Human Platelet-Rich Plasma Stimulates Migration and Chondrogenic Differentiation of Human Subchondral Progenitor Cells

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ABSTRACT: In cartilage repair, platelet-rich plasma (PRP) is used in one-step approaches utilizing microfracture and matrix-induced chondrogenesis procedures, bone marrow-derived cell transplantation, or intra-articular injection. The aim of our study was to evaluate the effect of human PRP on the migration and chondrogenic differentiation of human subchondral progenitors. Human progenitors were derived from subchondral cortico-spongious bone (CSP), were analyzed for their migration capacity upon PRP treatment in 96-well chemotaxis assays and cultured in high-density pellet cultures under serum-free conditions in the presence of 5% PRP. Chemotaxis assays showed that 0.1–100% PRP significantly ($p < 0.05$) stimulate the migration of CSP compared to untreated controls. Histological staining of proteoglycan and immuno-staining of type II collagen indicated that progenitors stimulated with PRP show significantly increased cartilage matrix formation compared to untreated progenitors. Real-time gene expression analysis of typical chondrocyte marker genes as well as osteogenic and adipogenic markers like osteocalcin and fatty acid binding protein showed that PRP induces the chondrogenic differentiation sequence of human progenitors in high-density pellet cultures, while osteogenic or adipogenic differentiation was not evident. These results suggest that human PRP may enhance the migration and stimulate the chondrogenic differentiation of human subchondral progenitor cells known from microfracture. J Orthop Res 30:845–852, 2012

Keywords: platelet-rich plasma; cartilage regeneration; microfracture; stem cells; chemotaxis; chondrogenesis

A commonly used first-line treatment option to restore cartilage is the microfracture technique.[1] In this bone marrow-stimulating technique, holes are drilled or introduced with a pick into the subchondral bone to induce bleeding. A blood clot evolves that will form a fibrous to hyaline-like repair tissue. The clinical outcome of this procedure is variable, the quality of the repair tissue is unpredictable and age as well as the activity level of the patient may influence the outcome.[2] To improve the microfracture technique, cell-free implants are suggested that recruit stem cells to the cartilage defect and guide them towards forming of hyaline cartilage repair tissue. In the ovine model, a cell-free polyglycolic acid (PGA)-hyaluronan implant immersed with autologous serum was implanted into the defect after microfracture. Subchondral mesenchymal progenitor cells migrated into the defect and formed cartilaginous repair tissue. Six months after implantation, histological staining showed the formation of cartilaginous repair tissue that was superior to the repair tissue formed after microfracture alone.[3,4] Recently, in first clinical cases, microfractured cartilage defects of the knee were covered with the cell-free PGA-hyaluronan implant. One- to 2-year follow-up showed good to excellent defect filling and the formation of hyaline-like cartilage repair tissue.[5,6]

Additional supporting information may be found in the online version of this article.

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Mesenchymal progenitor cells derived from the subchondral bone, cortico-spongious progenitors (CSP), are characterized by high proliferation capacity and the ability to differentiate into bone, cartilage, and fat. CSP show the typical cell surface markers known from mesenchymal stem and progenitor cell, such as CD 29, CD 73, CD 90, CD 105, and CD 166.[7–10] The migration and recruitment of such mesenchymal stem and progenitor cells in microfracture may be mediated by cytokines and growth factors, also present in varying amounts in human serum like, for example, interleukin 8, stromal-derived factor 12, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF1), and bone morphogenetic proteins (BMP)-4 and-7.[11–13]

In recent years, platelet-rich plasma (PRP) is often used in a variety of clinical applications, including spine and dental surgery as well as cartilage repair.[14] PRP contains high amounts of growth factors, which are available in the α-granules and can be delivered continuously by activation of the platelets.[15] Amongst others, the α-granules may release growth factors such as PDGF, transforming growth factor-beta (TGFB), IGF1, platelet-derived angiogenesis factor (PDAF), VEGF, and epidermal growth factor (EGF).[16,17] In addition, chemokines are released like CCL3, CCL5, CCL 7, CCL 17, or CXCL 1 and CXCL 12 that may lead to the migration of progenitor cells from the bone marrow.[18] In cartilage formation or chondrocyte differentiation, TGFB is known to induce chondrogenesis of mesenchymal progenitor cells, while PDGF helps chondrocytes to maintain the

hyaline-like chondrogenic phenotype and induces proliferation and proteoglycan synthesis.[19]

Taking the variety of autologous growth factors in PRP into account, cartilage repair strategies like microfracture treatment that addresses cell migration and chondrogenic differentiation may benefit from the application of PRP. PRP may have an impact on stem cell and/or mesenchymal progenitor cell in-growth and the development of these cells towards chondrocytes. Therefore, we hypothesize that PRP induces the effective migration and chondrogenic differentiation of human subchondral mesenchymal progenitor cells known from microfracture, in vitro.

MATERIALS AND METHODS

Isolation and Cultivation of Human Cortico-Spongious Progenitor Cells (CSP)

Human CSP were isolated from spongious bone of human tibia or femur head, post mortem ($n = 3$ donors; age 29–73 years) as described.[10] The ethics committee of the Charité-Universitätsmedizin Berlin approved the study. Bone was cut into small fragments that were digested for 4 h at 37°C using 256 U/ml collagenase XI (Sigma–Aldrich, St. Louis, MO). The fragments were placed in Primaria™ cell culture flasks (Becton Dickinson, Franklinlakes, NJ) and cultured in DME-medium (Biochrom, Berlin, Germany) containing 10% human serum (German Red Cross, Berlin, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml gentamycin, 0.1 μg/ml amphotericin B, 2 mM *N*-acetyl-L-alanyl-L-glutamine (all Biochrom) and 2 ng/ml human fibroblast growth factor-2 (PeproTech, Hamburg, Germany). Cells that reached 80–90% confluence were sub-cultivated using trypsin in PBS (0.05% v/v, Biochrom) and re-plated at a density of 6,000 cells/cm^2. Medium exchange was performed every 2 or 3 days.

Preparation of Human Platelet-Rich Plasma

PRP ($n = 5$) from normal, healthy blood donors was extracted by aphaeresis at the Department of Transfusion Medicine, Charité-Universitätsmedizin Berlin, using an automated blood collection system (Trima Accel®, CaridianBCT, Lakewood, CO) with ACD-A (anticoagulant citrate dextrose-A). PRP was prepared according to Weibrich and colleagues.[20] Number of platelets was 0.6–1.3 × 10^{10} per ml. Leukocytes were $<0.3 \times 10^4$ ml. Platelets concentrates (approx. 300 ml per sample) were frozen overnight at −30°C, thawed and centrifuged. The supernatant was taken and used for further analysis. Total protein content of PRP was determined using the bicinchoninic acid (BCA) assay (Sigma–Aldrich) according to the manufacturer's recommendations. Bovine serum albumin was used to prepare a standard curve. Samples were measured at 562 nm in a micro-plate reader (Synergy HT, BioTek, Bad Friedrichshall, Germany). The protein content was calculated according to the BSA standard and was adjusted with PBS to 40 mg/ml. PRP was stored at −20°C. Prior use, PRP was thawed slowly at 4°C, followed by centrifugation at 4°C at 1,600 × g for 10 min. The supernatant was used immediately.

Cell Migration Studies

The chemotactic effect of individual PRP preparations ($n = 5$) on progenitor cells (pool of $n = 3$ donors) was tested using a 96 multi-well chemotaxis assay (Neuro Probe, Gaithersburg, MD) as described previously.[21] In brief, PRP (0.1–100% PRP in DME medium containing 0.1% human serum (German Red Cross) was filled in the lower wells of the multi-well plate and covered with a porous (pore size 8 μm) polycarbonate membrane. In the upper compartment of each well 30,000 CSP in DME containing 0.1% human serum were given. The assay was performed in triplicates. DME medium containing 0.1% human serum was used as negative control, DME medium with 10% human serum served as positive control. After incubation at 37°C in humidified air for 20 h, non-responding cells were removed from the membrane and CSP that migrated through the pores to the underneath of the membrane were fixed in ice-cold methanol/acetone (1:1 v/v), followed by staining with Hemacolor® (Merck, Darmstadt, Germany). Enumeration of migrated CSP was done microscopically. Pictures were taken from four representative visual fields of each well, stained cells were counted using ImageJ (National Institutes of Health, Bethesda, MD) and the cell number was extrapolated to the size of one well.

Cell Differentiation Studies

Chondrogenic differentiation of CSP (passage 3) was performed under serum free conditions in high-density pellet cultures (CSP pool n = 3; 250,000 cells/pellet), as described previously.[22] Chondrogenesis was induced by adding 10 ng/ml TGFB3 (R&D Systems, Minneapolis, MN, USA) to DME-medium containing 1% ITS + 1 (Insulin-Transferrin-Selenium), 1 mM sodium pyruvate, 0.35 mM L-proline, 0.17 mM L-ascorbic acid-2-phosphate and 0.1 μM dexamethasone (all Sigma–Aldrich). To evaluate the influence of PRP on chondrogenic differentiation of CSP, pellets were treated with 5% PRP alone (pool of $n = 5$ PRP preparations) in complete DME medium ($n = 52$) or in combination with 10 ng/ml TGFB3 ($n = 52$). Pellets cultured in complete DME-medium without PRP or TGFB3 served as controls ($n = 52$). The medium was exchanged every 2–3 days and cells were maintained for up to 28 days.

Histology and Immunohistochemistry

At each point in time, cryo-sections (6 μm; n = 9) were prepared and stained using Alcian Blue 8GX (Roth, Karlsruhe, Germany) and counterstaining with nuclear fast red (Sigma–Aldrich) to evaluate presence of proteoglycans. Sections ($n = 9$) were stained with primary rabbit anti-human type II collagen antibody (Acris, Hiddenhausen, Germany) for 40 min, followed by colormetrical detection with 3-amino-9-ethylcarbazole (EnVision™; Dako, Glostrup, Denmark) and counterstaining with hematoxylin (Merck). Quantification of proteoglycan and type II collagen staining by image analysis with the Photoshop software was performed as described previously.[7] In brief, a standard color was defined that represents the particular color of the specific staining. The tools "magic wand" and "select similar" were used to select areas of that particular color. The amount of stained pixels in relation to the total amount of pixels of the section gives the percentage of the proteoglycan or type II collagen-stained area.

Osteogenic differentiation was analyzed by von Kossa staining of mineralized matrix components. Sections ($n = 9$) were incubated in the dark at room temperature with 5% silver nitrate for 30 min following incubation with 1.7 M sodium carbonate/10% (v/v) formalin for 5 min. After washing with tap water for 10 min, counterstaining was performed with nuclear fast red for 4 min. Adipogenic differentiation

($n = 9$ sections) with formation of lipid droplets was visualized using Oil Red O (Sigma–Aldrich) and counterstained with hematoxylin.

Polymerase Chain Reaction (PCR)

Total RNA (20 pellets per experimental condition and point in time) was isolated as described previously[23] and 1 μg RNA was reversely transcribed with iScript cDNA Synthesis Kit according to the manufacturer's recommendations (BioRad, Munich, Germany). The relative expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used no normalize the samples. Real-time RT-PCR was performed with 1 μl of each cDNA sample in triplicates using i-Cycler PCR system (Bio BioRad) with SYBR green PCR Core Kit (Applied Biosystems, Foster City, CA). Relative quantification of marker genes (Supplemental material Table 1) was performed and is given as percentage of the GAPDH product.

Statistical Analysis

Statistical analysis was performed with SigmaStat 3.5 (Systat Software, GmbH, Erkrath, Germany). For determining significant differences in cell migration and quantitative image analysis normal distribution and equal variance of the data were analyzed according to Kolmogorov–Smirnov. Subsequently, the parametric t-test or the non-parametric Mann–Whitney U-test was used. Differences were considered significant at $p = < 0.05$.

RESULTS

Chemotactic Activity of PRP

In the presence of 0.1% HS up to 200 CSP migrated through the pores of the membrane (Fig. 1A, the black arrow shows a membrane pore). 10% HS stimulated the migration of up to 865 cells (Fig. 1B, the white arrow shows a migrated cell). The highest number of migrated cells (up to 18,957 cells) was evident after stimulation of CSP with 5% PRP (Fig. 1C). Compared to CSP stimulated with 0.1% HS, the number of migrated cells was significantly increased ($^{*}p < 0.05$) by stimulation with 10% HS. Stimulation of CSP with PRP (100%, 50%, 25%, 10%, 5%, 2%, 1%, and 0.1%) significantly ($\#p < 0.05$) increased the number of migrated CSP, compared to CSP treated with 0.1% or 10% HS (Fig. 1D).

Histology of CSP Undergoing the Chondrogenic Differentiation

CSP treated with PRP, TGFB3, or PRP-TGFB3 developed a dense pellet, rich in viable cells (Fig. 2A–C,E,G). Controls not stimulated with PRP or TGFB3 developed a small fibrous pellet (Fig. 2D,H). High-density cultures stimulated with PRP (Fig. 2A) or TGFB3 (Fig. 2C) or PRP-TGFB3 (Fig. 2B) showed a proteoglycan-rich tissue. Deposition of type II collagen was evident in pellets treated with PRP (Fig. 2E), TGFB3 (Fig. 2G), or PRP-TGFB3 (Fig. 2F). CSP pellets not stimulated with PRP or TGFB3 showed a weak staining of proteoglycan (Fig. 2D) and no type II collagen (Fig. 2H).

Image analysis showed that CSP pellets cultured with PRP, TGFB3 or PRP-TGFB3 showed a steady increase of proteoglycan (Fig. 3A). At day 28, pellets treated with PRP, TGFB3 or PRP-TGFB3 showed proteoglycan in 91–95% of the section, while untreated controls showed only up to 0.9% positive staining. At day 7, a significant ($^{*}p < 0.005$) increase of proteoglycan was evident in CSP treated with TGFB3 compared to all other groups. At day 14, 21, and 28, CSP treated with PRP, TGFB3, and PRP-TGFB3 showed significantly ($\#p < 0.05$) increased proteoglycan staining, compared to the untreated controls. Type II collagen (Fig. 3B) was observed as early as at day 7 in CSP treated with TGFB3 (7% positive staining). At day 28,

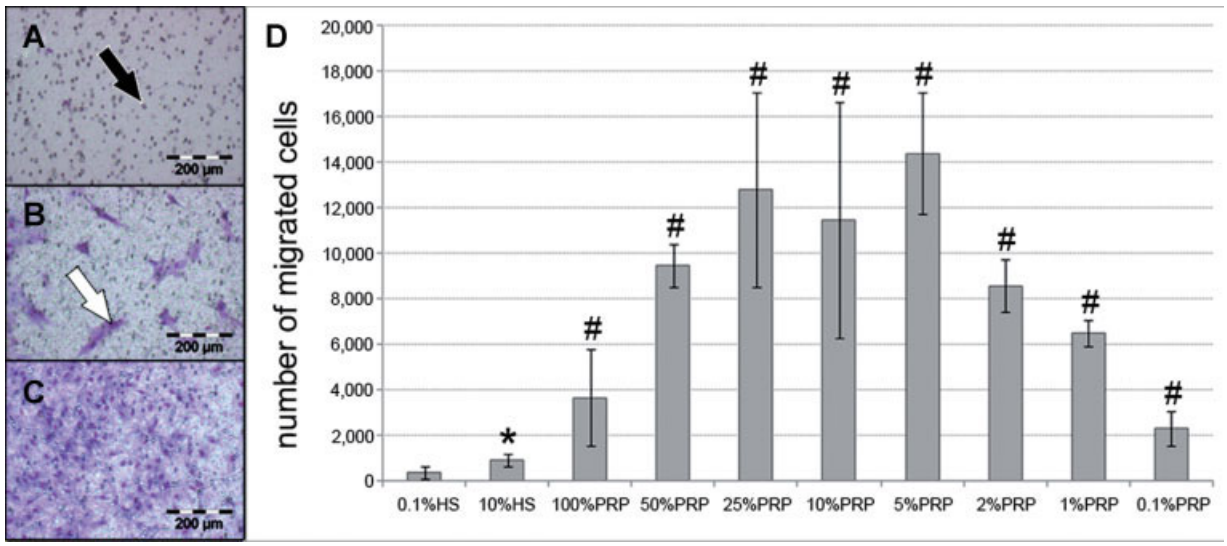

Figure 1. Quantification of the chemotactic activity of PRP on subchondral progenitors. (A) Medium with 0.1% human serum (negative control) showed virtually no migrated cells (A), the black arrow indicates a membrane pore. (B) Medium with 10% human serum (positive control) stimulated the migration of progenitor cells (white arrow). (C) Migrated progenitor cells upon stimulation with 5% PRP. (D) Number of migrated cells upon stimulation with rising amounts of PRP. *Significant ($p = < 0.05$) increase compared 0.1% human serum. #Significant ($p = < 0.05$) increase compared 0.1% and 10% human serum. The bars show the mean ($n = 5$) and the SD.

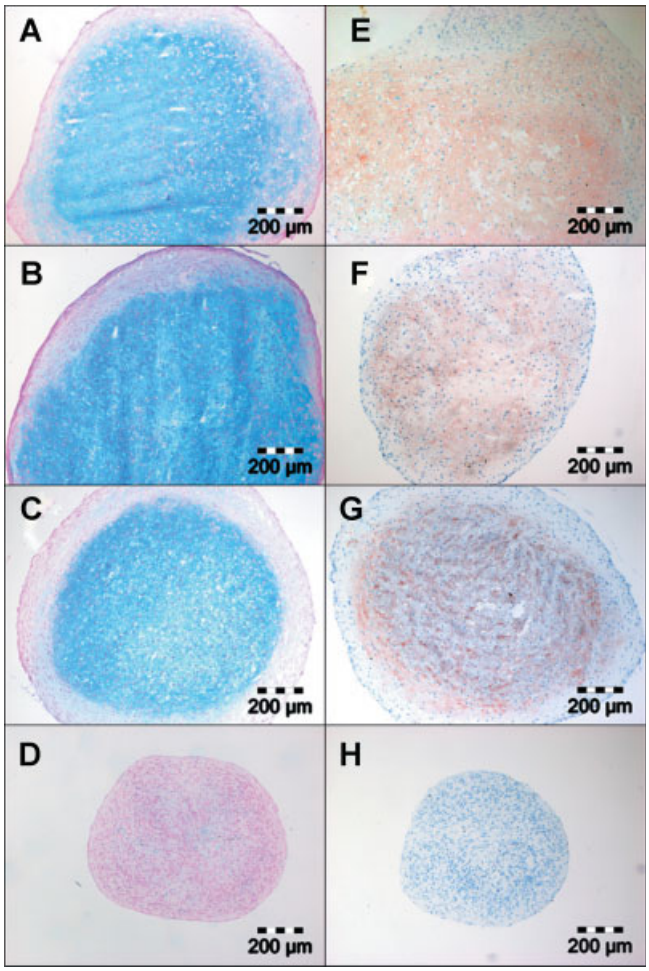

Figure 2. Histochemical analysis of CSP undergoing chondrogenic differentiation upon stimulation with TGFB3, PRP or PRP-TGFB3. At day 28, CSP stimulated with PRP (A), or PRP-TGFB3 (B) and TGFB3 (C) showed a strong staining of proteoglycan. Type II collagen was evident in CSP treated with PRP (E), PRP-TGFB3 (F) and TGFB3 (G). Control CSP showed a weak staining of proteoglycan (D) and absence of type II collagen (H).

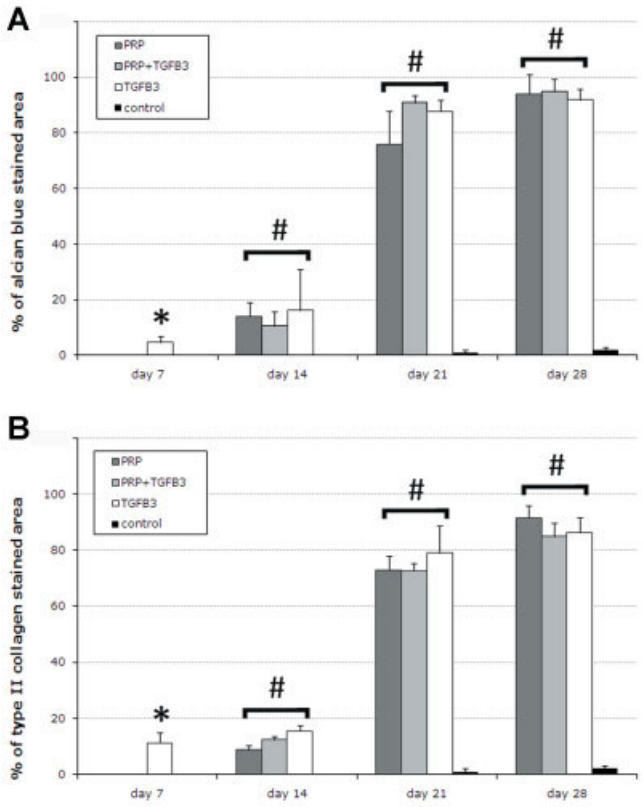

Figure 3. Quantification of histological and immune-histochemical staining. (A) Image analysis showed that the amount of proteoglycan was significantly ($^{*}p = < 0.05$) elevated in CSP pellets treated with TGFB3 compared to pellets stimulated with PRP-TGFB3 and PRP at day 7. At day 14, 21, and 28 CSP treated with TGFB3, PRP and PRP-TGFB3 showed significantly increased ($\#p = < 0.05$) amounts of proteoglycan compared to controls. (B) Type II collagen was significantly ($^{*}p = < 0.05$) enhanced in CSP treated with TGFB3 compared to CSP stimulated with PRP, PRP-TGFB3 and untreated controls at day 7. At day 14, 21, and 28, collagen type II was significantly ($\#p = < 0.05$) increased in CSP treated with PRP, PRP-TGFB3 and TGFB3, compared to untreated controls. The bars show the mean ($n = 9$) and the SD.

type II collagen was highly present in CSP treated with TGFB3 (86% positive staining), PRP (91%) and PRP-TGFB3 (85%). Type II collagen was weak in untreated controls (1.2% positive staining). As shown for proteoglycan staining, a significant ($^{*}p < 0.005$) increase of type II collagen was evident in CSP treated with TGFB3 at day 7. At day 14, 21, and 28, CSP treated with PRP, TGFB3 and PRP-TGFB3 showed a significantly ($\#p < 0.05$) increased type II collagen staining, compared to the untreated controls.

Gene Expression Analysis of CSP High-Density Cultures

To assess early effects of PRP treatment on progenitor cell differentiation, gene expression analysis for cartilage-related genes was performed at days 7 and 14 (Fig. 4). At day 14, PRP induced the chondrogenic marker genes *aggrecan* (from 8% to 65%), *type II collagen* (from 1% to 835%), *link protein* (from 658% to 6,320%) and *type IX collagen* (from 217% to 3,987%). TGFB3 as well as PRP-TGFB3 induced the marker genes *aggrecan* (from 8% up to 138%), *cartilage oligomeric matrix protein* (from 4% up to 90%), *type II collagen* (from 1% up to 915%), *link protein* (from 658% up to 6,993%), and *type IX collagen* (from 217% up to 6,968%), at day 14. The expression level of *type X collagen* in CSP treated with TGFB3 was up to 13,371%, in CSP treated with PRP-TGFB3 up to 4,103%, and after stimulation with PRP alone up to 853%. Untreated controls showed a type X collagen expression level of 80% relative to the expression level of GAPDH.

For evaluating adipogenic or osteogenic differentiation of subchondral progenitors upon PRP treatment, histological and gene expression analyses of typical adipogenic and osteogenic markers were performed (Fig. 5). At day 28, CSP stimulated with PRP or TGFB3 or PRP-TGFB3 showed no calcified matrix as assessed by von Kossa staining (Fig. 5A). The gene expression level of osteocalcin was low in CSP pellets treated with TGFB3, PRP and PRP-TGFB3 (Fig. 5B).

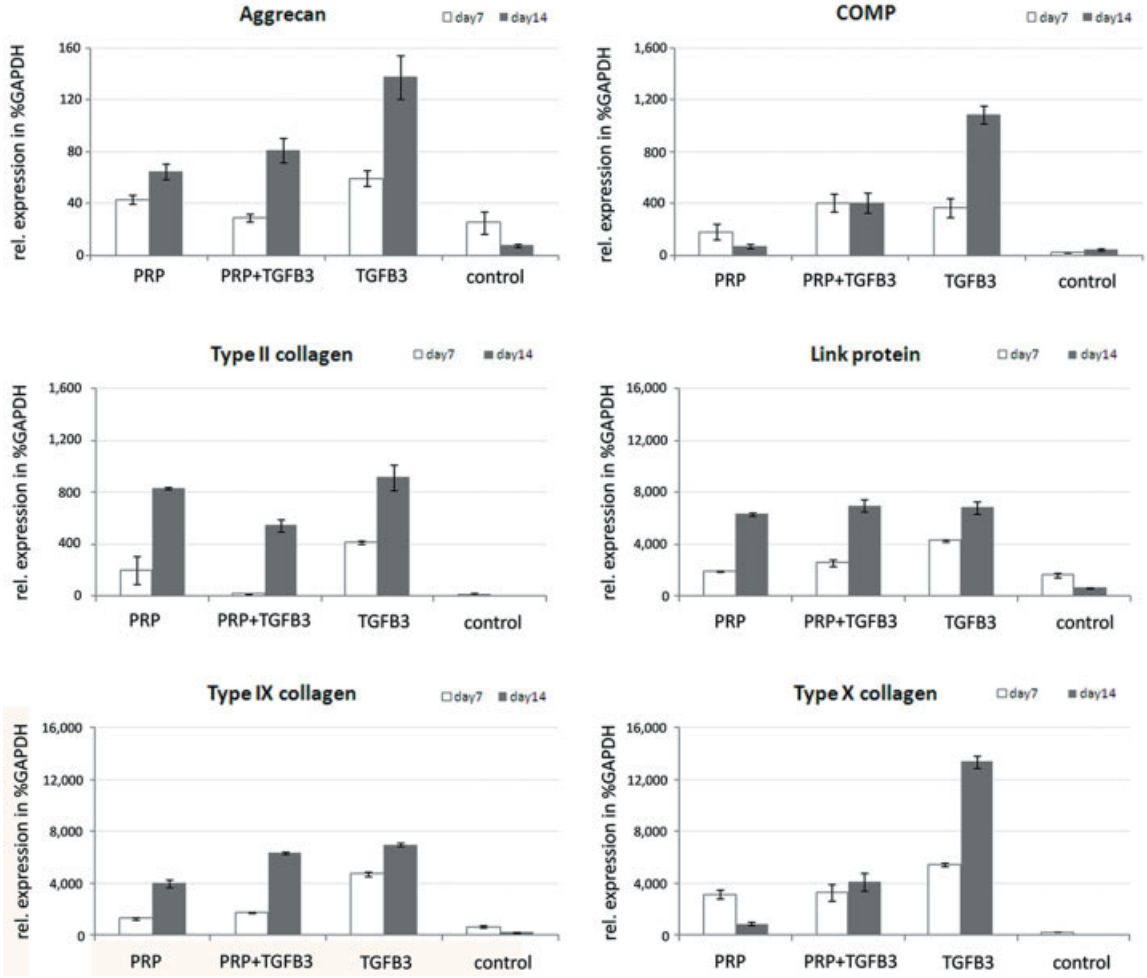

Figure 4. Semi-quantitative real-time gene expression analysis of CSP treated with TGFB3, PRP, or PRP-TGFB3.The expression level of typical chondrogenic marker genes such as aggrecan, cartilage oligomeric matrix protein (COMP), link-protein, type II, and type IX collagen was calculated as percentage of the expression level of the housekeeping gene GAPDH. For chondrocyte hypertrophy, the expression level of type X collagen was measured. The bars show the mean ($n = 3$) and the SD.

At day 28, CSP stimulated with PRP, TGFB3, or PRP-TGFB3 showed no lipid droplets (Fig. 5C) and compared to controls a decrease of the expression level of fatty acid-binding protein-4 (Fig. 5D).

DISCUSSION

In the present study, we demonstrated that human PRR stimulated the migration and the chondrogenic differentiation of human subchondral mesenchymal progenitors. Although the chondrogenic developmental sequence may be delayed compared to the TGFB3-mediated cartilage matrix formation, PRP effectively induced the formation of cartilage matrix rich in proteoglycans and type II collagen. Platelets play a key role in tissue homeostasis and repair. The alpha granules of platelets contain a variety of growth factors, cytokines and chemokines.[24–26] In particular PDGF, IGF, and chemokines may account for the stimulatory effect of PRP on subchondral progenitor cell migration. Among other growth factors, PDGFββ, IGF-I, and IGF-II have been shown to recruit human mesenchymal progenitor cells derived from bone marrow.[11,27] In recent years, special attention has been drawn to chemokines that stimulate the migration of human bone marrow-derived mesenchymal stem cells.[12,28,29] CSP that were used in the present study are actively recruited by PRP as shown here and by human synovial fluid containing the chemokines CCL25, CXCL10, and XCL1 that stimulate CSP migration in 96-well chemotaxis assays.[21,30] Therefore, it is most likely that PRP, respectively its particular growth factors and chemokines independently or synergistically may enhance the migration of human subchondral progenitor cells. Using human CSP in our serum-free high-density pellet culture system that favors chondrogenic differentiation,[10,22,31] PRP as well as TGFB3 induced the expression of typical chondrogenic markers genes like for example type II and IX collagens, cartilage link protein and aggrecan, while osteogenic and adipogenic marker gene expression remained low or has been repressed. Cartilage tissue formation with proteoglycan and type II collagen

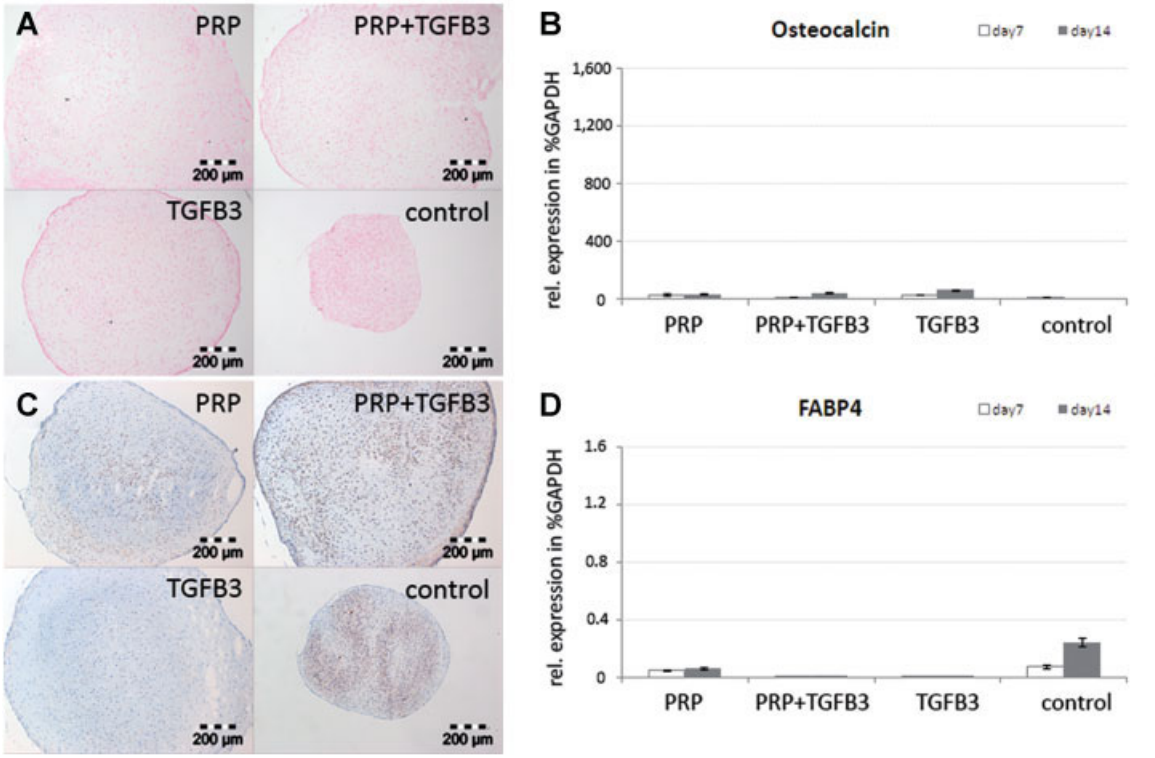

Figure 5. Histological analysis of matrix mineralization of CSP stimulated with TGFB3, PRP, and PRP-TGFB3. (A) At day 28, CSP co-stimulated with PRP, TGFB3, or PRP-TGFB3 showed no matrix calcification. (B) The real-time gene expression analyses showed a low expression of osteocalcin in CSP treated with TGFB3, PRP and PRP-TGFB3. (C) Histological analysis of lipid vacuoles of CSP stimulated with TGFB3, PRP, or PRP-TGFB3 showed no evidence for adipogenic differentiation. (D) The real-time gene expression analyses showed a marginal expression of fatty acid-binding protein-4 (FABP4) in CSP pellets treated with TGFB3, PRP, and PRP-TGFB3. The bars show the mean ($n = 3$) and the SD.

confirmed the chondrogenic effect of PRP on subchondral progenitor cells in high-density pellet culture. Interestingly, PRP or platelet supernatant stimulates the proliferation of articular chondrocytes, but has no effect on chondrocyte differentiation or even leads to dedifferentiation towards a fibroid phenotype as shown in bovine, ovine and human chondrocytes.[32–34] In contrast, PRP also stimulated the proliferation of mesenchymal stem cells but did not induce stem cell differentiation as shown in the ovine model.[34] In human mesenchymal stem cells, buffered PRP enhanced stem cell proliferation and induced the differentiation along the osteogenic and chondrogenic lineage as shown by the induction of the transcription factors Runx2 and Sox9 in monolayer cultures.[35] As shown here, in high-density pellet cultures, PRP induced the chondrogenic differentiation but not the development along the osteogenic or adipogenic lineage. Obviously, the cell culture or cell differentiation system (monolayer vs. 3D pellet culture) has to be taken into account when assessing the potential of putative chondrogenic inducers. In addition, the conflicting data about PRP effects in vitro and in clinical application may due to the various techniques for harvesting and activating PRP.[16] Since the release of growth factors depends on the activation of the platelets, validated methods and/or quantification of growth factors/platelet number are necessary to ensure standardized PRP preparations, in particular for clinical application.

PRP contains high amounts of TGFB (up to 260 ng/ml)[36] and since all isoforms of TGFB induce chondrogenesis of mesenchymal stem cells,[22,37] it is likely that TGFB available in PRP may induce chondrogenesis of human progenitor cells. The lack of knowledge about which growth factors and at which doses exert the PRP effect is a limitation of the study. Further studies have to elucidate which particular PRP growth factors and which minimal and/or maximal doses of PRP may mediate the migration and chondrogenic differentiation of subchondral progenitors. Such application-related (chondrogenic) factors should be measured in terms of quality control to ensure a standardized high quality PRP. Further limitation of the study is, due to human donor tissue limitations, the need for using a pool of PRP and progenitor cells. Future studies using individual PRP preparations should verify the robust chondrogenic effect of PRP in an autologous setting, favorable for clinical application. Thinking of cartilage repair, the in vitro system does not resemble the situation in a cartilage defect and does not take into account that additional factors released by the subchondral bone or present in the synovial fluid may influence the

chondrogenic developmental sequence of subchondral progenitors.

Recently, blood-derived additives and PRP found its way into cartilage repair approaches by its use in intra-articular injection or by enhancing the microfracture technique. PRP stimulated cartilage repair after microfracture in the ovine model, even more effectively when it was used as a gel.[38] The chondrotissue matrix of a resorbable polyglycolic acid-hyaluronan scaffold immersed with autologous serum improved the quality of microfracture-induced cartilage repair in ovine joint defects[4] and has been shown to be clinically effective at 1- and 2-year follow-up.[5,6] The injection of PRP in knees with chronic degenerative symptoms revealed a beneficial effect on pain reduction and improvement in knee function in younger patients at 1-year follow-up.[19] A PRP gel was used in five patients to fill microfractured cartilage defects covered with a type I/III collagen membrane and resulted in improvement of the patients' situation, while MRI showed incomplete filling in three out of five cases.[39] Using a scaffold-based one-step approach in 20 patients, PRP combined with bone marrow cells and transplanted with a hyaluronan scaffold has been shown to regenerate cartilaginous tissue and to improve the patient's situation significantly at 2-year follow-up compared to the preoperative situation as assessed by the IKDC and KOOS score.[40] This is in line, with our own clinical experience with PRP and the chondrotissue matrix in more than 50 patients that show clinical improvement after 1 and 2 years and the formation of hyaline-like cartilage (personal communication A. Siclari). Although speculative, PRP may be beneficial in cartilage repair, in particular in bone marrow-stimulating techniques such as microfracture or drilling. PRP may enhance the migration of subchondral progenitor cells and in turn the population of cartilage defects and/or scaffolds with stem and progenitor cells. In addition, PRP has obviously a chondrogenic effect on mesenchymal progenitor cells. Therefore, the application of PRP may accelerate the formation of cartilage repair tissue or may improve repair tissue quality towards a hyaline cartilage tissue. However, controlled clinical studies have to proof the beneficial effects of PRP in cartilage repair.

In conclusion, PRP is a source of autologous growth factors that is clinically applied for cartilage repair and may exert its effect by stimulating the migration and chondrogenic differentiation of mesenchymal progenitor cells as shown here for human subchondral mesenchymal progenitor cells cultured in high-density in the presence of human PRP.

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