Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis

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Summary

Objective: Platelet-rich plasma (PRP) is a fraction of plasma that contains high levels of multiple growth factors. The purpose of this study was to examine the effects of PRP on cell proliferation and matrix synthesis by porcine chondrocytes cultured in alginate beads, conditions that promote the retention of the chondrocytic phenotype, in order to determine the plausibility of using this plasma-derived material for engineering cartilage.

Design: PRP and platelet-poor plasma (PPP) were prepared from adult porcine blood. Adult porcine chondrocytes were cultured in the presence of 10% PRP, 10% PPP or 10% fetal bovine serum (FBS) for 3 days. Cell proliferation, proteoglycan (PG) and collagen synthesis were quantified, and the structure of newly synthesized PG and collagen was characterized.

Results: Treatment with 10% PRP resulted in a small but significant increase in DNA content (+11%, vs FBS; P < 0.01; vs PPP; P < 0.001). PG and collagen syntheses by the PRP-treated chondrocytes were markedly higher than those by chondrocytes treated by FBS or PPP (PG: +115% vs FBS; +151% vs PPP, both P < 0.0001; collagen; PRP: +163% vs FBS; +163% vs PPP, both P < 0.0001). Biochemical analyses revealed that treatment with PRP growth factors did not markedly affect the types of PGs and collagens produced by porcine chondrocytes, suggesting that the cells remained phenotypically stable in the presence of PRP.

Conclusion: PRP isolated from autologous blood may be useful as a source of anabolic growth factors for stimulating chondrocytes to engineer cartilage tissue.

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Key words: Platelet-rich plasma, Chondrocyte, Growth factor, Proteoglycan, Collagen.

Introduction

Articular cartilage provides a resilient, low friction, wear resistant joint surface. Properties inherent to adult articular cartilage, such as a lack of vascularity and a limited numbers of chondrocytes, restrict the tissue’s intrinsic capacity for healing after acute damage, especially if an injury does not penetrate into the subchondral bone1. In addition, the tissues formed by spontaneous repair are fibrocartilaginous2, having an abnormal biochemical composition and inferior biomechanical function3. The implantation of tissue-engineered cartilage fabricated in vitro shows clinical promise as a method for delivering reparative cells and preformed tissue into articular cartilage defects4-7.

Because human chondrocytes have a relatively low basal propensity for proliferating and producing extracellular matrix, methods to accelerate the formation of cartilaginous tissue during in vitro culture are of practical importance. The stimulation of cell proliferation and matrix deposition by exogenous growth factors during culture shortens the culture duration required for producing cell-laden tissue, and may also increase the mass of transplantable tissue.

Growth factors, applied singly or in combination with cells, have been shown to stimulate cell proliferation and matrix formation of tissue-engineered cartilage (reviewed by Hunziker8). However, in most of those studies, fetal bovine serum (FBS) has been used in combination with growth factors to enhance their stimulatory effects. Alternatively, without FBS, a mixture of growth factors, such as transforming growth factor-β (TGF-β), fibroblast growth factor-2 and platelet-derived growth factor (PDGF)9, can be used to stimulate cell proliferation and matrix formation9.
The use of autologous serum-derived stimuli for the cultivation of cells in vitro circumvents the possibility of disease transmission. Platelet-rich plasma (PRP) is a fraction of plasma that can be produced by centrifugal separation of whole blood. Because PRP contains multiple growth factors concentrated at high levels\textsuperscript{10–12}, PRP from patients is used as an autologous source of growth factors for soft tissue and bone repair in several clinical settings\textsuperscript{13–16}. PRP contains multiple growth factors, such as TGF-β\textsuperscript{17}, PDGF\textsuperscript{8}, epidermal growth factor (EGF)\textsuperscript{18} and insulin-like growth factor-1 (IGF-1), and these growth factors induce biological changes in cell proliferation and matrix metabolism of a variety of connective tissues\textsuperscript{10–12,13}. Because most growth factors found in PRP have been demonstrated to independently enhance the cell proliferation and/or matrix production of articular chondrocytes, we postulate that autologous PRP, a highly concentrated natural combination of growth factors, has the potential to stimulate cell proliferation and extracellular matrix metabolism by articular chondrocytes.

The purpose of this study was to examine the effects of PRP on cell proliferation and matrix synthesis by porcine chondrocytes cultured in alginate beads in order to determine the plausibility of using this plasma-derived material for engineering cartilage.

Materials and methods

ANIMALS—BLOOD AND CARTILAGE COLLECTION

Eight adult (1.0–1.5 years old) minipigs (Sinclair Research Center, Inc., Columbia, MO) were used to isolate PRP, platelet-poor plasma (PPP), and articular cartilage with the approval of the authors’ institutional Animal Care and Use Committee. Animals were first anesthetized by subcutaneous injections of Telazol (4.4 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and Xylazine (2.2 mg/kg, Xyla-Ject®, Phoenix Pharmaceuticals, Inc., St. Joseph, MO). Blood (15 ml/kg) was drawn from the popliteal vein with a 14G needle into a 60 ml syringe treated with an anticoagulant, citrate dextrose solution (Boehringer Laboratories, Inc., Norristown, PA). After collecting peripheral blood, the number of platelets in miniature swine whole blood from the elbow joints and pooled from two or three minipigs for each cell preparation. Chondrocytes were isolated from tissues by sequential enzyme digestion with 0.2% pronase (EMD Bioscience, La Jolla, CA) for 1 h, and 0.025% collagenase P (Roche Applied Science, Indianapolis, IN) for 16 h at 37°C, as previously described\textsuperscript{e1}. After several washes in Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (DMEM/F12: Mediatech, Herndon, VA), the cells were resuspended in 1.2% low-viscosity sterile pharmaceutical grade alginate (Keltone LV-(HM), a gift from ISP Alginate Inc., San Diego, CA) solution at 4 million cells/ml\textsuperscript{21}. The cultures, containing nine beads per well in a 24-well plate, were maintained throughout the 72-h treatment period (data not shown).

PREPARATION OF PRP AND PPP

PRP and PPP were isolated from fresh porcine blood (about 55 ml) using the SYMPHONY 2 Platelet Concentration System (DePuy Spine, Raynham, MA) according to the manufacturer’s protocol. Briefly, the blood treated with anticoagulant was separated into plasma and hemocyte (erythrocyte and leukocyte) fractions, and then the plasma was separated into PRP (containing a high number of platelets) and PPP (containing few platelets) by a continuous two-step sedimentation. PRP and PPP were clotted by adding a 10% thrombin solution (w/v, 1000 U/ml in 100 mM CaCl$_2$) to yield a final thrombin concentration of 100 U/ml. Soluble PRP and PPP releasates from the clotted preparations were isolated by centrifugation (1500 g for 5 min) and cleared by ultrafiltration (0.22 μm). These final soluble releasates preparations of PRP and PPP were frozen at −80°C until used.

PLATELET COUNT ANALYSIS AND QUANTIFICATION OF A GROWTH FACTOR

The number of platelets in miniature swine whole blood (before centrifugation) and in isolated PPP and PRP fractions was assessed using the Advia 120 Bayer Hematology Analyzer (Bayer Diagnostics, Tarrytown, NY). As an indicator for the efficacy of the concentration of growth factors in PRP, the level of TGF-β in serum made from the whole blood used to prepare PPP and PRP fractions and in PPP and PRP releasates was assessed. The PPP and PRP isolated fractions were clotted with thrombin and centrifuged to obtain releasates as described above. The level of TGF-β in serum and in PPP and PRP releasates was determined using an enzyme-linked immunosorbent assay method according to the manufacturer’s instructions (RD Mouse/Rat/Porcine/Canine TGF-beta 1 Quantikine ELISA Kit, R&D Systems, Inc., Minneapolis, MN).

CHONDROCYTE CULTURE IN ALGINATE BEADS

Following blood collection, articular cartilage was obtained from the elbow joints and pooled from two or three minipigs for each cell preparation. Chondrocytes were isolated from tissues by sequential enzyme digestion with 0.2% pronase (EMD Bioscience, La Jolla, CA) for 1 h, and 0.025% collagenase P (Roche Applied Science, Indianapolis, IN) for 16 h at 37°C, as previously described\textsuperscript{e1}. After several washes in Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (DMEM/F12: Mediatech, Herndon, VA), the cells were resuspended in 1.2% low-viscosity sterile pharmaceutical grade alginate (Keltone LV-(HM), a gift from ISP Alginate Inc., San Diego, CA) solution at 4 million cells/ml\textsuperscript{21}. The cultures, containing nine beads per well in a 24-well plate, were maintained in triplicate culture in 0.4 ml of complete medium containing 10% FBS (Hyclone, Logan, UT), 25 μg/ml ascorbic acid (Sigma-Aldrich, St. Louis, MO), 360 μg/ml L-glutamine (Mediatech) and 50 μg/ml gentamicin (Invitrogen, Carlsbad, CA) in a 5% CO$_2$,95% air incubator at 37°C. The medium in all cases was changed daily.

CULTURE PROTOCOL FOR THE STUDY OF THE EFFECTS OF PRP RELEASATE, PPP RELEASATE AND FBS ON CELL PROLIFERATION AND PROTEOGLYCAN (PG) AND COLLAGEN SYNTHESIS

After 7 days of culture in complete medium, the cells were precultured in serum-free medium (SFM), which consisted of DMEM/F12 with supplements as described above, for 24 h. The cells were then cultured for another 72 h under three different conditions: FBS (10% FBS in SFM), 10% PPP (10% PPP releasate in SFM) or PRP (10% PRP releasate in SFM). The cultures were incubated in 0.8 ml of the respective medium for 72 h without changing the medium during the treatment period. A preliminary experiment demonstrated that the rate of incorporation of $^{35}$S-sulfate into PGs by cells cultured under these conditions was constant throughout the 72-h treatment period (data not shown).

MEASUREMENT OF DNA CONTENT

To evaluate cell proliferation during the 72-h treatment period, the DNA content of alginate beads was determined using the bisbenzimidazole fluorescent dye (Hoechst 33258: Polysciences, Warrington, PA) method\textsuperscript{21} with calf thymus DNA (Sigma-Aldrich) used as a standard. These DNA values were used to normalize the values obtained from the following analyses.
MEASUREMENT OF PG SYNTHESIS

The incorporation of radiolabeled 35S into sulfated PGs was measured as an indicator of PG synthesis. During the last 4 h of the 72-h treatment period, the cells were cultured in the presence of 35S-sulfate (final concentration: 20 μCi/ml; Perkin—Elmer Life and Analytical Sciences, Boston, MA). After the 72-h treatments, the beads and culture media were collected and the cell-associated and further removed matrix compartments (CM and FRM, respectively) were separated by dissolving the beads with sodium citrate followed by mild centrifugation, as previously described. The CM fractions were extracted for 48 h at 4°C with 4 M guanidine-HCl in the presence of proteinase inhibitors. The amount of radiolabeled 35S-PGs in the CM and FRM fractions and in the medium was quantified by a rapid filtration assay following precipitation of the glycosaminoglycans with alcian blue.

CHARACTERIZATION OF 35S-LABELED PGS

The size of newly synthesized PGs was assessed using Sepharose CL-2B sieve chromatography under dissociative conditions, as previously described. Briefly, CM and FRM fractions extracted with 4 M guanidine-HCl were chromatographed on a Sepharose CL-2B column. The 35S-PGs in each fraction were quantified by liquid scintillation counting. The void volume (V0) and total volume (Vt) of the column were determined using a high-molecular-weight glucose polymer containing covalently bonded Reactive Blue 2 dye, blue dextran (Sigma-Aldrich), and free 35S-Sulfate, respectively. The partition coefficient (Kd) of PGs in each column fraction was calculated as follows: (Vt − V0)/(V0 − Vp), where Vp represents the elution volume for each column fraction. Proportions of large and small PGs were calculated by using a Peak Fit v4.12 software (SeaSolve Software, Inc., Framingham, MA) that performs nonlinear least squares curve-fitting using the Marquardt—Levenberg algorithm to find the minimum value of the sum of the squared deviations.

MEASUREMENT OF TOTAL PG CONTENT

After dissolving the alginate beads, the matrix compartments (CM and FRM) were separated and digested with papain (Sigma-Aldrich) at 60°C for 16 h. The total sulfated PG content was measured in each compartment using a modified dimethylmethylen blue dye-binding method in the presence of 0.03% alcian blue. The sum of the total sulfated PG contents of the CM and FRM was used to represent the total content of PG.

MEASUREMENT OF COLLAGEN SYNTHESIS

The incorporation of radiolabeled proline into pepsin-resistant protein was measured as an indicator of collagen synthesis. During the last 16 h of the 72-h treatment period, the cultures were radiolabeled with l-[2,3,4,5-3H]proline (final concentration: 50 μCi/ml; Amersham Biosciences Corp., Piscataway, NJ) in the presence of β-aminopropionitrile (100 μg/ml, Sigma-Aldrich). After separation of the CM and FRM, as described above, the CM, FRM and medium fractions were digested with 0.5 M acetic acid including pepsin (Sigma-Aldrich; 100 μg/ml) at 4°C for 16 h. An aliquot (25 μl) of each sample and 125 μl of 30% trichloroacetic acid (TCA; final concentration 25%; Sigma-Aldrich) were pipetted into a well of a 96-well MultiScreen filtration plate assembly with gentle agitation for 1 h at 4°C. The formed precipitate, which contained pepsin-resistant 3H-labeled protein, was collected on the filter membrane, and unincorporated 3H-proline was removed by washing each well three times with 200 μl of 10% TCA followed by vacuum filtration through the membrane. The membrane in each well was punched out into a scintillation vial with 500 μl of dissolving buffer (2% Sodium Dodecyl Sulfate (SDS) in 0.1 N NaOH) followed by gentle shaking overnight at 37°C. The radioactivity of the samples was counted after adding 2.5 ml of Hydrofluor scintillation fluid (National Diagnostics, Atlanta, GA). Although 3H-proline can be incorporated into other proteins, a major portion of the pepsin-resistant radiolabeled molecules (greater than 80%) was precipitated with ammonium sulfate at 30% saturation and identified as the α1 chain of collagen molecules when analyzed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and fluorography as described below in the characterization of 3H-labeled pepsin-resistant proteins.

CHARACTERIZATION OF 3H-LABELED PEPsin-RESISTANT PROTEINS

The same samples prepared for collagen synthesis were also used for the analysis of the types of newly synthesized pepsin-resistant protein. After pepsin digestion, as mentioned above, each sample was centrifuged at 12,000 g for 30 min at 4°C. The supernatants, containing the pepsin-resistant collagens, were precipitated by the addition of ammonium sulfate (176 mg/ml) and incubated overnight at 4°C. The samples were then centrifuged at 12,000 g for 30 min at 4°C and the pellet was recovered and resuspended in 0.4 M NaCl, 50 mM Tris. The samples were then dialyzed for 48 h at 4°C against 10 mM Tris containing CHAPS at 1 μg/ml and protein inhibitors (1 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, 5 mM EDTA). The radiolabeled collagens were separated by SDS-PAGE (Mini-gel, Bio-Rad) in a 7.5% acrylamide gel (reducing conditions) and detected by fluorography.

STATISTICAL ANALYSES

All values are reported as the mean ± standard deviation of the results of analyses of three separate cultures of nine beads. Experiments were repeated on at least three separate occasions and representative data are presented. Analysis of variance with Fisher’s Protected Least Significant Difference (PLSD) test as a post hoc test was used to assess the effect of treatment on DNA content, matrix synthesis and accumulation.

Results

PLATELET COUNT ANALYSIS OF PRP

Platelet count analysis was performed on whole blood and PPP and PRP releasates from eight miniature swine. The concentration of platelets of whole blood (baseline)
was 359 ± 83 × 10^3/ml (range: 210–506 × 10^3/ml); compatible with the range of normal values for miniature swine (217–770 × 10^3/ml)\(^2\). The platelet concentration system resulted in a 9.9-fold increase in the platelet count of the PRP fraction when compared to whole blood (PRP: 1399 ± 174 × 10^3/ml, \(P < 0.0001\), vs whole blood; PPP: 157 ± 80 × 10^3/ml, \(P < 0.05\), vs whole blood). These data were compatible with the data obtained from the manufacturer for human serum (4.7 fold).

QUANTIFICATION OF GROWTH FACTOR IN PRP

The level of TGF-\(\beta\) in whole blood and in PPP and PRP releasates was quantified in eight animals. The level of TGF-\(\beta\) was significantly higher (two fold) in the PRP releasates compared to the serum and/or PPP releasates (serum: 13.8 ± 0.9 ng/ml, PPP: 2.1 ± 0.3 ng/ml, \(P < 0.001\) vs serum; PRP: 29.1 ± 8.0 ng/ml, \(P < 0.001\), vs serum). The efficiency of concentration was lower than that reported in the human (six fold).

DNA CONTENT

To study the effects of PRP on cell proliferation, the cultures were treated with FBS, PPP or PRP for 72 h and the DNA content of the alginate beads was measured. Treatment with PRP resulted in a small but significant increase in DNA content when compared to the FBS and PPP groups (PRP: +11\%, \(P < 0.01\), vs FBS, +18\%, \(P < 0.001\); vs PPP, Fig. 1).

PG SYNTHESIS

The rate of PG synthesis (per \(\mu\)g DNA) by the PRP-treated chondrocytes was significantly higher than that by the FBS- or PPP-treated chondrocytes (PRP: +115\% vs FBS; +151\%, vs PPP; both \(P < 0.0001\), Fig. 2). When the data were expressed per nine beads, essentially the same effects were apparent (data not shown). There were no significant differences between PG synthesis by the FBS- or PPP-treated chondrocytes (PPP vs FBS, \(P = 0.27\)). When the distribution of newly synthesized \(^{35}\)S-PGs in the CM extracts, the FRM fractions and the media were quantified (see the Materials and Methods section). The rate of PG synthesis by the PRP-treated chondrocytes was significantly faster than that by the PPP- or FBS-treated cells. The numbers presented in each column indicate how much PG was present in the CM, FRM and medium as a percentage of the total \(^{35}\)S-PG in the beads.

PG ACCUMULATION

Chondrocytes cultured in alginate beads for 3 days in the presence of PRP accumulated significantly more sulfated PGs than chondrocytes in beads cultured in FBS or PPP (PRP: +15\% vs FBS, \(P < 0.001\); +26\% vs PPP; \(P < 0.0001\), Fig. 4). However, when the data were expressed per \(\mu\)g DNA, there was no significant difference in PG accumulation among the three experimental groups (data not shown). No significant differences in the distribution of PGs within the CM or FRM were observed (Fig. 4).
COLLAGEN SYNTHESIS

The amount of newly synthesized pepsin-resistant \(^{3}H\)-protein (per \(\mu\)g DNA), as a measure of collagen synthesis by PRP-treated cells, was remarkably higher than that by FBS- or PPP-treated cells (PRP: +163\% vs FBS; +163\% vs PPP; both \(P < 0.0001\), Fig. 5). Measurement of pepsin-resistant \(^{3}H\)-protein synthesis per nine beads yielded essentially the same results as the data expressed per \(\mu\)g DNA (data not shown). There were no significant differences in synthesis between FBS- and PPP-treated cells (PPP vs FBS; \(P = 0.1\)). The proportion of newly synthesized pepsin-resistant \(^{3}H\)-protein within the medium, CM or FRM did not change as a result of treatment (data not shown).

The profile of newly synthesized pepsin-resistant protein was evaluated by separation of the \(^{3}H\)-proline-labeled, pepsin-resistant proteins incorporated into the CM and FRM compartments using SDS-PAGE. The results of SDS-PAGE fluorography revealed that both compartments in each experimental group contained a similar prominent band corresponding to the \(\alpha\)1 chain of the collagen molecules at 95 kDa (Fig. 6). An \(\alpha\)2 chain, that would have indicated the presence of type I collagen, was not detected. This relative prominence of \(\alpha\)1 chains suggests that the majority of the collagen synthesized in the presence of FBS, PPP or PRP was type II collagen, as previously described\(^{29,30}\). This 95 kDa band was confirmed to be immunopositive to an anti-type II collagen antibody (data not shown) and did not react with an anti-type I collagen antibody (data not shown).

Discussion

The study presented here is the first to show that PRP, a "natural cocktail" of growth factors obtained using a point of care apparatus, has the potential to stimulate cell proliferation and matrix metabolism of porcine articular chondrocytes cultured in alginate beads. At the same concentration (10\% in SFM), the stimulatory effects of PRP were stronger than those of FBS or PPP. Furthermore, the major profiles of PGs and collagens synthesized by chondrocytes in the presence of PRP were similar to those seen in cells cultured in FBS.

A platelet contains the vast majority of biologically active molecules required for blood coagulation, such as adhesive proteins, coagulation factors and protease inhibitors, within cytoplasmic \(\alpha\)-granules\(^{31}\). In addition to the factors that coagulate blood, growth factors such as TGF-\(\beta\), PDGF, EGF, vascular endothelial growth factor and IGF-1 are released from \(\alpha\)-granules\(^{31,32}\) when platelets are activated. These growth factors are known to stimulate collagen deposition, accelerate epithelial regeneration, promote angiogenesis, and ultimately improve wound healing in skin and periodontal tissues\(^{33-36}\).

Recently, the use of patient autologous PRP, which serves as a source of growth factors able to induce tissue regeneration, has gained wide acceptance in several surgical applications, such as bone regeneration in periodontal and maxillofacial surgery\(^{37-39}\), orthopedic surgery\(^{40}\).
This study focused on the effect of PRP on cell proliferation or matrix metabolism by articular chondrocytes. Growth factors identified in PRP independently stimulate either cell proliferation or matrix metabolism by articular chondrocytes. This study focused on the effect of PRP on matrix synthesis and accumulation to test the practicality of using PRP as a tissue culture supplement.

Adult minipigs were used in this study as a source of both articular cartilage and blood. Due to the limitation in the number of cells that could be obtained from the elbow joint, single minipigs, cells from different animals were pooled to perform the experiments. Therefore, it was not possible to use autologous PRP, which would have been preferable. This notwithstanding, it should be noted that four different batches of PRP from individual animals were tested and yielded essentially similar results. Although our study to determine the concentration of growth factors was limited to TGF-β, a significant concentration of TGF-β (two fold, 29.1 ng/ml) was achieved. Using human blood, further studies on the effects of donor age, length and other conditions of storage of PRP, and biological characteristic variability should be performed. Thrombin, which has been shown to have some biological effects on cell metabolism, was used to form a clot in the preparation of PRP. Because PPP was also treated with thrombin in order to control for the effects of thrombin, it is unlikely that thrombin itself contributed to the differences between the effects of PRP and PPP on cell proliferation and matrix metabolism.

Although the PG accumulation per beads and PG synthesis per DNA were significantly upregulated by the PRP treatment, the PG accumulation per DNA was not significantly different among the treatment groups. This suggests that the increased sulfated PG accumulation in alginate beads containing chondrocytes cultured with PRP was mainly a result of increased cell proliferation induced by the PRP treatment for 3 days. In addition, incubation in the three different experimental conditions for a period of 3 days might not be long enough to induce a significant difference in the PG content in alginate beads that had been precultured for 6 days under identical conditions.

In porcine chondrocytes, the effects of PRP were more pronounced on collagen synthesis than PG synthesis, especially when compared with FBS. The essential role of growth factors released from platelets is to accelerate the wound-healing process of soft tissues (such as blood vessel walls, muscle and skin) or hard tissues such as bone after injury to those tissues; these tissues all contain large amounts of collagen. Thus, this ‘natural cocktail’ of growth factors included in PRP might have the ability to preferentially promote collagen synthesis. This is especially important because most tissue-engineered cartilage lacks sufficient collagen when formed in vitro.

Several investigators have previously reported that platelet lysates stimulate the cell proliferation of chondrocytes. However, the results on the effects of platelet lysates on matrix accumulation differ from our results with PRP, probably because of differences in isolation procedures.

In the study by Choi et al., the “platelet lysate” was isolated by centrifugation and repeated freeze–thawing, while the “PRP releasate” used in the present study was purified by a “point of care” procedure followed by clot formation with thrombin. These differences in isolation may result in differences in the proportions and/or concentrations of growth factors and other plasma components present in “platelet lysates” and “PRP”.

For clinical use, autologous chondrocyte transplantation (ACT) has been used to repair articular cartilage defects. The most popular and commercially available cell-based therapy for autologous chondrocyte implantation in the United States is Carticel® (Genzyme Biosurgery, Boston, MA), which uses FBS in the culture process. When utilizing FBS in culture systems for clinical use, there is a potential risk for the transmission of animal diseases. To avoid the risk of zoonotic transmission, the use of autologous serum from patients is preferable in in vitro cell-based culture.
systems. Because PRP has the potential to stimulate cell proliferation and matrix synthesis by articular chondrocytes, the application of autologous PRP from patients may be an attractive supplement for ACT, as well as tissue-engineered cartilage. Although the identification of factors involved in the effect of PRP on matrix metabolism of chondrocyte was outside the scope of this study, a study aimed at determining the concentration of growth factors responsible for these effects and changes of concentration with aging will provide important information about the limitations of this approach.

The use of PRP has several advantages over the use of recombinant growth factors or products of animal origin. First, as a point of care approach, the autologous preparation of PRP avoids the complex regulatory pathway. In addition, safety issues, such as immunologic reactions or carcinogenesis, are of much less concern. Finally, the costs of using PRP would be considerably less than those associated with the use of recombinant proteins. If 800 ml of autologous blood was collected from a patient, this would translate into 600 ml of culture medium containing 10% autologous blood containing platelet-derived growth factors or products of animal origin. Important information about the limitations of this approach. The effect of PRP on matrix metabolism of articular chondrocytes should be performed.

The results of this study provide evidence for the plausible use of PRP to stimulate the matrix metabolism of articular cartilage engineered in vitro. Further studies to establish the efficacy and reproducibility of PRP prepared from human blood, especially from donors of different ages, to stimulate chondrocyte metabolism should be performed to extend this simple and safe new approach to the engineering of cartilage constructs.

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