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Effect of Platelet-rich Plasma on the *in vitro* Proliferation and Osteogenic Differentiation of Human Mesenchymal Stem Cells on Distinct Calcium Phosphate Scaffolds: The Specific Surface Area Makes a Difference

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ABSTRACT: The *in vitro* effect of platelet-rich plasma (PRP) on cell loading, proliferation, and osteogenic differentiation of human mesenchymal stem cells (MSC) is assessed on distinct resorbable and synthetic calcium

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phosphate scaffolds. A high specific surface area scaffold composed of calcium-deficient hydroxyapatite (CDHA; $48\text{m}^2/\text{g}$) is compared with one made out of β -tricalcium phosphate (β -TCP; surface area $<0.5\text{m}^2/\text{g}$). Fivefold concentrated fresh PRP is applied to scaffolds loaded with 2×10^5 MSC ($n=5$). These constructs are kept in a medium with osteogenic supplements for 3 weeks. The addition of PRP leads to a higher cell loading efficiency of MSC on CDHA ($p=0.0001$), that reaches the values of β -TCP. Proliferation over 21 days is improved by PRP both on CDHA ($p=0.0001$) and β -TCP ($p=0.014$) compared to MSC/calcium phosphate composites. Without the addition of PRP, CDHA has a lower cell loading efficiency ($p=0.0001$) and proliferation ($p=0.001$) than β -TCP. The ALP activity is higher in the MSC/ceramics groups than in the monolayer controls ($p<0.05$). The addition of PRP does not significantly affect ALP activity. However, ALP activity varies considerably within the cell donors and different PRP-pools ($p=0.001$), while the cell numbers do not vary within these two parameters. PRP generates a positive effect on the loading efficiency of MSC on the high specific surface scaffold CDHA that thereby reaches the loading efficiency of β -TCP. PRP improved proliferation, but its osteogenic properties on both calcium phosphate scaffolds are weak.

KEY WORDS: platelet rich plasma, mesenchymal stem cells, calcium phosphate scaffolds, calcium-deficient hydroxyapatite, β -tricalcium phosphate, tissue engineering, bone regeneration, bone healing.

INTRODUCTION

Autogenous bone grafting is widely used in orthopedic and trauma surgery, usually to treat bone defects or to perform spondylodesis. Artificial bone-substitute materials have been introduced to avoid morbidity at the donor site or when large amounts of bone are needed [1]. A variety of degradable and osteoconductive biomaterials, such as β -tricalcium phosphates (β -TCP) is available and used clinically [2,3]. A new group of resorbable high specific surface area (SSA) ceramics is emerging that has been proven to be equally effective as, e.g., β -TCP, regarding osteogenic differentiation *in vitro* [4] and ectopic bone formation *in vivo* [5,6]. Calcium-deficient hydroxyapatite (CDHA) is one of the materials that are used to form high SSA scaffolds that exhibit a SSA between 20 and $80\text{m}^2/\text{g}$ depending on the manufacturing process. Its properties are close to those of human bone ($80\text{m}^2/\text{g}$) [4]. If the scaffolds are not precoated with proteins like e.g., fibronectin, cells adhere more easily to these high SSA mineral scaffold as compared to low SSA ceramic scaffolds, such as β -TCP (SSA $<0.5\text{m}^2/\text{g}$) [4].

Bone-substitute materials may be combined with mesenchymal stem cells (MSC) [7–11] and/or growth factors [12–15]. Both components are important for osteogenesis, and improve their ability to induce bone

formation (osteinduction). Among the growth factors that play a role in bone healing, bone morphogenic proteins seem to be strong osteoinductive agents [16,17]. Recently, the recombinant human bone morphogenic proteins 2 and 7 were approved for clinical use in the United States and the European Community for a narrow range of indications [18]. There are some major drawbacks to using recombinant human bone morphogenetic proteins in clinical applications. Apart from the few approved indications, the disadvantages include the high costs and the super-physiologic dose of a single growth factor. Especially, the latter might be problematic because it can cause voids in the newly formed bone [14]. Furthermore, it is well known that combinations of growth factors are more efficient. Instead of using recombinant human bone morphogenetic proteins, autogenous platelet-rich plasma (PRP) may be used as a source for growth factors, such as platelet derived growth factor (PDGF), transforming growth factor (TGF- β 1, TGF- β 2), vascular endothelial growth factor, and endothelial growth factor [19]. Basic research endorses the ability of PRP to promote bone healing [20–25]. In addition, clinical studies have shown that after implanting bone grafts to mandibular bone defects, the bone density and the maturation rate increased significantly when the grafts were used in combination with PRP compared to grafts alone [19,23]. Because of its autogenous origin, PRP does not hold any risk of transmissible diseases. Furthermore, it contains natural growth factors in a patient specific level and ratio. Nowadays, there are closed systems commercially available that allow for time- and cost-effective production of autogenous PRP in daily surgery. Previous studies demonstrated that there is an effect of PRP on human MSC after ectopic implantation *in vivo* [25]. The basic mechanisms and the interaction of growth factors and MSC on bone substitution materials are not well understood. This is, however, essential to appreciate the effect of PRP and assess its potential for bone healing.

Therefore, the aim of the present study was to evaluate the *in vitro* effect of PRP on cell-loading efficiency, proliferation, and osteogenic differentiation of bone marrow-derived human MSC on high SSA CDHA and low SSA β -TCP in a three-dimensional (3-D) *in vitro* culture.

MATERIAL AND METHODS

Calcium Phosphate Ceramics

Porous β -TCP and CDHA ($\text{Ca}_9(\text{PO}_4)_5(\text{HPO}_4)\text{OH}$) ceramic bodies were synthesized in an emulsion process and subsequent casting

(all by Dr. h.c. Robert Mathys Foundation, Bettlach, Switzerland) [2,26]. CDHA and β -TCP have only minor differences in porosity and interconnectivity: the overall porosity averages 84–85 vol% (macroporosity (pores \varnothing 0.2–0.6 mm) 54–55 vol% and microporosity (pores \varnothing $<5\ \mu\text{m}$) 29–31 vol%). However, they show a large difference in topography [6], i.e., the specific surface area ranged from $<0.5\ \text{m}^2/\text{g}$ for β -TCP to $48\ \text{m}^2/\text{g}$ for CDHA. The ceramic bodies were machined in half-round plates of 5 mm in diameter and a thickness of 3 mm ($29.5\ \text{mm}^3$).

Preparation and Characterization of Platelet-rich Plasma

Fresh allogeneic leukocyte-depleted PRP was obtained from the local blood bank. The PRP was prepared by centrifugation (10 min, $205 \times g$) of a pool of four buffy coats separated from whole blood donations that were anticoagulated with citrate-phosphate-dextrose (Compoflex, Fresenius HemoCare, Bad Homburg, Germany). PRP donations of four donors were pooled to compensate for individual differences in the level of growth factors. The platelet-rich supernatant was leukocyte-depleted via filtration (Fresenius Bio P Plus, Fresenius HemoCare). As a linear correlation of platelet count and growth factor levels was suggested in a certain range [27,28], the mean platelet count in the leukocyte-depleted PRP was adjusted to 0.8×10^9 – 1.3×10^9 platelets/mL corresponding to a four to fivefold increase of the platelet count in whole blood. The amount of growth factors, i.e., TGF- β 1 and PDGF-AB, was quantified by a commercial enzyme-linked immunoassay (ELISA) (R&D, Wiesbaden, Germany). The PRP was kept at room temperature and applied on to the scaffolds within 6–8 h after preparation as recommended previously [19,29].

Isolation and Cultivation of Human Bone Marrow Stromal Cells

Samples of human MSC were obtained from iliac crest or femoral bone marrow aspirates of donors receiving pelvic surgery under general anesthesia ($n=5$). All donors were hematologic healthy Caucasians (2 men, 3 women) ranging in age from 11 to 80 years (mean 38.6, standard deviation (SD) 32.2). The patients were operated for Perthes disease (1x), for congenital hip dysplasia (2x), and hip osteoarthritis (2x). Comorbidities affecting bone metabolism were not present. All procedures were approved by the institutional ethics committee (No. 42/2000, updated No. 11/2002) and all donors or their parents provided informed

written consent. MSC isolation and cultivation were performed as reported earlier [6]. Rapidly dividing cells in passages 2–4 equaling 16–19 population doublings were used.

Loading of Cells and 3-D Culture of Biocomposites

Both biomaterials were tested in duplicate with MSC of five different patients and four different PRP pools (Table 1). The same PRP pool was used with MSC of Donors 1 and 2 since the MSC of both donors were available at the same time. The scaffolds were incubated for 24 h in 40 $\mu\text{g}/\text{mL}$ fibronectin/PBS-solution (Calbiochem/Merck, Darmstadt) to improve cell adhesion and dried for 10 min under a sterile flow at room temperature. Half of the samples were statically loaded with 2×10^5 MSC in 10 μL culture medium. Then, 3.3 μL thrombin (0.8 I.E. activity)-calcium chloride (1 M) solution (1:1) (Tissucol-duo, Baxter, Unterschleißheim, Germany) was pipetted on the seeded scaffolds, followed by 10 μL fresh PRP. The other half of the samples did not receive PRP, but was seeded with MSC in the same manner, i.e., with all components including thrombin. Scaffolds bodies without MSC and MSC in monolayer culture (3.5×10^4 cells) served as controls. Lower cell numbers were used in the monolayer group to allow culturing in a 6-well plate over 21 days without the need to split the cells. All scaffolds and the monolayers were cultured for up to 3 weeks in an osteogenic induction medium containing DMEM (Gibco/Invitrogen, Karlsruhe, Germany), with the addition of 10% fetal calf serum (FCS), 0.1 μM dexamethasone (Sigma, Taufkirchen, Germany), 0.17 mM ascorbic acid-2-phosphate (Sigma), 10 mM β -glycerophosphate (Sigma), and 100 IE/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin (Biochrom, Berlin, Germany). Samples were harvested for evaluation at days 1, 7, and 21.

Biochemical Evaluation

The WST-1 test was used to determine the numbers of metabolically active cells in the biomaterial. Before usage, the cell proliferation reagent WST-1 (Roche Diagnostics GmbH, Mannheim, Germany) was diluted in a ratio of 1:10 with DMEM (Gibco/Invitrogen). The osteogenic induction medium was removed from all wells and the matrices were transferred into a 96-well plate; 250 μL of diluted cell proliferation reagent WST-1 was then pipetted into each emptied well (to determine the number of cells remaining in the wells, where the matrices had

been), to the matrices, and onto the monolayers. During a 60-min incubation period at 37°C, WST-1 was converted into a colored formazan salt by the metabolic activity of the living cells. To quantify the amount of formazan, 100 µL of each sample was put into a separate 96-well plate and measured using an ELISA plate-reader at 450 nm. A monolayer-standard curve was prepared, using different cell numbers, and measured in the same manner. A correction factor for each biomaterial was established to account for possible artifacts induced by the adsorption or quenching of the WST-1 reaction in triplicate. Briefly, 2×10^5 cells in monolayer were incubated with the cell proliferation reagent WST-1. The soluble formazan salt was measured in the ELISA plate reader as described above. Subsequently, empty scaffolds were incubated in the formazan-containing supernatant for 20 min at 37°C. Hundred microliters of each sample were measured in a separate 96-well plate using an ELISA plate-reader at 450 nm. By dividing the initial optical density (OD) of the colored supernatant by the OD that was measured after the empty scaffolds had been incubated in it, a correction factor was obtained for each biomaterial, that was added to the WST-1 values of the respective scaffold groups: 24.1% for CDHA, 30.2% for CDHA+PRP, 17.7% for β-TCP, and 31.5% for β-TCP +PRP (all by Dr. hc Robert Mathys Foundation, Bettlach, Switzerland).

For assessing the cell-loading efficacy, i.e., the number of cells that actually remained on the scaffold after one day, the scaffolds were transferred to another empty well and both the scaffolds and the well that were used for loading the scaffolds were assessed separately. Then, the number of cells on the biomaterial on day 1 was divided by the total amount of cells on the sample and next to the sample in the WST-1 test.

Osteogenic differentiation was assessed by measuring alkaline phosphatase (ALP) activity. Therefore, the matrices were incubated with 1 mL 0.01% Triton X-100 (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and homogenized mechanically to disintegrate the cells using a Polytron (dispersing and mixing technology, Model PT2000, Kinematica Inc., Cincinnati, OH, USA). The cell lysate was centrifuged ($460 \times g$ for 10 min) and the ALP enzyme activity was determined as reported earlier [6]. The amount of ALP was divided by the amount of viable cells as determined in the WST-1 test to normalize the specific amount of ALP product. Osteocalcin (OC) was quantified using an ELISA kit (N-MID™ Osteocalcin One Step ELISA Kit, Nordic Bioscience Diagnostics A/S, Herlev, Denmark) by measuring the photometric reaction at 450/650 nm. Again, correlation factors were established to account for calcium phosphate scaffold effects.

Morphologic Evaluation

The cell distribution within the ceramics was examined histologically by light microscopy for the first few patient's MSC/ceramic constructs only. Samples were fixed and embedded in paraffin wax (Leica, Bensheim, Germany) as published earlier [6]. Sections were stained with toluidine blue (Waldeck GmbH & Co. Division Chroma, Münster, Germany). At a defined depth of 0.75 mm, one section with a thickness of 5 μm was evaluated. The samples were examined at a magnification of 100 \times by two independent observers (A.K. and J.V.) to count the cell numbers within pores of a diameter of more than 200 μm . The average cell number in three pores of the scaffold was calculated for peripheral and central pores. In addition, from each group, representative specimens were analyzed by scanning electron microscopy (SEM, Cambridge S360) according to a standard protocol as published earlier [30].

Statistics

The primary outcome measures were the cell-loading efficiency on day 1, the cell proliferation and the normalized ALP activity over 21 days. The effects of the independent variables, i.e., 'biomaterial' (CDHA, β -TCP or monolayer), 'treatment' (empty biomaterial, +PRP, +MSC, +MSC and PRP, and monolayer), 'biomaterial and time' (biomaterials on day 1, 7, and 21) and 'cells' (nos. 1–5) were examined by multifactorial analysis of variance (ANOVA). Differences between the subgroups of the independent variables were checked in post-hoc tests. The alpha error was adjusted; $p < 0.05$ was considered significant. All tests were two-tailed. Data analysis was performed with SPSS for Windows 12.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Platelet-rich Plasma: Cell Count and Growth Factor Content

The mean platelet count in the leukocyte-depleted PRP ranged from 0.8×10^9 to $1.3 \times 10^9/\text{mL}$. PDGF-AB averaged 126 ng/mL (SD 41) and TGF- β 1 245 ng/mL (SD 72) (Table 1).

Cell-loading Efficiency

The addition of PRP led to a higher cell loading efficiency of MSC on CDHA ($p = 0.0001$), but not on β -TCP (Figure 1). β -TCP exhibited

a higher cell loading efficiency ($p = 0.0001$) without PRP compared to CDHA. After the addition of PRP, no significant differences between the calcium phosphate scaffolds could be observed.

Proliferation

Proliferation and metabolically active cells were significantly improved by the addition of PRP both on CDHA ($p = 0.0001$) and β -TCP ($p = 0.014$)

Table 1. Age/gender of the MSC donors, growth factor content of the platelet-rich plasma pools and ALP activity after 21 days of osteogenic differentiation.

	Experiment				
	No.1	No. 2	No. 3	No. 4	No. 5
Age and gender of MSC donor	11y male	80y female	66y female	24y female	14y male
TGF- β 1 in PRP pool (ng/mL)	206.2	206.2	285.9	323.4	165.1
PDGF-AB in PRP pool (ng/mL)	75.8	75.8	175.5	128	123.8
ALP activity*** (p -nitrophenol/cell number) MV \pm SD	2.3 \pm 3.5	9.8 \pm 12.4	10.7 \pm 12.9	1.5 \pm 1.8	2.1 \pm 3.8

y = year old, *** $p = 0.001$ between the donors.

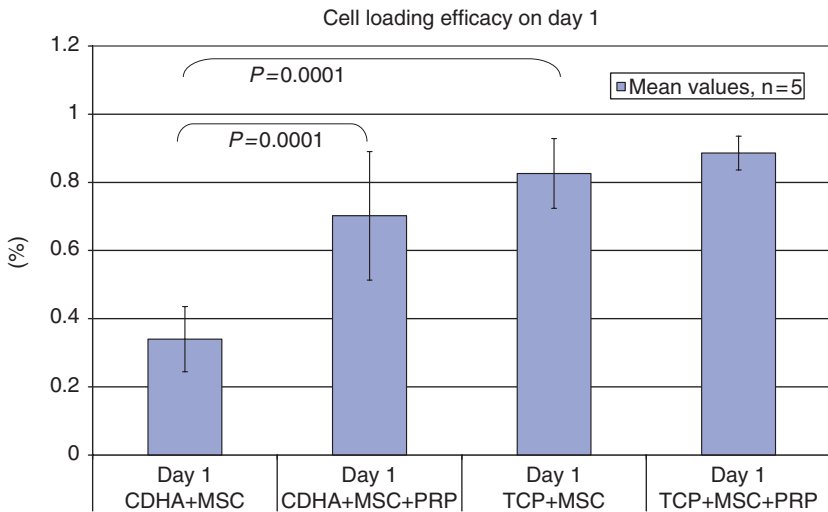


Figure 1. The addition of PRP led to a higher cell loading efficiency of MSC on CDHA, but not on β -TCP. The term cell-loading efficacy describes the amount of cells that were on the scaffold on day 1 in relationship to the total amount of cells that were applied. Mean values and SD are shown.

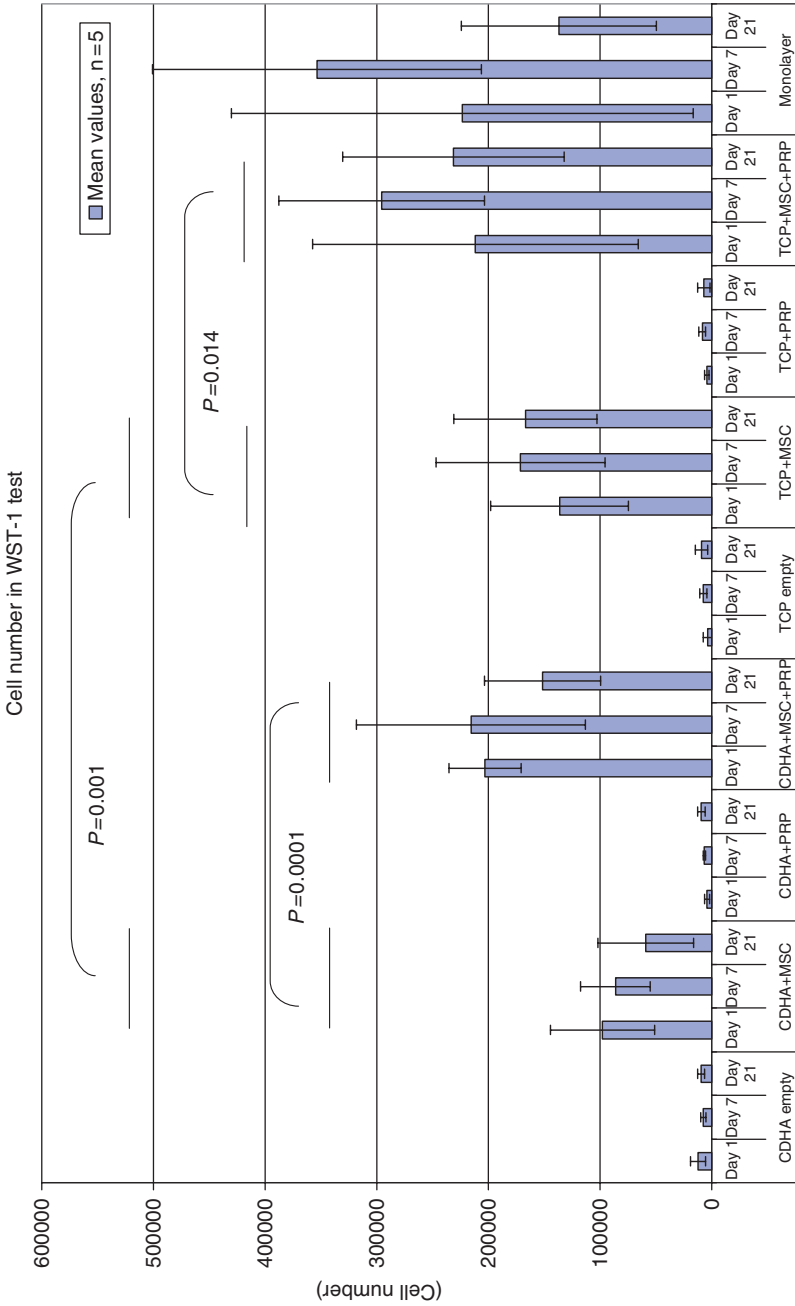


Figure 2. Without PRP, significantly higher cell numbers could be observed on the low surface scaffold β -TCP when compared with CDHA. By the addition of PRP proliferation and metabolically active cells were significantly improved both on CDHA and β -TCP when compared to MSC/calcium phosphate-composites that did not receive PRP. Mean values and SD are shown. All time points of one group were compared with the respective time points of the other groups by ANOVA.

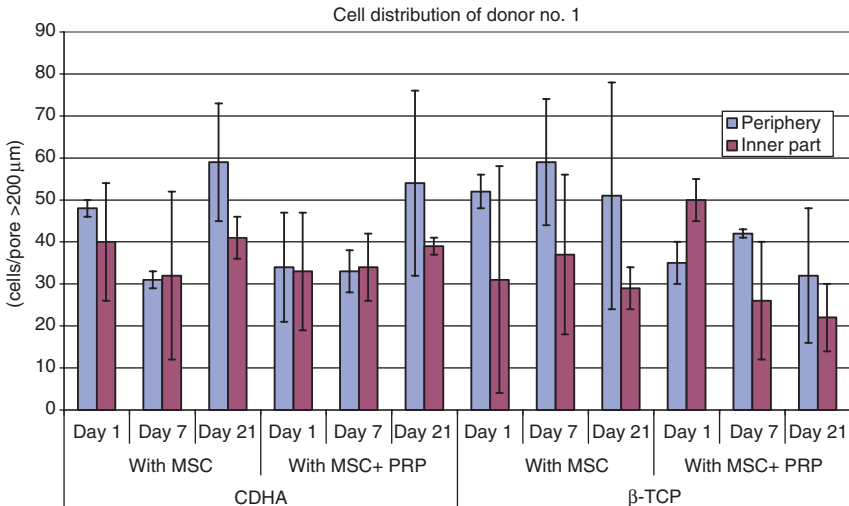


Figure 3. Looking for the penetration and survival of cells in the central part of the scaffolds, histologic specimens were analyzed for the amount of cells in peripheral and central pores of one scaffold. There was a trend towards higher cell numbers in peripheral pores that did not reach significance.

including all time points when compared to MSC/calcium phosphate-composites that did not receive PRP (Figure 2). Without PRP, significantly higher cell numbers could be observed on the low surface scaffold β -TCP when compared with CDHA ($p=0.001$). After the addition of PRP, however, no significant differences between the two scaffolds were present anymore. All MSC/scaffold groups had significantly higher values than the empty scaffolds with and without PRP ($p=0.0001$) which shows that the increase is truly attributed to the metabolic activity of cells. During the course of osteogenic differentiation there were no significant differences of metabolically active cells on the scaffolds. In the 2-D culture, however, there was a significant reduction of metabolically active cells from Day 7 to 21 ($p=0.022$). The cell numbers of active cells did not differ significantly among the five experiments. Histological cross-sections revealed no significant differences in cell numbers for the peripheral and the central zone of the scaffolds (Figures 3 and 4). This may indicate a sufficient interconnectivity of the pores of the scaffolds with a volume of 29.5 mm^3 . The scanning electron micrographs showed both the similar macroporosity (Figure 5 (a)) and the distinct microporosity of the scaffolds (Figure 5(b) and (c)).

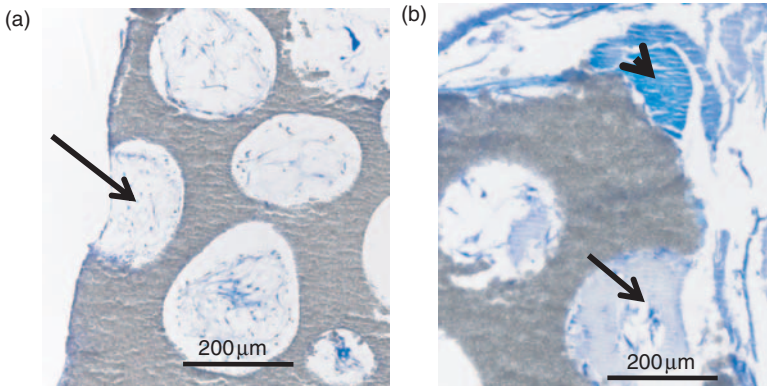


Figure 4. (a) This representative β -TCP specimen without PRP shows the cells' distribution after 7 days of osteogenic differentiation. The arrow marks the MSC within a peripheral pore with a fibroblastic phenotype ($100\times$ magnification) and (b) the addition of PRP to this CDHA scaffold resulted in a gel-like structure (arrowhead) around the biomaterial and the MSC (arrow) that were still present after 7 days of osteogenic differentiation ($100\times$ magnification).

Osteogenic Differentiation

The addition of PRP did not affect ALP activity on both CDHA and β -TCP scaffolds (Figure 6), and there was no significant difference between the two scaffolds. ALP activity was higher in the MSC/CDHA ($p=0.041$) and MSC/ β -TCP groups ($p=0.019$) when compared with the monolayer controls including all time points. This emphasizes the importance of 3-D constructs for osteogenic differentiation of MSC. Cell donors and PRP-pools varied considerably with regard to ALP activity ($p=0.001$) which is reflected by the high standard deviation. This variability may be mainly attributed to the MSC donors. The age did not seem to be a major prognostic factor for osteogenic differentiation in the samples. Interestingly, ALP values were higher with cells of Donor 2 (80 years old) than of Donor 1 (11 years old) (Table 1), although the same PRP pool was used and the experiments were performed in parallel. Furthermore, the PRP pool in Experiment 4 had the highest TGF- β 1 values (which is known to be osteoinductive) and was used with the MSC of a young, 24-year-old donor, but ALP activity was still inferior (Table 1). The highest ALP activity was found in MSC of donors 66 years and older (Table 1).

A statistical analysis of osteocalcin was not performed since the levels of OC values were below the detection limit of 0.5 ng/mL.

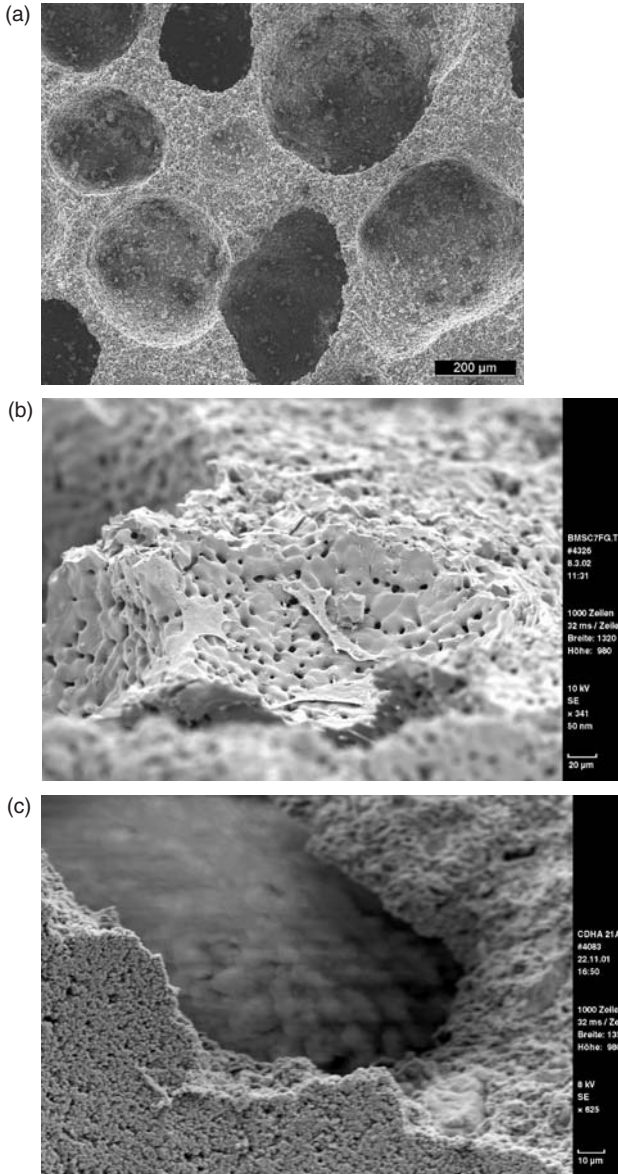


Figure 5. (a) A scanning electron micrograph of a β -TCP specimen without cells at $100\times$ magnification demonstrates the macroporosity with pores averaging $200\text{--}60\ \mu\text{m}$, (b) the $250\times$ magnification of this β -TCP specimen shows cells adhering to the smooth surface of the biomaterial after 21 days, and (c) the higher ('rougher') surface area of a CDHA scaffold without cells can be seen at the $500\times$ magnification.

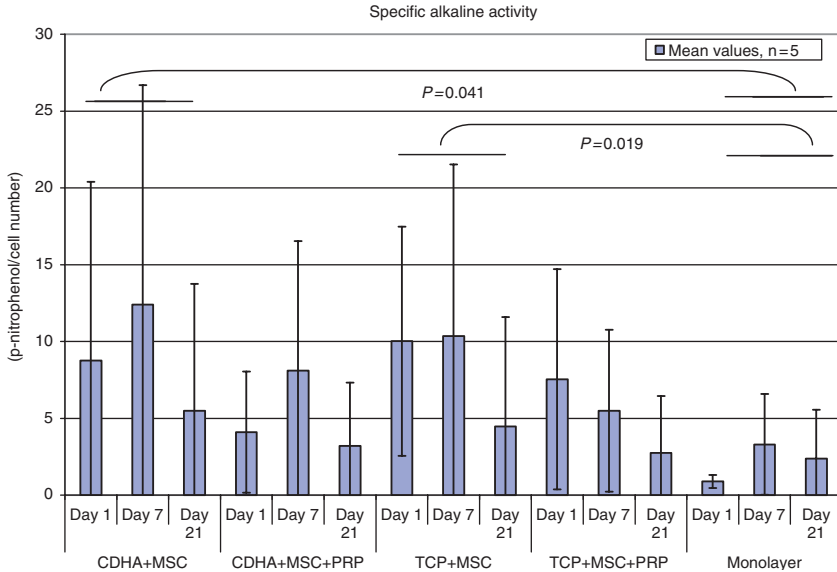


Figure 6. The addition of PRP did not affect ALP activity on both CDHA and β -TCP scaffolds, and there was no significant difference between the two scaffolds. ALP activity was higher in the MSC/CDHA and MSC/ β -TCP groups when compared with the monolayer controls including all time points. The high standard deviations reflect a significant donor variability. Mean values and SD are shown. All time points of one group were compared with the respective time points of the other groups by ANOVA.

DISCUSSION

The purpose of the present study was to evaluate the effect of fresh PRP on the cultivation and differentiation behavior of MSC loaded on high SSA scaffolds and low SSA scaffolds. Generally, the type of biomaterial can significantly influence the interaction with MSC *in vitro* [4,11]. Furthermore, particle size, pore size (macro-, micro-, and nanopores), pore-to-pore interconnectivity, and the resorption rate of the biomaterial are important characteristics [10,31]. While the two ceramic materials used in this experiment were similar in porosity and interconnectivity, they substantially differed in their SSA [6] and resorption characteristics. The approximate hundredfold difference in the SSA can affect protein adsorption. The high SSA of CDHA may allow more extensive and longer absorption of PRP, and the rough surface of CDHA may cause stronger degranulation and release of the growth factors from the platelets.

The results suggest that there is a distinct characteristic for high and low SSA scaffolds when PRP is combined with MSC. Scaffolds with a high SSA benefit to a higher extent from the addition of PRP regarding cell-loading. Without the addition of PRP, the cell-loading efficiency onto CDHA is inferior to β -TCP scaffolds, but with PRP equal cell-loading properties are found. This is very important since the retention of cells within the ceramic is a crucial step for optimization of MSC-initiated bone formation. According to the cell-loading protocol, all biomaterials are immersed in fibronectin prior to applying the cells. Previous studies showed that the adhesion of cells to biomaterials was improved and even more important, that osteogenesis was increased *in vivo* which is in contrast to the effect e.g., human serum [32,33]. However, the pretreatment with fibronectin before loading of the cells was not as effective on CDHA as on β -TCP. Therefore, PRP is an ingenious additive if MSC are to be implanted on CDHA. A possible explanation for the improvement by PRP might be the fact that PRP coagulates on the biomaterial and entraps the MSC in a favorable environment.

The data revealed positive effects of PRP on the proliferation of MSC on both calcium phosphate scaffolds, but no stimulation or impairment of osteogenic differentiation of MSC. This is partly in accordance with a previous study in which Gruber and co-workers [34] reported that the supernatants of activated platelets can increase migration and proliferation of mesenchymal progenitor cells in monolayers, but decrease osteogenic differentiation. Possible explanations for these divergent findings may be the 3-D culture of MSC on calcium phosphate scaffolds which is different from the monolayer culture of Gruber and co-workers. Furthermore, they used a distinct expansion protocol for MSC. A 3-D culture is known to be beneficial for osteogenic differentiation and there are data showing that the expansion protocol of MSC can significantly influence their differentiation capacity [32,35]. Another study showed that addition of PRP to the expansion medium instead of FCS improved expansion of human MSC which retained their differentiation and *in vivo* bone formation capacity [36]. Summarizing the data of the studies mentioned above leads to the conclusion that PRP is a potent mitogenic agent, but does not have strong osteogenic properties *in vitro*. Whether this characteristic is sufficient to significantly improve bone healing has to be tested *in vivo*. For example, PRP was tested as an additive to MSC on CDHA and β -TCP in an ectopic mouse model [25]. In this setting, the effect of PRP depended on the type of ceramic and the differentiation status of the MSC, and enhanced ALP activity of MSC on the high surface scaffold CDHA only, but PRP did not improve osteogenesis [25]. These results, however, have to be

interpreted with caution because the ectopic subcutaneous implantation lacks the mechanic stimulation and the local environment of an osseous defect. To draw a final conclusion further animal studies with an orthotopic implantation of PRP and MSC grafts with a standardized quality of PRP are needed.

This seems to be very important as the method of preparing PRP that can significantly influence the concentrations of platelets and growth factors [37], and consequently their osteogenic capacity [38]. Recommendations for increasing the platelet concentrations in PRP range from three to fivefold of its original concentration in whole blood [19,39]. Higher platelet concentrations do not necessarily correlate with higher levels of growth factors, such as TGF- β 1 [28]. Some groups performed an eightfold increase in platelet concentration in the PRP compared with whole blood, however, the respective TGF- β 1 levels were lower (120 ng/mL) as compared to TGF- β 1 levels in the study (245 ng/mL, SD 72) [40]. The PRP used in this study was prepared by the local blood bank with the standard centrifugation technique (buffy coat-pooling) resulting in four to fivefold PRP concentration. The previously mentioned PRP concentration technique was used because it is standardized and comes closest to the clinical application. It must be pointed out that the levels of growth factors vary in platelets. Therefore, the levels of PDGF-AB and TGF- β 1 were measured in the pool fraction to allow for an easier comparison with other studies (Table 1). The levels of PDGF-AB and TGF- β 1 in the present study were similar to the ones reported by Weibrich et al. [37]: they found 134 ± 46 ng/mL and 269 ± 71 ng/mL for PDGF-AB and TGF- β 1, respectively, in comparison to 126 ± 41 ng/mL and 245 ± 72 ng/mL in the study. Yet, the ideal concentration of growth factors in PRP for improving mitogenesis and osteogenesis is unknown [39]. Other issues are the time delay between preparation and application of the PRP, the temperature, and the storage. Recommendations are for the time delay 4–6 h, storage at room temperature, and avoidance of agitation to prevent degradation of the growth factors [19,41]. These parameters were respected in the present setting.

The variability among the MSC cells of the five donors and the PRP pools was considerable, resulting in large differences in ALP activity. This illustrates that the osteogenic potential between individual patients' MSC may vary tremendously, and it confirms findings of other groups [42–44]. The samples that had a high ALP activity came from very old patients. Although it is known that the quantity of MSC decreases with age [45], there is little data about changes of osteogenic potential of MSC related to age. Some animal studies report stronger

or equal osteogenic potential of MSC from elder MSC donors [46,47], other studies do not [48,49]. The data of the present study show that older patients have MSC with osteogenic potential; however, because of the small sample size a general conclusion is avoided.

CONCLUSIONS

The use of PRP revealed a positive effect on the loading efficiency of MSC on the high surface scaffold CDHA that afterwards reached the loading efficiency characteristics of β -TCP. PRP improved proliferation of MSC on both calcium phosphate scaffolds, but had a weak influence on osteogenic properties.

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