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Can Platelet-Rich Plasma Enhance Tendon Repair?

A Cell Culture Study

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Background: Autologous platelet-rich plasma (PRP) application appears to improve tendon healing in traumatic tendon injuries, but basic knowledge of how PRP promotes tendon repair is needed.

Hypothesis: Platelet-rich plasma has a positive effect on cell proliferation and collagen production and induces the production of matrix-degrading enzymes and endogenous growth factors by human tenocytes.

Study Design: Controlled laboratory study.

Methods: Human tenocytes were cultured 14 days in 2% fetal calf serum medium complemented with 0%, 10%, or 20% vol/vol platelet-rich clot releasate ([PRCR] the active releasate of PRP) or platelet-poor clot releasate (PPCR). At day 4, 7, and 14, cell amount, total collagen, and gene expression of collagen Iα1 (COL1) and IIIα1 (COL3), matrix metalloproteinases ([MMPs] MMP1, MMP3, and MMP13), vascular endothelial-derived growth factor (VEGF)-A, and transforming growth factor (TGF)-β1 were analyzed.

Results: Platelet numbers in PRP increased to 2.55 times baseline. Growth-factor concentrations of VEGF and platelet-derived growth factor (PDGF)-BB were higher in PRCR than PPCR. Both PRCR and PPCR increased cell number and total collagen, whereas they decreased gene expression of COL1 and COL3 without affecting the COL3/COL1 ratio. PRCR, but not PPCR, showed upregulation of MMP1 and MMP3 expression. Matrix metalloproteinase 13 expression was not altered by either treatment. PRCR increased VEGF-A expression at all time points and TGF-β1 expression at day 4.

Conclusion: In human tenocyte cultures, PRCR, but also PPCR, stimulates cell proliferation and total collagen production. PRCR, but not PPCR, slightly increases the expression of matrix-degrading enzymes and endogenous growth factors.

Clinical Relevance: In vivo use of PRP, but also of PPP to a certain extent, in tendon injuries might accelerate the catabolic demarcation of traumatically injured tendon matrices and promote angiogenesis and formation of a fibrovascular callus. Whether this will also be beneficial for degenerative tendinopathies remains to be elucidated.

Keywords: platelet-rich plasma; tendon; growth factors; collagen; matrix metalloproteinases

Traumatic tendon injuries and tendinopathies are a growing problem in sports medicine and orthopaedic practice.[4,17] Most tendons have the ability to heal after injury, but the repair tissue is functionally inferior to normal tendon tissue and is accompanied by increased risk of further injury.[25] The poor vascularization seems to be a major reason for this limited healing capacity.[14,28] Treatment of tendon lesions, either primary traumatic or degenerative tendinopathies, is often hampered by contradictory descriptions of the underlying pathologic changes, with a limited repertoire of successful and evidence-based treatments.[31] New treatment strategies, such as the use of platelet-rich plasma (PRP), might improve healing.

Clinical applications of autologous PRP in human medicine include periodontal and maxillofacial surgery, plastic surgery, treatment of bone fractures, and treatment of chronic skin and

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soft-tissue ulcers.[3,10,15,20,23,27] Numerous publications on PRP reported excellent clinical outcomes.[3,10,15,20,23,27,32] The only published cohort study in tendon research reported 93% reduction of pain for PRP-treated patients with chronic elbow tendinosis.[24] However, this was a pilot study with a small number of patients and without a randomized control group.

Platelets actively participate in healing processes in the body.[4] Platelets contain different growth factors, such as platelet-derived growth factor (PDGF),[4] transforming growth factor (TGF)-β,[4,27] insulin-like growth factor (IGF),[27] epidermal growth factor (EGF),[27] vascular endothelial growth factor (VEGF),[27] and fibroblast growth factor (FGF),[27] which are released from their α-granules upon platelet activation and delivered to the injured site to facilitate healing.[4] Platelet-rich plasma is, by clinical definition, a volume fraction of the plasma, having a platelet concentration above baseline (whole blood).[27] In activated PRP, compared with activated whole blood, significant increases of growth factors can be observed, for example, VEGF (6.2-fold), PDGF-BB (5.1-fold), EGF (3.9-fold), and TGF-β1 (3.6-fold).[12] Specific roles of growth factors in tendon and ligament healing have been studied before. Platelet-derived growth factor, peaking shortly after tendon damage, plays a central role in the healing process[12,25] by chemotaxis, proliferation of fibroblasts, collagen synthesis, and the stimulation of TGF-β1 and VEGF. Transforming growth factor-β1 increases collagen production and cell viability.[25] Vascular endothelial growth factor is a powerful stimulator of angiogenesis.[25]

To summarize, platelets rapidly release a variety of growth factors, and PRP might provide an autologous source of these growth factors that play a key role in tendon repair mechanisms.[25] Not only controlled clinical studies, but even more in vitro studies are required to investigate in detail the effects of PRP on human tendon cell metabolism.

In in vitro tendon research, the effects of culturing equine flexor digitorum superficialis tendon explants with 100% PRP (vol/vol) and other blood products were examined.[30] Enhanced anabolic gene expression patterns (collagen types Iα1 [COL1A1] and IIIα1 [COL3A1] and cartilage oligomeric matrix protein), with no concomitant increase in catabolic genes (matrix metalloproteinase [MMP] 3 and MMP13), after 3 days of 100% PRP (vol/vol) treatment were reported. The only study with human tendon cells (tenocytes) cultured in 20% PRP (vol/vol) reported an increase in cell proliferation and in VEGF and hepatocyte growth factor (HGF) production.[4] Effects of PRP on collagen production and degradation of human tenocyte cultures remain to be elucidated.

The purpose of this study was to investigate the effects of releasates from 10% (vol/vol) and 20% (vol/vol) PRP and platelet-poor plasma (PPP) on human tenocytes in culture. We examined whether PRP releasate affects cell proliferation, collagen production, and production of matrix-degrading enzymes and endogenous growth factors by human tendon cells.

MATERIALS AND METHODS

Isolation of Tendon Cells

Human tendon-derived cells were explanted from hamstring tendon tissue of 3 children (age, 13-15 years) undergoing hamstring-tendon release for treatment of knee contractures. Approval was obtained from the Medical Ethical Committee of the Erasmus MC University Medical Center Rotterdam. After removal of the peritendineum, the tendon was cut into 3-mm^3 sections, transferred into 6-well plates (Corning Inc, Corning, NY), and cultured in expansion medium (Dulbecco's modified Eagle's medium, 10% fetal calf serum [FCS], 50 g/mL gentamycin, and 1.5 g/mL fungizone [all from Invitrogen, Paisley, Scotland, United Kingdom]). Cultures were maintained at 37 C in a humidified atmosphere of 5% CO_2 for 10 days. During this time, fibroblasts migrated out of the tissue and adhered to the bottom of the culture dish. Cells were subcultured and trypsinized at subconfluency. Cells from the fifth and sixth passage were used.

Plasma Preparations

After informed consent, whole blood (500 mL) from 9 healthy, male donors (median age, 47 years; range, 31-69) was collected in 70 mL of anticoagulants (citrate-phosphate-dextrose [CPD], Sanquin Blood Supply Foundation, Amsterdam, the Netherlands) and processed within 24 hours as a nonautologous source of platelets. None of the donors used medication that is known to influence platelet function. Processing protocols for PRP and PPP were adopted from the literature[4-6] and tested in different combinations. The best combination of these protocols (with the highest platelet counts and lowest blood cell counts) was used in this study.

Briefly, whole blood was centrifuged at 300*g* for 10 minutes. The supernatant was centrifuged at 4500*g* for 12 minutes, to obtain a superficial layer of PPP. The buffy coat of the first centrifugation was centrifuged at 300*g* for 10 minutes again to separate it into PRP (supernatant) and erythrocytes and leucocytes (bottom layer). To increase the platelet concentration, PRP was then centrifuged at 480*g* for 20 minutes to precipitate the platelets. Half the superficial plasma layer was removed, and the platelet pellet was suspended in the remaining half of the plasma volume. Clotting upon addition of 22.8 mM $CaCl_2$ at 37°C for 1 hour activated platelets to release their growth factors. The soluble releasate from the clotted preparations (platelet-rich clot releasate [PRCR] and platelet-poor clot releasate [PPCR]), containing growth factors, was aspirated, stored at 4°C, and used within 2 weeks. Concentrations of platelets and red and white blood cells were measured on an ABC animal blood counter (Scil, Viernheim, Germany) in samples of whole blood, PRP and PPP (before clotting), and PRCR and PPCR (after clotting). For growth factor measurements, 1 mL of freshly prepared PPCR and PRCR was collected separately and immediately centrifuged at 1000*g* for 10 minutes at 4°C and stored at –80°C until further use. Growth factor concentrations in PRCR and PPCR were measured in triplicate using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits for VEGF and PDGF-BB (R&D Systems, Abingdon, Oxfordshire, United Kingdom) according to the manufacturers' protocol.

Cell Culture Experiment

Trypsinized tenocytes were plated at a density of 4000 cells/cm^2 and maintained in 10% FCS for 24 hours prior to

replacement by medium with 2% FCS and 0.1 mM L-ascorbic acid 2-phosphate (Sigma-Aldrich, St Louis, Mo), with or without (1) PPCR 10% (vol/vol), (2) PRCR 10% (vol/vol), (3) PPCR 20% (vol/vol), or (4) PRCR 20% (vol/vol). Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 for 14 days. Medium was refreshed at day 4, 7, and 11. At day 4, 7, and 14, the amount of cells (deoxyribonucleic acid [DNA] assay), total collagen (hydroxyproline assay), and gene expression of COL1 and COL3, MMP1, MMP3, and MMP13, VEGF-A, and TGF-β1 were analyzed.

DNA Assay

Cells were suspended in 0.1% phosphate-buffered saline/Triton X-100 (Sigma-Aldrich). Samples were sonificated for 10 seconds and incubated with 200 L of 8.3 IU/mL heparin solution (Leo Pharma BV, Breda, the Netherlands) and 100 L of 0.05 mg/mL ribonuclease A for 30 minutes at 37°C. This was followed by adding 100 L ethidium bromide solution (25 g/mL) (Sigma-Aldrich). Quantification of incorporated dye was performed in triplicate on the Wallac 1420 Victor2 (PerkinElmer, Wellesley, Mass) using an extinction filter of 340 nm and an emission filter of 590 nm.[11] For standards, calf thymus DNA (Sigma-Aldrich) was used.

Hydroxyproline Assay

Cells were suspended in milli-Q, hydrolyzed at 108°C for 18 to 20 hours in 6 M HCl and dried and redissolved in 100 L water. Hydroxyproline contents were measured by colorimetric method[8] (extinction, 570 nm), with chloramine-T and dimethylaminobenzaldehyde as reagents and hydroxyproline as standard (Merck, Damstadt, Germany).

Gene Expression Analysis

Cells were suspended in RNA-Bee (TEL-TEST, Friendswood, Tex). Downstream processing and real-time polymerase chain reaction (PCR) are described elsewhere.[9] Briefly, ribonucleic acid (RNA) was purified using RNeasy Micro Kit (Qiagen, Hilden, Germany), and 1 g of total RNA of each sample was reverse-transcribed into complementary DNA (cDNA) using RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, St Leon-Rot, Germany). Primers were designed to meet TaqMan or SYBR Green requirements and ensure gene specificity. COL1[9] and COL3[9] were studied as markers for collagen production. MMP1,[9] MMP3, and MMP13[9] were used as indicators of collagen degradation. Also, gene expression of growth factor VEGF-A (forward: 5′-CTTGCCTTGCTGCTCTACC-3′; reverse: 5′-CACACAGGATGGCTT GAAG-3′) and TGF-β1 (forward: 5′-GTGACAGCAGGGATAACACACTG-3′; reverse: 5′-CATGAATGGTGG CCAGGTC-3′; probe: 5′-ACAT CAACGGGTTCACTACCGGC-3′) were assessed. Amplifications were performed as 20- L reactions using TaqMan Universal PCR MasterMix (ABI, Branchburg, NJ) or qPCR Mastermix Plus for SYBR Green I (Eurogentec, Maastricht, the Netherlands) according to the manufacturer's guidelines on an ABI PRISM 7000 with SDS software version 1.7. Data were normalized to 18SrRNA (forward: 5′-AGTCCCTGCCCTTTGTACACA-3′; reverse: 5′-GATCCGAG GGCCTCACTAAAC-3′; probe: 5′-CGCC-CGTCGCTACTACCGATTGG-3′), which was stably expressed across samples (not shown). Relative expression was calculated according to the $2^{-\Delta CT}$ method.[21]

Statistical Analysis

The experiment was performed in duplicate for all 3 donor explants (n = 6). A 2% FCS condition without PRCR or PPCR was used as control and was set to 1 at each time point. All conditions were expressed as n-fold difference from the control at the corresponding timepoint. Statistical analysis was performed using SPSS 13.0 software (SPSS Inc, Chicago, Ill). A Kruskal-Wallis H test and post hoc Dunn multiple comparison test were used; $P < .05$ was considered to indicate statistically significant differences.

RESULTS

Plasma Preparations

Whole blood baseline platelet concentrations were all within physiological range (119×10^6 to 195×10^6 platelets/mL) (Table 1). The platelet concentration procedure increased platelet numbers in the PRP group on average to 2.55 times baseline concentration in whole blood. The PPP group showed an average decrease in platelet number to 0.02 times baseline. After clotting PRP and PPP, platelet numbers decreased to 0.08 and 0.05 times baseline, respectively. In addition, the centrifugation and clotting procedure decreased white blood cell counts to 0.02 and red blood cell counts to 0.01 in PRCR, and in PPCR to 0.02 and 0.00 times baseline, respectively.

Vascular endothelial growth factor concentration in PPCR was below the detection limit of 20 pg/mL in all preparations. On the other hand, VEGF concentration in PRCR was 107 ± 83 pg/mL (mean ± standard deviation [SD]). Platelet-derived growth factor–BB concentration in PPCR was 123 ± 151 pg/mL (mean ± SD); in PRCR, it was 3114 ± 2709 pg/mL (mean ± SD).

Cell Morphology

All cells at day 1 exhibited a spindle-shaped, fibroblast-like appearance. Control cells maintained their fibroblast-like appearance during the experiment, but in all PRCR and PPCR conditions, the cells altered their appearance toward a more stretched, oblong shape during the 14 days of culture (Figure 1).

DNA Assay (Amount of Cells)

Deoxyribonucleic acid content under control conditions increased in time. Both 20% PRCR (vol/vol) and 20% PPCR (vol/vol) significantly increased DNA content compared with control cultures ($P < .05$) (Figure 2). The effects of PRCR and PPCR were dose related. No significant changes between PRCR and PPCR were found.

TABLE 1
Platelet (PLT), White Blood Cell (WBC), and Red Blood Cell (RBC) Numbers in 5 Different Preparations From 3 Donors

Plasma Preparation	Donor	Concentration ($\times 10^6$/mL)			N-Fold Change in PLT Concentration (Preparation/Whole Blood) (Mean ± Standard Deviation)
		PLT	WBC	RBC	
Whole blood	1	186	5.00	4430	1.00 ± 0.25
	2	195	4.17	4050	
	3	119	3.63	4313	
Platelet-poor plasma (PPP)	1	4.33	0.10	0.00	0.02 ± 0.00
	2	5.00	0.10	0.00	
	3	3.00	0.10	10.00	
Platelet-rich plasma (PRP)	1	451	14.47	2570	2.55 ± 0.16
	2	488	4.27	1750	
	3	324	8.90	2437	
Platelet-poor clot releasate (PPCR)	1	6.00	0.10	10.00	0.05 ± 0.02
	2	11.00	0.10	10.00	
	3	8.00	0.10	10.00	
Platelet-rich clot releasate (PRCR)	1	7.00	0.10	10.00	0.08 ± 0.07
	2	7.00	0.10	10.00	
	3	19.00	0.10	90.00	

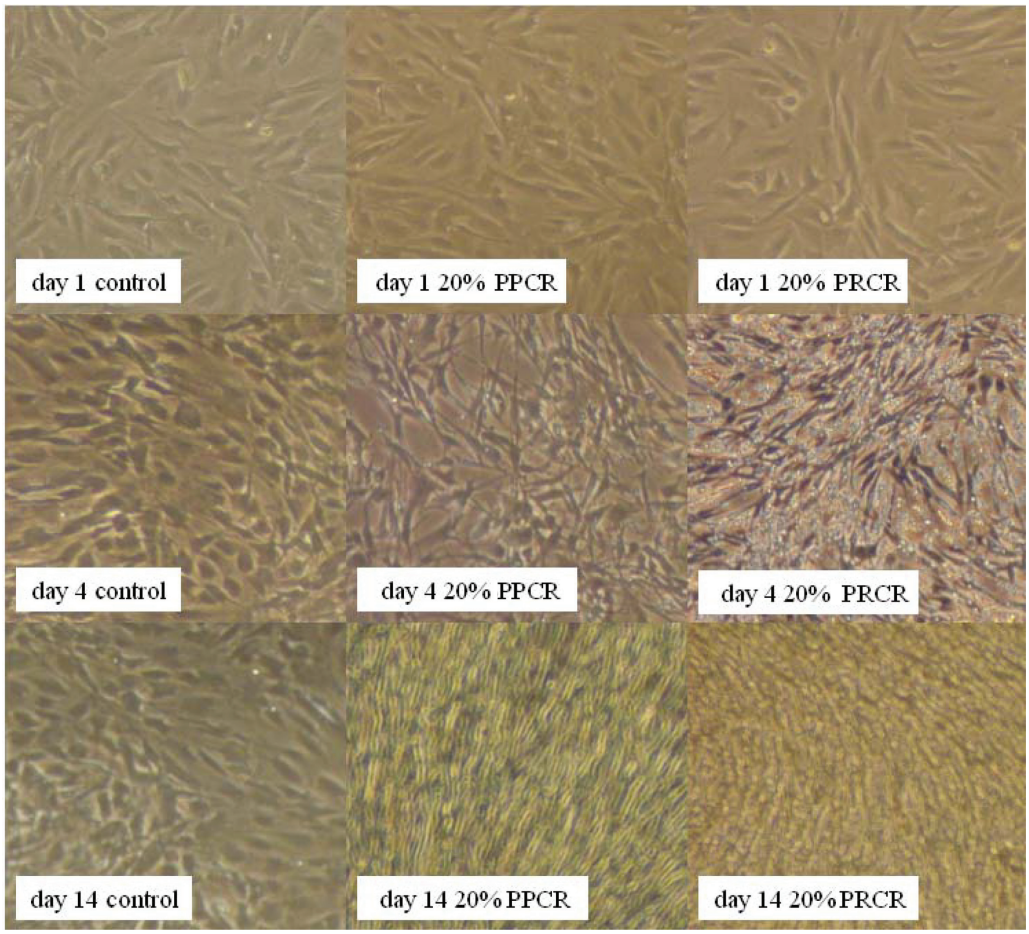

Figure 1. Photomicrographs of tenocyte cultures with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Representative photomicrographs (200× magnification) are shown of control, 20% PPCR, and 20% PRCR conditions on days 1, 4, and 14 of the experimental culture period.

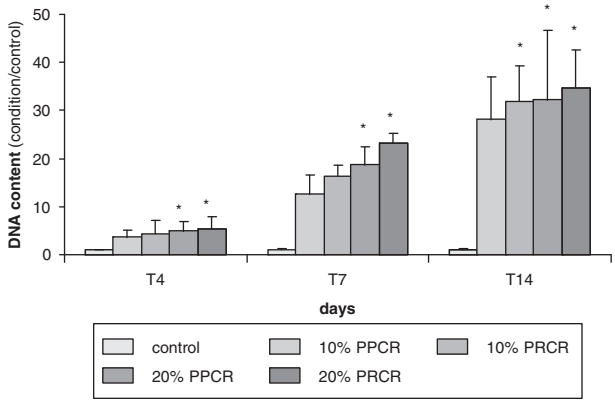

Figure 2. Deoxyribonucleic acid (DNA) content of tenocyte cultures with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Cells were harvested at day 4, 7, and 14. Control cultures (2% fetal calf serum) are set to 1 at each time point. The DNA content for each condition is expressed as the n-fold difference from control cultures at the corresponding time point. Results represent mean ± standard deviation (n = 6). *$P < .05$ as compared with control.

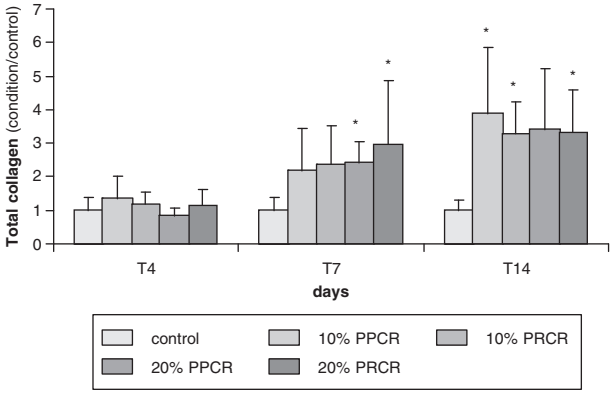

Figure 3. Total amount of collagen synthesized by tenocytes cultured with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Collagen was measured at day 4, 7, and 14. Total amount of collagen for each condition is expressed as the n-fold difference from control cultures at the corresponding time point. Control is set at 1, and results represent mean ± standard deviation (n = 6). *$P < .05$ as compared with control.

Hydroxyproline Assay (Amount of Collagen)

The total amount of collagen in the control condition increased with time. From day 7 on, PRCR and PPCR both increased the total amount of collagen, up to at least 3.3 times the control at day 14. However, only the 20% (vol/vol) conditions reached significance at day 7 (Figure 3). No differences were found between PRCR and PPCR, although at day 14, the 20% PPCR did not reach significance but the 20% PRCR did.

Gene Expression

Gene expression of COL1 significantly decreased with PRCR, but not PPCR, treatment at day 7 and 14 (Figure 4A). Differences between PRCR and PPCR were not significant. The COL3 gene expression was not significantly decreased upon addition of PPCR until day 14, but PRCR decreased COL3 gene expression at all points in time with the exception of 20% PRCR at day 4 (Figure 4B). The apparent difference between PRCR- and PPCR-treated groups at days 4 and 7 did not reach significance. The COL3/COL1 ratio did not significantly change in any condition.

Platelet-rich clot releasate significantly upregulated MMP1 expression at day 7 and 14 in the 20% condition (Figure 5A). The apparent dose-related response in MMP1 expression on days 7 and 14 reached significance only at day 14. The PRCR also increased MMP3 gene expression (significant at day 4) (Figure 5B). No significant differences in MMP1 and MMP3 gene expression were found with PPCR treatment. Neither PRCR nor PPCR affected MMP13 gene expression significantly (Figure 5C).

Platelet-rich clot releasate highly increased VEGF-A gene expression at all time points (significantly for the 10% PRCR condition at day 7 and for the 20% PRCR condition at days 4 and 14). This increase reached up to

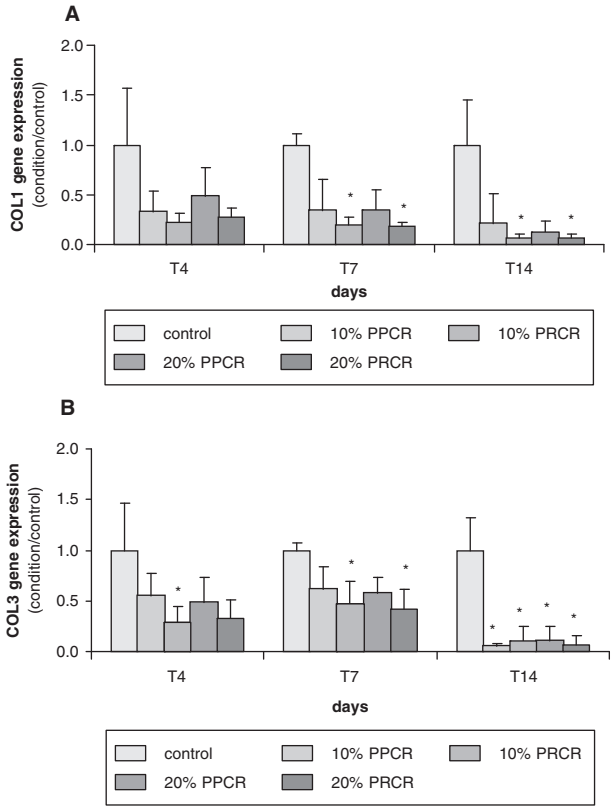

Figure 4. Gene expression levels of collagen Iα1 (COL1) (A) and collagen IIIα1 (COL3) (B) in tenocytes cultured with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Cells were harvested at day 4, 7, and 14. Gene expression was normalized to 18SrRNA and expressed as the n-fold difference from control cultures at the corresponding time point. Control is set at 1, and results represent mean ± standard deviation (n = 6). *$P < .05$ as compared with control.

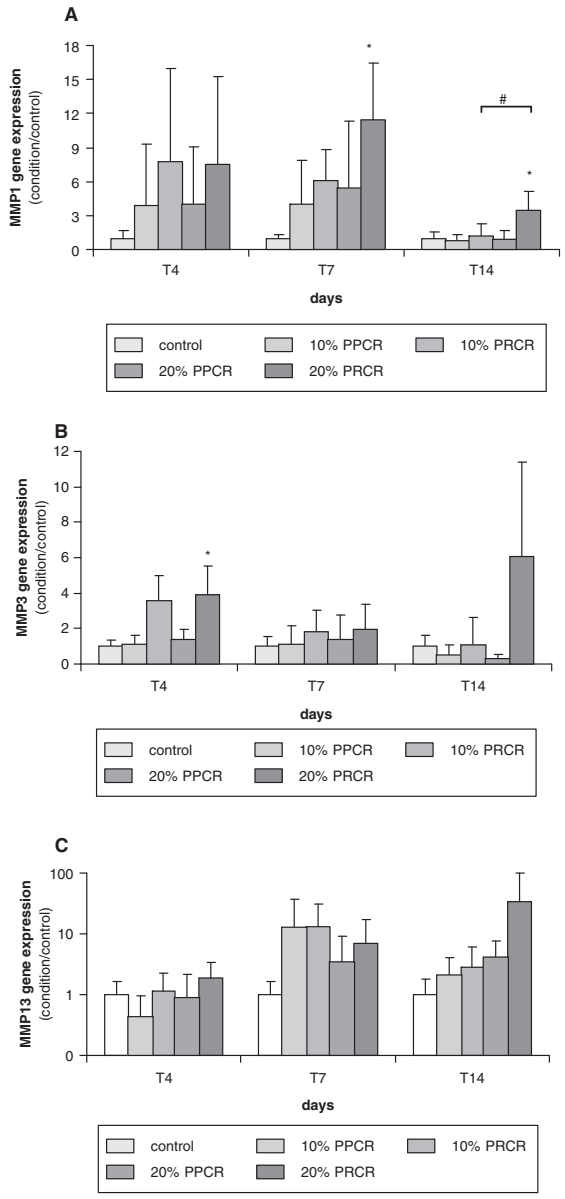

Figure 5. Gene expression levels of matrix metalloproteinase (MMP) 1 (A), MMP3 (B), and MMP13 (C) in tenocytes cultured with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Cells were harvested at day 4, 7, and 14. Gene expression was normalized to 18SrRNA and expressed as the n-fold difference from control cultures at the corresponding time point. Control is set at 1, and results represent mean ± standard deviation (n = 6). **P* < .05 as compared with control; #*P* < .05 comparison between 2 specified groups.

30 ± 14 times the control level at day 14 in the 20% PRCR condition (Figure 6A, note the logarithmic scale). Vascular endothelial growth factor–A expression in PRCR-treated cells appeared higher than in PPCR-treated cells, but this difference did not reach significance. Transforming growth factor–β1 gene expression was significantly increased only at day 4 in the 20% PRCR condition (Figure 6B).

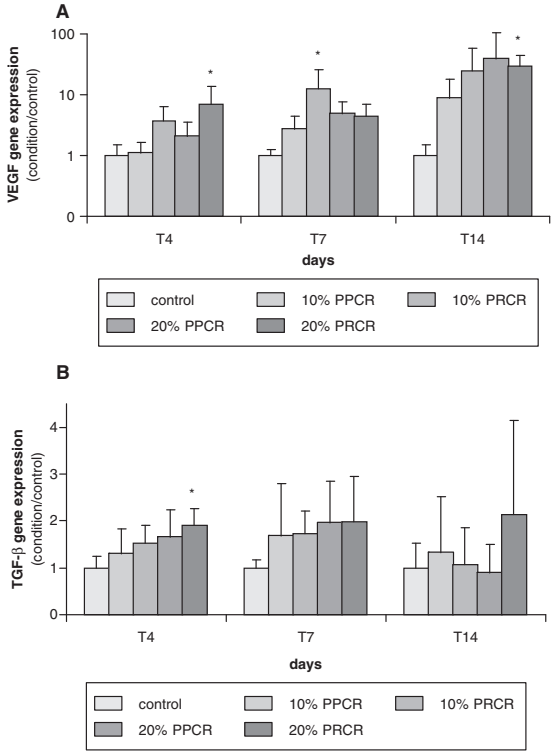

Figure 6. Gene expression levels of vascular endothelial growth factor (VEGF)-A (A) and transforming growth factor (TGF)-β1 (B) in tenocytes cultured with or without platelet-rich clot releasate (PRCR) or platelet-poor clot releasate (PPCR). Cells were harvested at day 4, 7, and 14. Gene expression was normalized to 18SrRNA and expressed as the n-fold difference from control cultures at the corresponding time point. Control is set at 1, and results represent the mean ± standard deviation (n = 6). VEGF expression in Figure 6A is presented on a logarithmic scale. **P* < .05 as compared with control.

DISCUSSION

In this in vitro study, we tested our hypothesis that the releasate from PRP, PRCR, has a positive effect on proliferation and matrix metabolism of human tendon cells to enhance tendon repair. Our results show that PRCR, but also PPCR, enhances cell proliferation and total collagen production by human tendon cells in culture, despite a possible decrease of collagen production per cell. Platelet-rich clot releasate, but not PPCR, slightly increases the expression of matrix-degrading enzymes and endogenous growth factors. In vivo, these effects of PRP, but also of PPP to a certain extent, on tenocyte behavior might accelerate the catabolic demarcation of traumatically injured tendon matrices and promote angiogenesis and the formation of a fibrovascular callus. Whether these working mechanisms will also be beneficial in cases of degenerative tendinopathies remains to be elucidated.

Examining the effects of PRCR and PPCR on human tendon cells in culture provides an interesting model to study the cooperative effects of a mixture of growth factors. We applied PRCR and PPCR to our cultures in 2 concentrations that are most frequently used in in vitro studies with

PRP,[1,4,5] namely 10% and 20% (vol/vol), allowing comparison of our results with the literature.[4] We did not apply a 100% concentration of the plasma product to our cultures[30] because we believe that this might be less comparable to the concentration of PRP reached during in vivo administration. Upon injection of 100% PRP into a tendon in vivo, it is unlikely that tendon cells are exposed for more than several minutes to a 100% PRP concentration because the PRP will be diluted in extracellular fluids immediately after injection. However, a major problem might be the fact that not only the platelets counts, but even more so the growth factor concentrations in the respective releasates, depending on the platelet activation, cannot be standardized, rendering comparison of experimental results rather complicated.

The only in vitro study with human tendon cells reported that, in contrast to unclotted PPP, both 20% PRCR and 20% PPCR stimulated tendon cell proliferation.[4] In line with their results, we also found that PRCR and PPCR increased cell numbers as well as the total amount of collagen, the latter probably being a direct consequence of increased cell numbers. The COL1 and COL3 transcripts decreased with both treatments similarly, suggesting a decrease in collagen production per cell. The COL3/COL1 ratio of the tendon cells is known to shift toward COL3 in case of tendinosis, in early stages of tendon repair, and in tendon scarring.[13,22] No significant changes were found in the COL3/COL1 ratio in this study, suggesting no negative side effects of PRCR and PPCR on this ratio.

Expression levels of MMP1 and MMP3 were upregulated by PRCR in some conditions, while no significant differences were found with PPCR treatment. Neither PRCR nor PPCR changed MMP13 expression. Gene expression of MMP1 shows no change in chronic Achilles tendinosis[16,18] but increases in ruptured Achilles and supraspinatus tendons.[18,29] While expression levels of MMP3 decrease in both degenerative and ruptured Achilles tendon,[2,16,18,29] in most studies, the gene expression of MMP13 does not change significantly. However, conclusions on MMP activity based on gene expression must be drawn only with utmost precaution. With current knowledge, it is difficult to state whether increased gene expression levels of MMP1 and MMP3, as found in our experiment, will be of benefit in degenerative or ruptured tendons. The secretion of MMPs facilitates the ingrowth of neovessels by dissolution of the extracellular matrix.[7] Angiogenesis contributes, on one hand, to the repair and remodeling of the injured tendon, but, on the other hand, the proteolysis of the extracellular matrix by invading endothelial cells results in impaired mechanical stability.[26] Therefore, the application of PRP in already degenerative tendons needs further investigation as progressive weakening of the matrix might predispose for spontaneous rupture.[19]

The amount of growth factors VEGF and HGF synthesized by the tendon cells is significantly higher with PRCR than with PPCR treatment, as demonstrated by Anitua et al.[4] In addition to this, we found that PRCR highly increased VEGF-A gene expression by tendon cells. Vascular endothelial growth factor is active after inflammation, most notably during proliferation and remodeling phases, where it has been shown to be a powerful stimulator of angiogenesis.[25] Increased VEGF expression and concentration could be an intrinsic mechanism for inducing angiogenesis as part of a tissue repair process.[33] Furthermore, TGF-β1 expression increased by PRCR at day 4. Transforming growth factor–β1 is thought to play an important role in the initial inflammatory response to tissue damage, having a positive effect on collagen production and viability of tendon cells.[25]

In our experiment, we aimed for a PRCR product with the following characteristics: (1) high platelet numbers before clotting as well as a high level of platelet activation, (2) content of mainly growth factors released from the platelets, and (3) absence of leucocytes to minimize risk of graft versus host reaction in our cultures (even more for the reason that the platelet concentrate used in this experiment was heterologous). After the concentration procedure, platelet numbers in PRP were 2.55 times baseline (whole blood), which corresponds well to the platelet concentration in the study of Anitua et al,[4] who also applicated their product in 20% (vol/vol) concentration on human tendon cells in vitro. After clotting, the platelet numbers in PRCR decreased to 0.08 times baseline, which indicates that most platelets were actually trapped in the clot. The higher concentrations of VEGF and PDGF-BB measured in PRCR compared with PPCR indicate that the platelets were not only trapped in the clot but were also activated to release their growth factors. A possible release of growth factors from white blood cells scarcely affected our results because up to 98% of the white blood cells was already eliminated from our preparations before clotting. This simultaneously minimized the risk of a graft versus host reaction in our experiments.

We found that not only PRCR but also PPCR affected the outcome parameters when compared with the control condition. This might be caused by the many unavoidable handling procedures performed to obtain PPCR and PRCR, such as drawing and centrifugating the blood, which could theoretically have activated some of the platelets, leading to untimely release. In this way, PPCR could also contain a small but sufficient amount of growth factors, which might have induced the effects that we found in PPCR conditions. However, other studies examining the difference in growth factor concentrations between whole blood, PRCR, and PPCR indicated that this is unlikely. Concentrations of TGF-β1 and PDGF-BB significantly increased in PRCR compared with PPCR and whole blood, at least 2-fold[4,30] and 3-fold,[4,30] respectively; in addition, growth factor concentrations in PPCR were not significantly different from whole blood.[30] A more plausible explanation for why both PRCR and PPCR affected the outcome parameters compared with control conditions therefore seems to be the fact that all experimental conditions basically consisted of adding extra serum (in the quality of 10% or 20% clot releasates) compared with the control cultures. The higher serum concentrations could account for the higher cell amounts in the experimental conditions because cells proliferate faster in higher serum concentrations. Notwithstanding the aforementioned observation that PPCR already exerted a considerable effect on the tendon cells, the effects on MMP and growth factor expression in particular were still more pronounced in PRCR conditions than in their equivalent PPCR conditions. These additive PRCR effects might be attributed to higher concentrations of

growth factors present in PRCR compared with PPCR. This is supported by the results of our growth factor concentration measurements in PPCR and PRCR.

Our tendon donors were of relatively young age, which might be a limitation of our study. Although unlikely, it is not known whether adult tendon tissue might respond very differently from adolescent tendon tissue regarding the effects of PRP on tendon cell behavior. Also, the behavior of explanted and passaged tendon cells in an artificial culture environment cannot be considered identical to the behavior of tendon cells in their natural matrix environment in vivo. Therefore, one should always be cautious about translating culture data directly to the in vivo situation.

Finally, the PRP in our study was applied heterologously to the tendon cell cultures, although in clinical settings, this plasma product is usually prepared from autologous blood. For this reason, we aimed to and succeeded in minimizing the number of leucocytes so that no graft versus host reactions occurred in our cultures.

Autologous PRP application appears promising in healing of traumatic tendon injuries and tendinopathies, but how PRP might improve or accelerate the tendon repair process remains to be elucidated. We found that PRP clot releasate stimulates cell proliferation and collagen deposition and enhances the gene expression of matrix-degrading enzymes and endogenous growth factors by human tendon cells in vitro. This suggests that in vivo PRP application could lead to accelerated remodeling and angiogenesis in the injured matrix, which may promote repair of traumatic tendon injuries.

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