, compared with TSCs treated with stretching alone (control). (A) Cell number and (B) protein concentration in cell lysates. *P < 0.05, **P < 0.01 and ***P < 0.001 versus control; one-way analysis of variance followed by Fisher’s protected least significant difference post hoc test.](image)
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10% PRCR treatment ($P < 0.05$ and $P < 0.001$, respectively; Fig. 5D).

Discussion

Tendinopathy is a generic term for the clinical conditions that develop in and around tendons as a result of chronic mechanical loading stress. Tendinopathy is a complex clinical problem requiring lengthy management, with patients often responding poorly to treatment. Genetic factors and ageing can contribute to tendinopathy, but chronic mechanical stress is considered to be the major cause of tendon inflammation and degeneration. Several previous *in vitro* studies have explored the pathogenic mechanisms of mechanical loading on the development of tendinopathy. Cell proliferation of stretch-treated TSCs has been shown to be enhanced by incubation with PRCR, a blood fraction known to contain a variety of...
growth factors that can promote wound healing.\textsuperscript{20} PRCR treatment in the present study markedly increased levels of type I and III collagen, suggesting that PRCR activated tenocyte differentiation or proliferation. PRP has previously been shown to induce chondrogenic differentiation of human mesenchymal stem cells,\textsuperscript{21} indicating an influence on cell fate. It is not known, however, whether PRCR treatment simply facilitated the differentiation of progenitor cells already committed to the tenocyte lineage, or if PRCR-derived growth factors stochastically induced some pluripotent TSCs to differentiate into tenocytes.

Flow cytometry analysis revealed that PRCR dose-dependently decreased the relative numbers (cell fractions) of PPARγ-positive, SOX-9-positive, and RUNX2-positive cells in the present study, indicating that PRCR reduced TSC differentiation to adipocytes, chondrocytes and osteocytes, respectively. This was accompanied by a reduction in lipid accumulating TSC-derived adipocytes, mucoid forming TSC-derived chondrocytes and tissue calcifying TSC-derived osteocytes. PRP has been shown to enhance tendon and ligament healing.\textsuperscript{7,8} In addition, PRCR has been shown to increase human tenocyte proliferation, collagen production and synthesis of VEGF and human growth factor \textit{in vitro},\textsuperscript{9,22,23} and is considered to be a safe treatment for injured tendons.\textsuperscript{7,22} The results of the present study suggest that PRCR treatment promotes tenocyte differentiation \textit{in vitro}. These differentiated tenocytes became activated to produce abundant collagen, which is necessary for the structural growth of injured tendons. Furthermore, PRCR inhibited the differentiation of adipocytes, chondrocytes and osteocytes that may impede proper healing. \textit{In vivo} studies have begun to explore the efficacy of PRP.
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Platelet-rich clot releasate contains many growth factors (including VEGF, human growth factor, basic fibroblast growth factor, PDGF, IGF1 and TGF-β1) with complex actions on TSCs. Individual factors may enhance proliferation, migration and synthesis of matrix proteins such as types I and III collagen. While VEGF production was not significantly enhanced by PRCR at 2% or 10%, PRCR did significantly increase TGF-β1 production in the present study. These results suggest that TGF-β1 may have an important role in reducing TSC differentiation to adipocytes, chondrocytes and osteocytes, thus reducing the lipid accumulation, mucoid formation and tissue calcification known to exacerbate tendinopathy following detrimental mechanical stretching.

In conclusion, the present study has demonstrated that PRCR can inhibit TSC differentiation following mechanical stretching \textit{in vitro}, resulting in a reduction in lipid accumulation, mucoid formation and...
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These data show that PRCR treatment after mechanical stress can promote TSC proliferation and TGF-β₁ secretion, which may be related to a reduction in TSC differentiation towards nontenocyte lineages. PRCR may promote tendon healing both by facilitating the proliferation and differentiation of tenocytes, and by actively suppressing the differentiation of cell types known to impede the healing process.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

References

1. Kannus P, Józsa L: Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg Am 1991; 73: 1507 – 1525.
2. Bi Y, Ehirchiou D, Kilts TM, et al: Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 2007; 13: 1219 – 1227.
3. Wang JH, Thampatty BP: Mechanobiology of adult and stem cells. Int Rev Cell Mol Biol 2008; 271: 301 – 346.
4. Zhang J, Wang JH: Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy. J Orthop Res 2010; 28: 639 – 643.
5. Dahlgren LA, Mohammed HO, Nixon AJ: Temporal expression of growth factors and matrix molecules in healing tendon lesions. J Orthop Res 2005; 23: 84 – 92.
6. Tsai RY, McKay RD: A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. Genes Dev 2002; 16: 2991 – 3003.
7. Fleming BC, Spindler KP, Palmer MP, et al: Collagen–platelet composites improve the biomechanical properties of healing anterior cruciate ligament grafts in a porcine model. Am J Sports Med 2009; 37: 1554 – 1563.
8. Sahni A, Francis CW: Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. Blood 2000; 96: 3772 – 3778.
9. de Mos M, van der Windt AE, Jahr H, et al: Can platelet-rich plasma enhance tendon repair? A cell culture study. Am J Sports Med 2008; 36: 1171 – 1178.
10. Tsubone T, Moran SL, Amadio PC, et al: Expression of growth factors in canine flexor tendon after laceration in vivo. Ann Plast Surg 2004; 53: 393 – 397.
11. Visser LC, Arnoczky SP, Caballero O, et al: Growth factor-rich plasma increases tendon cell proliferation and matrix synthesis on a synthetic scaffold: an in vitro study. Tissue Eng Part A 2010; 16: 1021 – 1029.
12. Pyczak T: Use of animals in scientific procedures – annotations on the future administrative procedure base on the provisions of Directive 2010/63/EU. Berl Munch Tierarzt Wochenschr 2011; 124: 376 – 381 [in German, English abstract].
13. Sharma P, Maffulli N: Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am 2005; 87: 187 – 202.
14. Riley G: The pathogenesis of tendinopathy. A molecular perspective. Rheumatology (Oxford) 2004; 43: 131 – 142.
15. Archambault JM, Wiley JP, Bray RC: Exercise loading of tendons and the development of overuse injuries. A review of current literature. Sports Med 1995; 20: 77 – 89.
16. Almekinders LC, Temple JD: Etiology, diagnosis, and treatment of tendonitis: an analysis of the literature. Med Sci Sports Exerc 1998; 30: 1183 – 1190.
17. Almekinders LC, Banes AJ, Ballenger CA: Effects of repetitive motion on human fibroblasts. Med Sci Sports Exerc 1993; 25: 603 – 607.
18. Tsuzuki M, Bynum D, Almekinders L, et al: ATP modulates load-inducible IL-1, COX 2, and MMP-3 gene expression in human tendon cells. J Cell Biochem 2003; 89: 556 – 562.
19. Wang JH, Jia F, Yang G, et al: Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E₂ and levels of cyclooxygenase expression: a novel in vitro model study. Connect Tissue Res 2003; 44: 128 – 133.
20. Bao P, Kodra A, Tomic-Canic M, et al: The role of vascular endothelial growth factor in wound healing. J Surg Res 2009; 153: 347 – 358.
21. Mishra A, Tummala P, King A, et al: Buffered platelet-rich plasma enhances mesenchymal
stem cell proliferation and chondrogenic differentiation. *Tissue Eng Part C Methods* 2009; 15: 431 – 435.

22 Murray MM, Spindler KP, Ballard P, et al: Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen–platelet-rich plasma scaffold. *J Orthop Res* 2007; 25: 1007 – 1017.

23 Anitua E, Andia I, Sanchez M, et al: Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *J Orthop Res* 2005; 23: 281 – 286.

24 Murray MM, Spindler KP, Abreu E, et al: Collagen–platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. *J Orthop Res* 2007; 25: 81 – 91.

25 Zhang J, Wang JH: Platelet-rich plasma releasate promotes differentiation of tendon stem cells into active tenocytes. *Am J Sports Med* 2010; 38: 2477 – 2486.

26 Marx RE: Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 2004; 62: 489 – 496.

27 Anitua E, Andia I, Ardanza B, et al: Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004; 91: 4 – 15.

28 Bennett NT, Schultz GS: Growth factors and wound healing: part II. Role in normal and chronic wound healing. *Am Surg* 1993; 166: 74 – 81.

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