METHODS ARTICLE

An *In Vitro* Investigation of Platelet-Rich Plasma-Gel as a Cell and Growth Factor Delivery Vehicle for Tissue Engineering

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Platelet-rich plasma (PRP) has been used for different applications in human and veterinary medicine. Many studies have shown promising therapeutic effects of PRP; however, there are still many controversies regarding its composition, properties, and clinical efficacy. The aim of this study was to evaluate the influence of different platelet concentrations on the rheological properties and growth factor (GF) release profile of PRP-gels. In addition, the viability of incorporated bone marrow-derived human mesenchymal stem cells (MSCs) was investigated. PRP (containing 1000×10^3 , 2000×10^3 , and $10,000 \times 10^3$ platelets/ μL) was prepared from human platelet concentrates. Platelet activation and gelification were achieved by addition of human thrombin. Viscoelastic properties of PRP-gels were evaluated by rheological studies. The release of GFs and inflammatory proteins was measured using a membrane-based protein array and enzyme-linked immunosorbent assay. MSC viability and proliferation in PRP-gels were assessed over 7 days by cell viability staining. Cell proliferation was examined using DNA quantification. Regardless of the platelet content, all tested PRP-gels showed effective cross-linking. A positive correlation between protein release and the platelet concentration was observed at all time points. Among the detected proteins, the chemokine CCL5 was the most abundant. The greatest release appeared within the first 4 h after gelification. MSCs could be successfully cultured in PRP-gels over 7 days, with the highest cell viability and DNA content found in PRP-gels with 1000×10^3 platelets/ μL . The results of this study suggest that PRP-gels represent a suitable carrier for both cell and GF delivery for tissue engineering. Notably, a platelet concentration of 1000×10^3 platelets/ μL appeared to provide the most favorable environment for MSCs. Thus, the platelet concentration is an important consideration for the clinical application of PRP-gels.

Introduction

PLATELETS ARE KNOWN TO play an important role in hemostasis and in the process of tissue healing. Upon activation, they release numerous growth factors (GFs) and cytokines, such as transforming growth factor- β -1 (TGF β -1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor I (IGF-I), basic fibroblast growth factor (bFGF), endothelial growth factor (EGF), and hepatocyte growth factor (HGF).¹ Platelet-released GFs are not only known to enhance recruitment, proliferation, and differentiation of cells^{2–4} but also thought to play a role in angiogenesis^{5–7} and inflammation.^{8,9} As such, platelet-rich plasma (PRP) has gained popularity in tissue repair and regeneration and serves as a

treatment option for various medical conditions in human and veterinary medicine.^{10–13} Despite many reports discussing the clinical application of PRP, there are still controversies regarding its preparation, delivery method, and clinical efficacy. Many protocols and commercially available systems have been used for the production of PRP, although each of these methods generates a different final product, where the concentration of platelets and leukocytes and the amount of released protein vary,^{14,15} often leading to conflicting results.^{16,17} The optimal amount of platelets in PRP is still unknown, and it is not clear whether its specific formulation correlates with levels of released GFs.^{18,19} Many proteins released from platelets possess inflammation-modulating properties; hence, the potential benefit they may exert at the injury site is likely to be tissue dependent.

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PRP can be produced using whole-blood centrifugation or apheresis. Several commercial centrifugation systems are available and offer a simple, minimal manipulative preparation of autologous PRP; however, the platelet counts and enrichment levels are variable (ranging from physiological platelet concentrations to 18-fold enrichment), and only few systems allow adjustments by the user (according to the manufacturer's brochures and Mazzucco *et al.*²⁰). Platelet concentrates from whole-blood centrifugation contain erythrocytes and leukocytes. In contrast, apheresis allows the exclusion of components, which are undesired, since the accumulation of leukocytes might be deleterious for the healing process.^{21,22}

The form in which PRP is used depends on the application and target tissue. Many clinicians use PRP in an inactivated liquid form that can be easily injected. After application, platelets are activated by contact with the surrounding tissue collagen.²³ Alternatively, an exogenous activator, such as chitosan, batroxobin, thrombin, calcium chloride, or a combination of the latter two, can be used to form a PRP-gel.^{24–28} PRP-gels have been used as an autologous hydrogel containing bioactive molecules, locally enhancing tissue healing.^{29–34} The composition of PRP-gels in these studies was, however, highly variable, mainly because of differences in PRP preparation and activation methods, which in turn leads to significant differences in platelet concentration, leukocyte content, and GF release.²⁰ Previous studies have reported a successful culture of cells incorporated into PRP-gels, suggesting that the gel environment enhances viability and proliferation of encapsulated cells.^{33,35} Kawasumi *et al.* encapsulated rat bone marrow (BM) mesenchymal stem cells (MSCs) in PRP-gels containing different platelet concentrations, ranging from 0.05 to 4.4×10^6 platelets/ μL . The authors demonstrated a platelet concentration-dependent stimulation of cell proliferation *in vitro* as well as enhanced bone formation in a rat osteotomy *in vivo*.³⁵

Although previously applied for various applications *in vivo*, in our opinion, PRP-gels have not been sufficiently characterized. PRP may be homemade or obtained using clinical systems, and all these protocols/systems result in different platelet and leukocyte concentrations, with additional variability introduced by donor variations.³⁶ In this study, we investigated three different platelet concentrations in PRP-gels (1000×10^3 , 2000×10^3 , and $10,000 \times 10^3$ platelets/ μL), referring to as 5 $\times$, 10 $\times$, and 50 $\times$ platelet enrichment, assuming an average concentration of 200×10^3 platelets/ μL in healthy human donors. Most studies using PRP-gels, particularly in the context of bone repair, have used similar concentrations (e.g., $48\text{--}4358 \times 10^3$ platelets/ μL ³⁵ and 1040×10^3 platelets/ μL ³⁴), while other studies do not indicate the platelet concentration in their PRP preparations. The aims of this study were to systematically characterize different compositions of PRP-gels created from platelet concentrates, to compare these gels with respect to their rheological properties, the content, and release profile of GFs, and finally to assess the viability of incorporated MSCs.

Materials and Methods

Preparation of PRP and PRP-gels

PRP was produced from human leukocyte-depleted platelet concentrates (blood bank; Kantonsspital Graubünden, Chur, Switzerland) obtained by apheresis and collected into trans-

fusion bags containing acid citrate dextrose (ACD-A), with a thrombocyte concentration of 2.4×10^{11} per 240 mL of plasma (1000×10^3 thrombocytes/ μL) and containing less than 5×10^5 leukocytes. Based on the assumption that the average platelet count in healthy human adults is 200×10^3 / μL , this platelet concentrate was considered to be five times enriched. To produce PRP with higher platelet concentrations, the platelet concentrates were centrifuged at 2000 *g* for 20 min, and the resulting pellet was resuspended in the appropriate volume of platelet-depleted plasma. PRP was sonicated for 15 min and stored at -20°C until use. If not stated otherwise, PRP-gels were produced by pipetting 3 μL of human thrombin (final concentration 5 U/mL, TISSEEL; Baxter) into a well of a 48-well cell culture plate (Falcon). The plate was placed on an orbital shaker, and 312 μL of PRP was slowly added. Gels were incubated at room temperature for 15 min for gelification.

Rheology

The viscoelastic properties of PRP-gels were investigated through oscillatory rheology. Discs of PRP-gels of 25 mm diameter and 0.2 mm thickness were prepared with a dedicated mold. Thrombin concentrations were 5, 8, and 16 U/mL. All rheological measurements were performed using an Anton Paar MCR-302 rheometer equipped with a 25 mm parallel plate, a Peltier temperature control system, and an insulating thermostatic hood. First, the samples were screened with an amplitude sweep at 10 rad/s at 20°C . This measurement allows the individuation of a suitable deformation range, which should be within the linear viscoelastic region. A strain of 1% was verified to be within this limit for all tested gels and further used for each sample. A frequency sweep (measure of viscoelastic shear moduli as a function of the frequency of deformation) between 0.1 and 25 rad/s at 20°C was finally performed. For each specimen, three independent freshly prepared samples were measured, and the results are reported as mean $\pm$ standard error of the mean (SEM).

GF release from PRP-gels

GF release was measured from PRP-gels incubated with 800 μL of phosphate-buffered saline (PBS) at 37°C at different time points after gelification (30 min–168 h). At every time point, 400 μL of conditioned PBS was collected for analysis, and removed volume was replaced with a new 400 μL of PBS. Enzyme-linked immunosorbent assays (ELISAs) were performed to quantify CCL5/RANTES (DuoSet[®]; R&D Systems), TGF β -1, VEGF, PDGF-AB, and PDGF-BB (all Quantikine[®]; R&D Systems) according to the manufacturer's instructions. Samples were diluted according to the platelet concentration of the gels. Absorbance was measured at 450 and 560 nm to eliminate background signal using a VICTOR3[™] plate reader (PerkinElmer).

The presence of inflammatory proteins in conditioned PBS from different PRP-gels (pooled samples from two PRP donors, collected 12 h after gelification) was investigated using a human inflammation protein array (RayBio[®], C-Series AAH-INF-3-4) according to the manufacturer's instructions. Briefly, the membranes were incubated with 1 mL of PRP supernatant overnight at 4°C on an orbital shaker. Chemiluminescence (20-min exposure time) was detected

using the ChemiGenius Bio-Imaging System (Syngene). Semi-quantification was performed by averaging values from duplicated spots and measuring signal intensity using ImageJ (Rasband, NIH). Data are presented as relative abundance following normalization to the RayBio intrinsic positive control.

MSC isolation, culture, and encapsulation in PRP-gels

BM aspirates were obtained from the iliac crest or vertebral body of patients undergoing elective orthopedic surgery (mean age: 56.5 years, range: 26–89 years; one male and eight females). Informed consent was given by all patients, and approval from local ethical authorities was obtained (KEK Bern 126/03). Mononucleated cells (MNCs) were isolated from BM aspirates by density centrifugation with Ficoll™ (Histopaque-1077; Sigma). MNCs were seeded in tissue culture flasks at a density of 5×10^4 cells/cm² in an alpha-minimum essential medium (α MEM; Gibco) containing 10% fetal bovine serum (FBS; PAN) and 5 ng/mL bFGF (R&D Systems). After 4 days in culture, non-adherent hematopoietic cells were removed. MSCs selected by adherence to cell culture plates were further expanded with a change of medium every 3 days. Cells were passaged when 80% confluency was reached. All experiments were performed with cells at the second passage. MSCs used as monolayer controls were seeded at a density of 3×10^3 cells/cm² in six-well tissue culture plates.

For the incorporation of MSCs into PRP-gels, PRP aliquots were thawed, and to avoid donor variability, three PRP donors with the same blood group were pooled. MSCs were seeded in PRP-gels at a density of 1.25×10^5 cells per gel in a total volume of 315 μ L. Gelification was performed using a final concentration of 5 U/mL thrombin as described above. Gels were incubated at 37°C in α MEM containing 10% FBS, 5 ng/mL bFGF, 100 U/mL PenStrep, and 5 μ M ϵ -aminocaproic acid (Sigma).

Viability of MSCs

The viability of MSCs encapsulated in PRP-gels was determined at days 0 (3 h after seeding), 3, and 7. PRP-gels were stained with a solution of 100 pg/mL calcein AM and 1 pg/mL ethidium homodimer-1 (both Sigma) in 1 mL of α MEM for 30 min at 37°C and visualized with an inverted phase contrast microscope (Olympus CK40) equipped with a fluorescence lamp.

For semiquantitative analysis of cell viability following recovery from PRP-gels, 1 h and 3 and 7 days after cell encapsulation, PRP-gels underwent enzymatic digestion in type II collagenase (600 U/mL; Worthington). The number of retrieved viable cells was determined by trypan blue (Sigma) exclusion. Cell viability was further determined by propidium iodide (PI; 4 μ g/mL; Sigma) staining and flow cytometry (BD FACSaria III). To define the forward scatter and side scatter region for the analysis, a PRP-gel without cells was used to exclude platelet debris.

DNA content

At days 0 (5 h after seeding), 3, and 7, PRP-gels were subjected to digestion in proteinase K (0.5 mg/mL; Roche) for 16 h at 56°C. The DNA content was measured using

an ultrasensitive fluorescent nucleic acid stain PicoGreen® (Invitrogen), which allows quantitating double-stranded DNA at concentrations as low as 25 pg/mL. A calf thymus DNA standard (Invitrogen) was used to create a five-point standard curve from 2 ng/mL to 2 μ g/mL. Measurements were performed using a VICTOR3 (PerkinElmer) plate reader at 485 nm excitation and 535 nm emission wavelengths.

Statistical analysis

All values are shown as mean $\pm$ SEM. Statistical analysis was performed with the Kruskal–Wallis test using the GraphPad Prism. *p*-Value <0.05 was considered as statistically significant.

Results

Formation of PRP-gels

All gel formulations could be cultured for 7 days without evident visual changes in shape or size (Fig. 1). Rheological measurement revealed that for all tested gels, the G' was over G'' , demonstrating an effective cross-linking of samples. Neither the platelet concentration (Fig. 2A) nor the thrombin concentration (Fig. 2B) significantly affected the viscoelastic properties of the gels. However, we found that PRP-gels with 2000×10^3 platelets/ μ L were slightly stiffer than those with $10,000 \times 10^3$ or 1000×10^3 platelets/ μ L (Fig. 2A), with the lowest values observed for the lowest platelet concentration. When testing the influence of different thrombin concentrations on PRP-gels with the same platelet content, we found that the lowest final thrombin concentration (5 U/mL) showed the highest values of storage and loss moduli and was therefore chosen for further experiments (Fig. 2B).

GF release from PRP-gels

First, the release of GFs that are known to be important signaling molecules in tissue repair was examined.¹ TGF β -1, PDGF-AB, PDGF-BB, and VEGF could be detected in conditioned PBS by PRP from all tested PRP-gels from all platelet concentrations (Fig. 3). Additionally, the cumulative and temporal release of GFs from PRP-gels was investigated (Fig. 3). A positive correlation between protein secretion and platelet concentration was observed at all time points

FIG. 1. Platelet-rich plasma (PRP) with 2000×10^3 platelets/ μ L forms an elastic cross-linked gel releasing growth factors. Macroscopic view of PRP-gels prepared from 315 μ L of PRP and activated with 5 U/mL thrombin. Scale bar depicts 30 mm. Color images available online at www.liebertpub.com/tec

FIG. 2. Rheological analysis of PRP-gels. Storage (G') and loss (G'') moduli as function of the angular frequency showing effective cross-linking of gels. (A) Influence of different platelet concentrations at a constant thrombin concentration of 5 U/mL on gel properties and (B) influence of different thrombin concentrations on properties of PRP-gels with 2000×10^3 platelets/ μ L. Error bars represent standard error of the mean (SEM), $n \geq 3$. Color images available online at www.liebertpub.com/tec

and platelet concentrations. Overall, most of the release of GFs appeared within the first 4 h after gelification. A comparatively lower sustained release of GFs was observed at later time points (Fig. 3). Twelve hours after gelification, the cumulative release of activated TGF β -1 (Fig. 3A, right) showed the highest values in PRP-gels with $10,000 \times 10^3$ platelets/ μ L reaching 13.08 ± 3.48 ng, followed by 2000×10^3 platelets/ μ L PRP-gels with 4.07 ± 0.61 ng and 1000×10^3 platelets/ μ L PRP with 2.62 ± 0.26 ng. Compared to TGF β -1, the values of cumulative release of PDGF-AB (Fig. 3B) were lower for the highest (9.34 ± 1.9 ng) and middle (3.54 ± 0.84 ng) platelet concentrations but higher (3.81 ± 0.29 ng) for PRP-gels with $10,000 \times 10^3$ platelets/ μ L. Release of PDGF-BB at 12 h was clearly lower for all PRP-gels reaching 0.23 ± 0.03 , 0.34 ± 0.04 , and 1.05 ± 0.25 ng in 1000×10^3 , 2000×10^3 , and $10,000 \times 10^3$ platelets/ μ L gels, respectively. VEGF release was the lowest compared to the other GFs.

PRP-gels release inflammatory proteins

Because of existing controversies regarding possible pro-inflammatory effects of PRP, a screening array was performed to identify the most abundant inflammatory proteins released from PRP-gels. A total of 28 proteins known to play a role in inflammation were detected (Fig. 4). The

chemokine CCL5 was released in the highest amounts compared to other factors, followed by PDGF-BB, interferon-gamma-induced protein 10 (IP10), interleukin-6 soluble receptor (IL-6sR), macrophage inflammatory protein 1-beta (MIP-1 β), metalloproteinase inhibitor 2 (TIMP2), and intracellular adhesion molecule (ICAM) (Fig. 4A). Other proteins, such as tumor necrosis factor-beta (TNF β), interferon-gamma (INF- γ), chemokine I-309 (I-309), and eotaxin-2, were found at comparably lower levels, while some factors, including TNF α , interleukin 2 (IL-2), IL-3, IL-5, and IL-16, were detected only in supernatants from PRP-gels with $10,000 \times 10^3$ platelets/ μ L. Overall, the relative abundance of inflammatory proteins positively correlated with increasing platelet concentration in PRP-gels. Based on these findings, the release of CCL5 over time was investigated by ELISA (Fig. 4B, C). The total amount of secreted CCL5 was significantly higher compared to TGF β -1, PDGF, and VEGF, also measured by ELISA. After 12 h postgelification, the cumulative release of CCL5 (Fig. 4C) showed the highest values in $10,000 \times 10^3$ platelets/ μ L gels (211.2 ± 97.5 ng), followed by 2000×10^3 platelets/ μ L gels (77.1 ± 6.8 ng), with lowest values in 1000×10^3 platelets/ μ L gels (37.3 ± 3.1 ng). The peak of CCL5 release appeared within the first 2 h after gelification and was sustained at moderate levels over the next 5 days (Fig. 4B).

FIG. 3. Growth factor release from different PRP-gels. Temporal (*left*) and cumulative (*right*) release of transforming growth factor- β -1 (TGF β -1; **A**), vascular endothelial growth factor (VEGF; **B**), platelet-derived growth factor (PDGF)-AB (**C**), and PDGF-BB (**D**) measured in the supernatants (phosphate-buffered saline) of different PRP-gels using enzyme-linked immunosorbent assay (ELISA). Measurements were taken at indicated time points after gelification. Data are represented as mean $\pm$ SEM. Color images available online at www.liebertpub.com/tec

FIG. 4. PRP-gels release inflammatory proteins. Supernatants from PRP-gels from two donors were collected after 12 h of incubation and pooled, and the relative abundance of inflammatory proteins was analyzed using an antibody protein array (A). Temporal (B) and cumulative (C) release of CCL5 from PRP-gels measured by ELISA. Error bars indicate SEM, $n=3$. Color images available online at www.liebertpub.com/tec

Platelet concentration in PRP-gels influences MSC behavior

MSCs could be successfully cultured for 7 days in all PRP-gels, regardless of the platelet concentration. A difference in DNA content between different platelet concentrations was observed directly after cell encapsulation (Fig. 5A). The highest DNA content was found in 1000×10^3 platelets/μL gels, followed by 2000×10^3 and $10,000 \times 10^3$ platelets/μL gels. The same trend was noticed at days 3 and 7. Overall, the DNA content at day 7 in all tested PRP-gels was approximately twofold higher compared to that at day 0.

PI staining revealed an average percentage of viable cells at day 0 (3 h after seeding) of $98.3\% \pm 0.14\%$, $93.3\% \pm 2.43\%$, and $93.2\% \pm 1.70\%$ in 1000×10^3 , 2000×10^3 , and $10,000 \times 10^3$ platelets/μL gels, respectively (Fig. 5B). At day 3, the highest cell viability was detected for PRP-gels with 1000×10^3 platelets/μL ($96.8\% \pm 0.07\%$) and significantly different ($p < 0.05$) compared to $89.8\% \pm 2.14\%$ in $10,000 \times 10^3$ platelets/μL gels. At day 7, the cell viability in gels containing the lowest concentration of platelets was the highest, reaching $92.4\% \pm 0.88\%$. For PRP-gels with 2000×10^3 and $10,000 \times 10^3$ platelets/μL, the viability was $86.73\% \pm 2.46\%$ and $87.13\% \pm 2.75\%$, respectively. Live-dead staining showed a change in the cell morphology over time, with rounded

cells on day 0 to a spindle-shaped morphology at days 3 and 7. The density of cells seeded in gels increased over time (Fig. 5C).

Discussion

An *in vitro* characterization of PRP-gels created from platelet concentrates, activated by human thrombin, and composed of different platelet concentrations was performed. It could be shown that the platelet concentration within PRP is a crucial factor determining gel properties and influencing behavior of encapsulated cells.

To the best of our knowledge, no study has investigated the viscoelastic properties of PRP-gels. For the PRP-gels prepared in the present study, a prevalence of storage over loss modulus was observed for every platelet and thrombin concentration. This is a direct consequence of the cross-linking at the molecular level. Macroscopically, this means that the material tends to maintain its shape rather than creeping and spreading like a viscous fluid. This property, in combination with a composition that is more than 95% water, allowing nutrition and waste transport, makes hydrogels effective cell carriers. In this study, it was shown that the highest storage modulus was achieved by PRP-gels with 1000×10^3 and 2000×10^3 platelets/μL gelified with

FIG. 5. Proliferation and viability of mesenchymal stem cells (MSCs) in PRP-gels. (A) Proliferation of MSCs incorporated in different PRP-gels assessed by DNA quantification. Error bars are SEM, $n=3$. (B) Cells were enzymatically retrieved from PRP-gels and subjected to propidium iodide staining, and cell viability analyzed using flow cytometry. Statistical significance at $*p < 0.05$ was determined using Kruskal–Wallis test. Error bars are SEM, $n=3$. (C) Fluorescent microscope images of MSCs encapsulated in PRP-gels after staining with calcein (green, living cells) and ethidium homodimer (red, dead cells). Scale bar represents 500 μm. Color images available online at www.liebertpub.com/tec

5 U/mL thrombin. Higher platelet content in PRP-gels did not result in increased stiffness. A possible explanation for this is that a proper balance between concentration of platelets and thrombin has to be reached to effectively gelify PRP; otherwise, some of the platelets stay in inactivated form and do not contribute to gel formation. Furthermore, a dense matrix of PRP-gels containing highly concentrated platelets may prevent a proper diffusion of thrombin within the gel. Additionally, platelets at a higher number may act as external bodies hindering the contact between the fibrinogen strands and their effective cross-linking. Despite the efficient cross-linking, PRP-gels do not have a stiffness of a load-bearing structure; hence, their mechanical properties are very limited. However, previous reports indicate that stiffness of hydrogels may influence behavior and differentiation potential of incorporated MSCs.^{37,38}

The importance of the platelet activator is not only limited to induction of platelet aggregation; an exogenous triggering factor is also necessary to ensure a complete release of GFs.³⁹ In the current study, thrombin was used, and it was hypothesized that this will result in a rapid gel formation, degranulation of platelets, and an immediate release

of GFs. It was demonstrated that the majority of GFs were, indeed, released within the first 6 h after the gel has formed. The results of this study agree with previous reports indicating that most of the release occurs between 1 and 6 h after clot formation.^{40,41} Interestingly, even 12 h after gelification, GF release could be detected, however, at clearly lower concentrations. One possible explanation for this is that some of the GFs remain captured in the PRP-gel and as a consequence are secreted at later time points. It is likely that GFs located peripherally in the gel may diffuse faster than the centrally situated ones. Furthermore, some GFs may be initially captured within the fibrin mesh of PRP-gel; hence, their release occurs at later time points. In addition, the presented *in vitro* investigation does not completely reflect the *in vivo* situation, where tissue-specific factors, proteins, and body fluids would interact with the applied material. In addition, various cells migrating into the PRP-gel may further influence the process of gel degradation and GF release. Rademakers *et al.* studied PRP degradation (platelet concentration $\sim 1000 \times 10^3$ platelets/μL) in an animal study and found that the weight of autologous platelet clots was reduced to 16% of the initial weight after 30 min of incubation

in the pericardial sac.⁴² *In vivo* tissue-related factors regulate the secretion of GFs from platelets. In addition, recruited or cotransplanted cells may also secrete GFs.⁴³ Another question is whether the released amount of GFs is physiologically relevant. Further studies are necessary to investigate the optimal concentration of GFs at the injury site and whether lower amounts still have a positive effect on tissue repair. Interestingly, a study investigating the mRNA profile of human platelets suggests that platelets may synthesize some cytokines and GF *de novo*.⁴⁴

The results of this study support the hypothesis that release of proteins from PRP-gels is correlated with the platelet concentration. Similar results have been reported previously³⁹; however, also opposite findings, indicating a poor correlation between platelet number and GF content, have been reported.^{36,45} Among the screened GFs in supernatants from our PRP-gels, CCL5 was released in the greatest amounts, followed by TGF β -1. TGF β has been proposed for the treatment of chronic inflammatory diseases, soft and hard tissue regeneration, and autoimmune diseases.⁴⁶ It has been shown that TGF β signaling enhances maturation of osteoblasts and bone matrix deposition.⁴⁷ Accordingly, several studies suggest positive effects of TGF β when used for bone repair.^{48–51} PDGF-AB and PDGF-BB were other GFs found in supernatants from PRP-gels in this study. PDGFs are known to promote the proliferation of bone cells and influence osteoblastic mitogenesis.⁵² Based on this, PRP-gel may serve as a local delivery system of TGF β and PDGF into bone defects. Considering the proangiogenic effects of PDGF and VEGF,⁶ PRP-gels may further enhance bone healing by supporting revascularization.

In supernatants from PRP-gels, many chemokines and mediators that are known to possess proinflammatory properties could be detected in this study. CCL5 was released at the highest levels, peaking at 1 h after platelet activation and still detectable up to 5 days after. Interestingly, in a study investigating the release of GFs from equine platelets, it was found that secretion of CCL5 was not associated with platelet activation and persisted longer than other GFs, suggesting that CCL5 release may be regulated differently.⁴¹ CCL5 may have deleterious effects on the healing process since it attracts activated leukocytes to the injury site and their accumulation may abolish beneficial effects of GFs.^{53,54} Increased infiltration of leukocytes may lead to tissue damages and a persistent inflammatory phase.^{55,56} In contrast, an early inflammatory phase is an essential component initiating the healing process, even though many mediators can either stimulate or suppress tissue repair during inflammation.⁵⁷ Furthermore, it has been shown that CCL5 is a main chemoattractant released by degenerated discs, and its presence may enhance the migration of MSCs and progenitor cells toward injury sites.⁵⁸ Another study investigating the role of CCL5 in mouse wound healing suggests that this protein induces migration of endothelial progenitor cells.⁵⁹ These findings are particularly interesting for tissue engineering strategies because application of PRP-gels that locally release CCL5 may intensify cell mobilization, enhance vascularization, and therefore improve the healing process.

In this study, MSCs could be successfully cultured in all tested PRP-gel compositions. The rationale behind encapsulating MSCs into gels was to investigate whether PRP-

gels could be used as a scaffold and delivery system of GFs and cells at the injury site. From a clinical point of view, it could be potentially beneficial in patients suffering from impaired wound healing and tissue loss, such as bony defects or cartilage lesions, when GFs and cells are needed to enhance repair. We found that cell survival and proliferation were greatest in gels containing the lowest platelet concentration (1000×10^3 platelets/ μ L). A study investigating the proliferation of MSCs and adipose tissue-derived stem cells encapsulated into PRP-gels has shown a three to fivefold increase of the cell number after 7 days of culture.³³ In that study, the platelet number in PRP-gels was sixfold higher than the average concentration in the whole blood. Similar findings were reported in a study investigating the effect of platelet concentration on bone healing, suggesting that a moderate platelet concentration (two to sixfold concentrated) gives optimal results.¹⁸ These studies along with our results suggest that a lower platelet content in PRP seems to be more favorable for incorporated cells. Thus, most of the commercially available systems based on whole-blood centrifugation usually yield to 2 $\times$ to 3 $\times$ platelet enrichment levels compared to baseline when used in standard settings.²⁵ Therefore, our preparation method of PRP-gels allows obtaining optimal platelet concentration in a clinically feasible manner.

In summary, the results of this study demonstrate that PRP-gels are stable hydrogels, which can effectively deliver bioactive substances and MSCs for various clinical applications. Importantly, the platelet concentration seems to determine the efficacy and properties of PRP-gels. It was shown that a concentration of 1000×10^3 platelets/ μ L in PRP provides an optimal environment for encapsulated MSCs. With regard to clinical application, the concentration and balance of pro- and anti-inflammatory substances should be further investigated to maximize efficacy.

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Disclosure Statement

No competing financial interest exists.

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