Bone Regeneration in Standardized Bone Defects with Autografts or Bone Substitutes in Combination with Platelet Concentrate: A Histologic and Histomorphometric Study in the Mandibles of Minipigs

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Purpose: *To evaluate the effect of the addition of platelet concentrate (PC) to autografts or bone substitutes on bone regeneration in standardized bone defects.* ***Materials and Methods:*** *Three standardized bone defects were prepared in both mandibular angles of 12 adult minipigs. The defects were grafted with autograft, anorganic bovine bone, or synthetic β-tricalcium phosphate (β-TCP). PC was added to only 1 side. The animals were divided into 4 groups, which were sacrificed at 4 different time points (1, 2, 4, and 8 weeks) for histologic and histomorphometric analysis. The concentrations of platelets and growth factors were measured to identify correlation to the histologic and histomorphometric results.* ***Results:*** *No correlation was found between platelet count in whole blood and platelet count in PC (r_p = 0.36). Furthermore, no correlation could be demonstrated between the platelet count of the PC and the concentrations of PDGF-AB (r_p = −0.27) and TGF-β (r_p = 0.34). There were no signs of a stimulating effect of PC on bone formation in combination with autografts or bone substitutes at any time point (*P = .89*). Addition of PC did not alter the pattern of graft degradation.* ***Discussion:*** *The present study underlines the need for further investigation to identify the optimal concentrations of platelets and combinations of growth factors to achieve a predictable stimulatory effect on bone regeneration. One of the first steps to achieve this goal will be the development of a reliable method for the procurement of PC.* ***Conclusion:*** *PC had no impact on bone formation and graft degradation in standardized bone defects in the mandibles of minipigs.* Int J Oral Maxillofac Implants 2005;20:703–712

Key words: *autogenous bone grafting, bone regeneration, bone substitutes, growth factors, platelet concentrates, platelet-rich plasma*

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In the past few years, utilization of platelet concentrate (PC), also called "platelet-rich plasma" (PRP), has been increasingly recommended in patients undergoing osseous reconstruction to enhance bone regeneration. PC has gained much attention since Marx and coworkers published promising results in regard to bone density achieved by adding PC to iliac cancellous cellular bone marrow grafts in the reconstruction of mandibular continuity defects.[1] It has been speculated that the stimulating effect of PC is the result of the accumulation of autogenous platelets, providing a high concentration of platelet growth factors with a well-documented impact on bone regeneration.[2,3] Platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) are both known to play major roles in bone healing,[4] and both have been identified in the α-granules of platelets. The concept of using autoge-

nous growth factors is attractive, since there is no risk of disease transmission, and it is relatively inexpensive compared to growth factors produced by recombinant techniques.

Since Marx and coworkers published these results in 1998, only 1 additional human clinical study has been published. Wiltfang and coworkers performed 45 sinus grafting procedures in 39 patients.[5] In 23 cases, the sinus was grafted with β-tricalcium phosphate (β-TCP) alone, and in 22 cases PC was added. Six months after implant placement, bone biopsies showed a statistically significant increase (8% to 10%) in bone regeneration in the PC group as compared to the non-PC group. The limited effect of adding PC in this study (38% versus 30% bone density) compared to the study by Marx and associates[1] may be the result of the fact that no autogenous bone was added to the bone substitute. Marx stated that PC must be combined with an autogenous bone graft for PC to have a beneficial effect, since the autogenous platelet growth factors stimulate viable osteogenic target cells only.[6]

A series of clinical case reports and case series have presented the use of PC in different applications,[7–20] leading to divergent recommendations. Documentation of a possible accelerating or stimulating effect of PC in bone regeneration procedures must be obtained from controlled clinical studies or well-designed experimental studies.

Data from experimental studies evaluating the addition of PC to bone graft materials have been conflicting.[21–33] A wide range of different well-documented animal models and PC preparation techniques have been used in these studies. Therefore, the results are difficult to compare. Moreover, none of the studies have analyzed the growth factor content in the applied PC. A recent investigation[34] analyzed the influence of PC platelet concentration in an in vivo model. They achieved the most positive biologic effect on bone regeneration with a platelet concentration of about 1,000,000 platelets/uL (a 3- to 5-fold increase compared to whole blood). The addition of PC with a lower concentration (ie, PC with a 0- to 2-fold increase) did not affect bone regeneration. There seemed to be an inhibitory effect on bone regeneration when higher concentrations were used (6- to 11-fold increased concentration compared to whole blood).[34]

PC can be procured by the discontinuous cell separation method. Typically, 400 to 450 mL whole blood is withdrawn from a central vein catheter.[1,20] The procurement takes place preoperatively in a transfusion institute[20] or intraoperatively.[1] After preparation of the PC, the red blood cells are returned to the circulation. Alternatively, a few systems that allow the surgeon to produce smaller amounts of PC have recently become commercially available.[35] Using these methods, approximately 50 mL of whole blood is withdrawn from a peripheral vein on the day of surgery. These latter "point-of-care" preparation methods are less time-consuming, have lower costs, and can usually be performed on an outpatient basis. The large amount of blood required for PC obtained by the discontinuous cell separation method limits the use of this technique in medically compromised patients and in the elderly because of the high levels of cardiovascular stress it causes.

The purpose of the present investigation was to conduct a well-designed animal experiment to

- Evaluate the impact of PC on bone regeneration in standardized bone defects from the time of the initial organization of the blood clot to the point at which complete bone fill was obtained
- Evaluate whether the presence of osteoprogenitor cells in the autograft is a prerequisite for PC to have a beneficial effect
- Analyze the content of platelets, leukocytes, PDGF-AB, and TGF-β in the applied PC
- Correlate the PC content with the observed bone regeneration, qualitatively and quantitatively

MATERIALS AND METHODS

The study protocol was approved by the Committee for Animal Research, State of Bern, Switzerland (approval no. 107/01). Twelve adult Gottingen minipigs (mean weight ± SD 43.3 ± 7.8 kg) were used in this study.

Surgical Procedure

The surgeries were performed in the Surgical Research Unit of the Department of Clinical Research and Clinic for Large Animals, University of Bern, Switzerland. The animals received no food for at least 12 hours before premedication with an intramuscular injection of ketamine 20 mg/kg body weight (Narketan 10; 100 mg/mL; Vétoquinol, Bern, Switzerland) and xylazine 2 mg/kg body weight (Xylapan; 20 mg/mL; Vétoquinol). General anesthesia was induced by an intravenous infusion of atropine 0.05 mg/kg body weight (1 mg/mL; Sintetica, Mendrisio, Switzerland) and midazolam 0.5 mg/kg body weight (Dormicum, Roche Pharma, Basel, Switzerland) through an ear vein. Endotracheal intubation was performed, and the animal was kept at a controlled respiration frequency of 12 breaths/min. Isofluran with a volume of 10 mL/kg body weight 1.0% to 1.5% (Forene; Abbott, Baar, Switzerland) was added to a mixture of oxygen and N_2O (ratio 1:3).

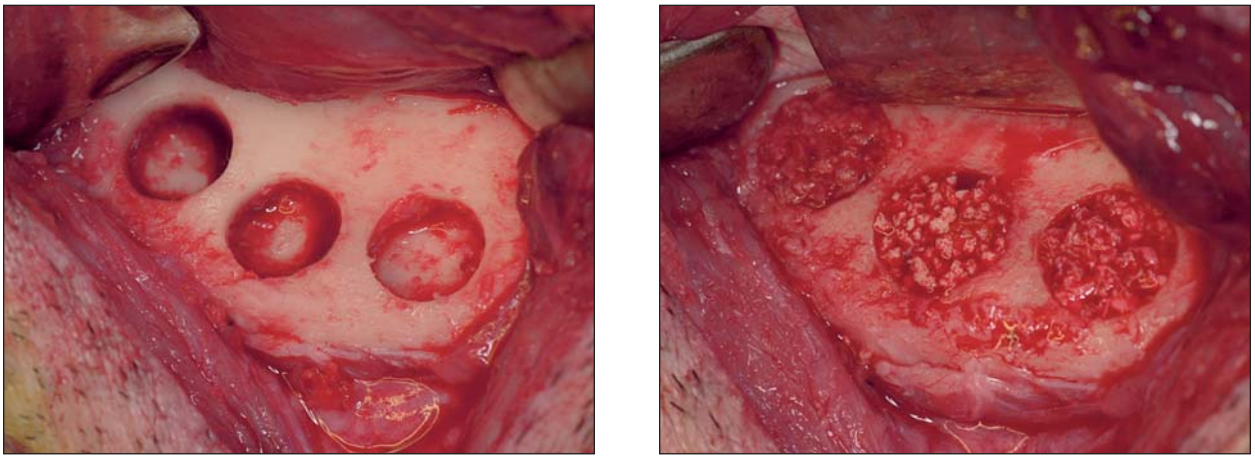

Fig 1 *(a)* Three standardized defects were prepared on each side of the mandible. *(b)* The defects were grafted identically on each side, apart from the addition of PC. *(From left)* In this sample, autograft, β-TCP, and anorganic bovine bone can be observed.

Prior to surgery, the animals were given 1 g of prophylactic amoxicillin (Clamoxyl; GlaxoSmithKline, London, UK) intramuscularly. A paramedian incision was made anteriorly on the neck to get access to the external jugular vein. Through a central vein catheter, 45 mL of whole blood was procured. PC was prepared using the Platelet Concentrate Collection System (PCCS; 3i/Implant Innovations, Palm Beach Gardens, FL) according to the manufacturer's instructions.

Through a subangular incision, the lateral portion of the mandibular body and ramus were sufficiently exposed to prepare 3 standardized intraosseous defects using a trephine with copious saline irrigation (Fig 1a). The defects measured 9 mm in diameter and 5 mm in depth. The corticocancellous blocks were ground to a particulate size of 1 to 2 mm using a bone-mill (R. Quetin, Leimen, Germany). Additional cancellous bone chips were harvested using a chisel and were mixed with the particulated autogenous bone, aiming at a 1:1 ratio of cortical bone to cancellous bone. Each defect was filled with a different grafting material: autogenous bone chips; anorganic bovine bone with a particle size of 1 to 2 mm (Bio-Oss; Geistlich, Wolhusen, Switzerland), ie, a deproteinized cancellous bovine bone substitute; or synthetic β-TCP, with a particle size of 0.7 to 1.4 mm (CEROS TCP; Robert Mathys, Solothurn, Switzerland) (Fig 1b).

In the first animal, the augmentation materials were assigned at random to the 3 defects. In the subsequent animals, the order of placement was always changed 1 defect distally. The augmentation material was mixed with blood and placed into the osseous defects on 1 side. On the contralateral side, 1 mL of PC was added to approximately 1 g of augmentation material in combination with a few drops of calcium chloride ($CaCl_2$) 10% (Streuli, Uznach, Switzerland) for initiating the coagulation process. All 3 defects were covered with a Gore-Tex membrane (GT9; W. L. Gore & Associates, Newark, DE) stabilized with 4 fixation screws (Memfix Plus; Institut Straumann, Waldenburg, Switzerland). The surgical site was closed in multiple layers using resorbable sutures (Vicryl 3-0 and 2-0, Johnson & Johnson/Ethicon, Somerville, NJ). A fentanyl plaster (Durogesic TTS 50 μg/h; Johnson & Johnson/Janssen-Cilag, Baar, Switzerland) was applied for postoperative pain control, and the animals received antibiotics for 7 days postoperatively (Ilcocillin PS200,200, 1 mL/25 kg body weight; Novartis, Basel, Switzerland). The animals were checked daily for the first postoperative week for signs of infection.

Laboratory Analysis

A sample of whole blood and PC was analyzed for platelet count and white blood cell count at the Hämatologisches Zentrallabor (Inselspital, Bern, Switzerland). Another PC sample was analyzed for content of PDGF-AB and TGF-β using Quantikine ELISA kits DB100 and DHD00 (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions. Growth factor analysis was carried out at the Institute of Clinical Chemistry and Laboratory Medicine, Johannes Gutenberg University, Mainz, Germany.

Healing Periods

The 12 animals were divided into 4 groups of 3 animals. The animals in each group were allowed 1 week, 2 weeks, 4 weeks, or 8 weeks to heal. At the end of each designated healing period, the animals were sacrificed by induction of deep anesthesia followed by withdrawal of the entire blood volume. The mandibles were bilaterally cut just above the mandibular foramen and anterior to the masseter to harvest 2 blocks each containing 3 defects for histologic preparation.

Table 1 Laboratory Values

	Mean	SD	Range
PC (mL)	6.75	0.62	5.5 to 7.5
Platelet count in whole blood ($\times 10^9$/L)	421	82	346 to 639
White blood cell count in whole blood ($\times 10^9$/L)	8.6	1.6	6.4 to 11.8
PDGF-AB concentration in PC (pg/mL)	565	210	370 to 1,165
TGF-β concentration in PC (ng/mL)	69	15	50 to 97
Platelet count in PC ($\times 10^9$/L)	1870	317	1486–2420
White blood cell count in PC ($\times 10^9$/L)	16.5	6.4	5.2–25.5

Histologic Preparation

The harvested mandibular blocks were fixed in 10% neutral buffered formalin combined with 1% $CaCl_2$ for 2 weeks. A radiograph was obtained to identify the exact location of the individual defects, whereupon each block was dehydrated and embedded in methylmethacrylate resin. Using an EXAKT low-speed diamond saw with copious cooling (EXAKT Apparatebau, Norderstedt, Germany), each defect was sectioned in the buccolingual direction, yielding 7 to 8 consecutive, nonrandomized, undecalcified sections (about 500 μm in thickness). The sections were mounted on opaque acrylic plastic with acrylic glue and ground to a final thickness of about 80 um. Finally, the sections were superficially stained with toluidine blue.[36]

Histomorphometric Evaluation

The 3 most central sections per defect were histomorphometrically analyzed by an experienced examiner (BH) who was blinded to the treatment modalities. The 1-week sections were dominated by the presence of a coagulum throughout the defects. Histomorphometric evaluation of the sections from this earliest healing period was excluded, and only qualitative descriptions were performed.

In the 2-, 4-, and 8-week specimens, the volume fractions (%) of newly formed bone, residual grafting material, and soft tissue/marrow space occupying the defects were determined by point counting directly in the microscope, using a square grid (distance between 6 × 6 testlines = 255 μm) at a magnification of 6.35.[37]

Statistical Analysis

Standard regression and analysis of variance with due regard to "pairing" (measurements on the same animal) were used, leading to standard Student *t* testing of effects of interest. The level of significance used was 5%. For the assessment of the effect of using PC on the amount of bone formed, the volume percentage data could be used without any need for scale transformation. For the analysis of correlations between laboratory values, Pearson correlation coefficients (r_p) were calculated.

RESULTS

Clinical Evaluation

All minipigs remained healthy during the healing period and healed uneventfully.

Laboratory Analysis of PC

Laboratory values are summarized in Table 1. The platelet concentration in PC was on average 4.4 times (range, 3.5 to 5.8) higher than the concentration in whole blood. It was not possible to demonstrate any statistically significant correlation between the initial platelet concentration in whole blood and the final platelet yield in PC ($r_p = 0.36$).

No statistically significant correlations could be identified between platelet count in PC and concentrations of PDGF-AB ($r_p = -0.27$) or TGF-β ($r_p = 0.34$). No statistically significant relationships were found between white blood cell counts in PC and concentrations of PDGF-AB ($r_p = 0.12$) or TGF-β ($r_p = 0.09$).

Histology

1 Week. All defects, irrespective of grafting material, were dominated by the presence of a blood clot. Granulation tissue could be seen proliferating from the defect walls, leaving a convex central portion with only grafting material and a fibrin network (Fig 2). In a few sections, a limited amount of woven bone could be seen growing from the floor of the defects, especially in areas where the defects comprised the cancellous portion of the mandible. There were no indications of a more extensive vascular ingrowth or advanced soft tissue maturation in defects where PC had been added.

2 Weeks. At 2 weeks, the blood clot had been replaced by granulation tissue that extended all the way to the bioinert barrier membrane. Woven bone was consistently formed only from the defect walls.

Some of the defects in the cancellous portion of the mandible that were grafted with autografts showed woven bone formation almost to the level of the membrane (Fig 3), whereas others that were placed in more compact bone exhibited limited bone formation (Fig 4). In defects filled with the 2 bone substitutes, much less bone formation could be observed. The differences in bone formation seemed to be independent of the addition of PC.

4 Weeks. All defects filled with autografts showed bone formation to the level of the membrane, and all

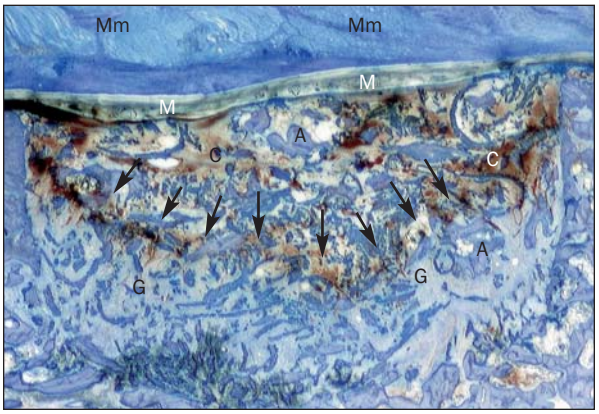

Fig 2 Defect grafted with autogenous bone with PC after 1 week. A convex central portion is seen occupied by autograft (A) and coagulum (C). Granulation tissue (G) proliferated from the defect walls. The borderline between granulation tissue and coagulum is marked with black arrows. An expanded polytetrafluoroethylene (e-PTFE) membrane (M) delineates the defect and separates the grafted area from the masseter muscle (Mm) (toluidine blue; original magnification ×3).

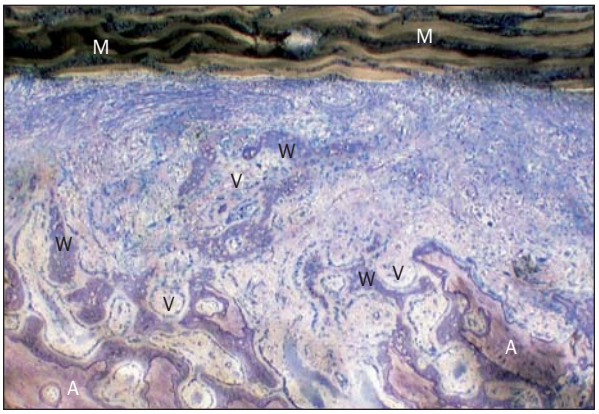

Fig 3 Defect grafted with autogenous bone (A) with PC after 2 weeks. Woven bone (W) proliferated in proximate relation to the vessels (V), almost to the level of the e-PTFE membrane (M) (toluidine blue; original magnification ×125).

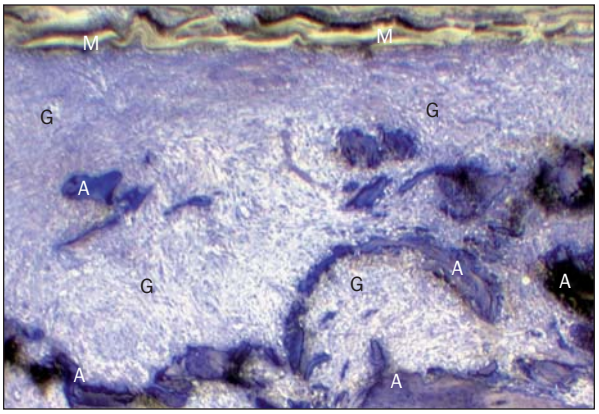

Fig 4 Defect grafted with autogenous bone (A) with PC after 2 weeks. In contrast to Fig 3, no woven bone can be observed in the area beneath the e-PTFE membrane (M). Instead, granulation tissue surrounded the autograft particles (toluidine blue; original magnification ×100).

graft particles were embedded in newly formed bone (Fig 5). Defects filled with anorganic bovine bone and β-TCP also contained newly formed bone throughout the defect, but in smaller amounts and with less maturity than seen in sites with autografts (Figs 6 and 7). β-TCP granules and small autograft particles had almost disappeared and were substituted by bone. The anorganic bovine bone particles remained stable, although osteoclast-like cells often could be identified on the surfaces. No differences could be observed in bone maturation or degradation of grafting material in relation to the addition of PC.

8 Weeks. All defects showed trabecular bone fill with a fully developed bone marrow. In most defects, more compact bone was newly established beneath the membrane, leading to a new cortex formation (Fig 8). Only a few remnants of β-TCP granules (Fig 9) and of the larger autograft particles (Fig 8) could be identified in the defects. These were predominantly embedded in newly formed bone. Anorganic bovine bone particles showed osseous integration as well (Fig 10), but with a slow substitution rate. Identical healing patterns were still observed in both defects where PC was used and those where it was omitted.

Histomorphometry

The results of the histomorphometric analysis are presented in Figs 11 through 13. Whether bone formation, residual graft material, or soft tissue occupying the defects (vol%) was analyzed, the PC effect exhibited no significant heterogeneity over graft materials or time points. In fact, the difference between results with and without PC (averaged over the 3 animals that held information about a given material at each time point) varied less than had been expected (the 3 F values were < 1). As there were no differences in PC response between time points or grafting materials, the assessment of the overall PC effect was reduced to a simple paired comparison: Likewise, it was nonsignificant (estimated effect: 0.17% [SE = 1.2%, P = .89]). Comparison of the grafting materials, including patterns of degradation and osteoconduction, will be presented in a separate publication.

Figure 14 illustrates the differences of bone formation on the PC side versus the non-PC side. It is noteworthy that no pattern for the material as a whole could be seen for the 3 different grafting materials or for the 3 healing periods.

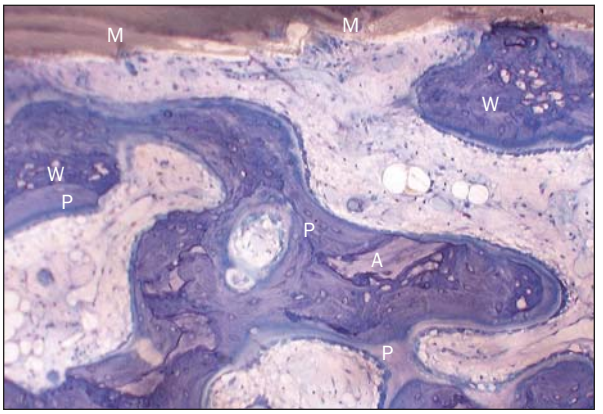

Fig 5 Bony incorporation of autograft (A) without PC after 4 weeks. A scaffold of woven bone (W) and parallel-fibered bone (P) connected the remnants of the autogenous particles (A) throughout the defect to the level of the e-PTFE membrane (M) (toluidine blue; original magnification ×250).

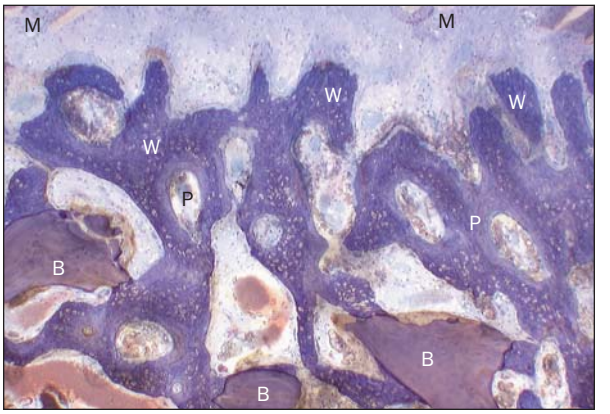

Fig 6 Bony incorporation of anorganic bovine bone (B) with PC after 4 weeks. A scaffold of woven bone bridges the bovine bone granules throughout the defect. In a few places, the reinforcement of the woven bone with parallel-fibered bone can be seen. The e-PTFE membrane (M) is seen at the top of the section (toluidine blue; original magnification ×160).

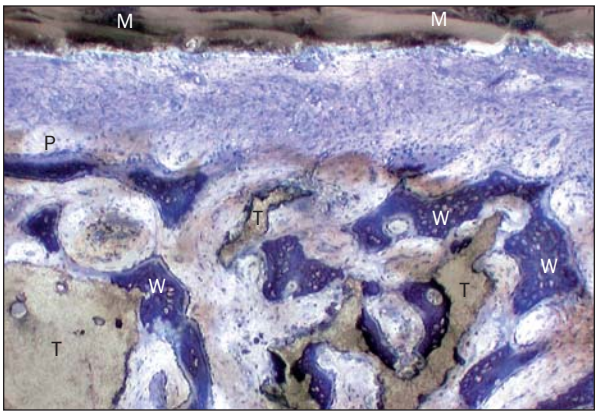

Fig 7 Bony incorporation of β-TCP (T) without PC after 4 weeks. Predominantly woven bone (W) was observed around remnants of β-TCP. The presence of osteoblastic seams indicates early deposition of parallel-fibered bone (P). The e-PTFE membrane (M) is seen at the top of the section (toluidine blue; original magnification ×160).

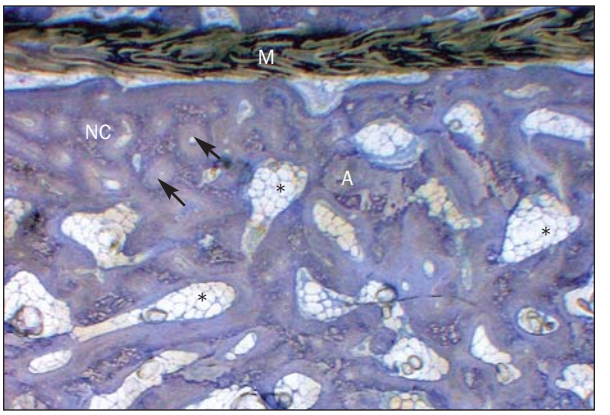

Fig 8 Defect grafted with autogenous bone without PC after 8 weeks. A new cortex formation (NC) was developing just beneath the e-PTFE membrane (M) with primary osteons *(arrows)*. Remnants of an autograft particle can be observed surrounded by both newly formed bone and mature marrow (*) (toluidine blue; original magnification ×63).

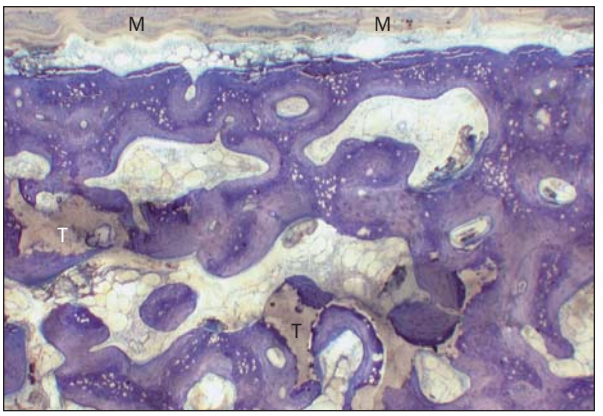

Fig 9 Defect grafted with β-TCP with PC after 8 weeks. A few remnants of graft particles (T) almost completely covered by new bone can be observed (toluidine blue; original magnification ×125).

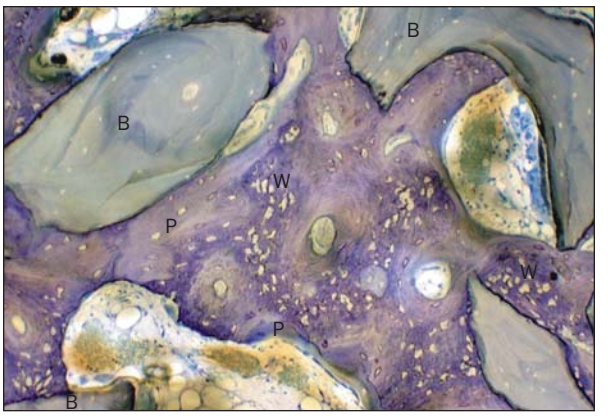

Fig 10 Defect grafted with anorganic bovine bone without PC after 8 weeks. Graft particles (B) are seen fully integrated in newly formed parallel-fibered (P) and woven bone (W) (toluidine blue; original magnification ×250).

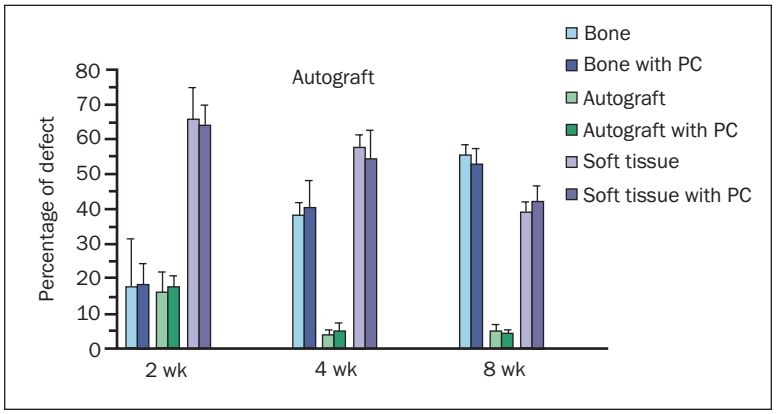

Fig 11 Percentage of newly formed bone, residual grafting material, and soft tissue occupying the defects grafted with autogenous bone with and without PC (SD indicated with vertical bars).

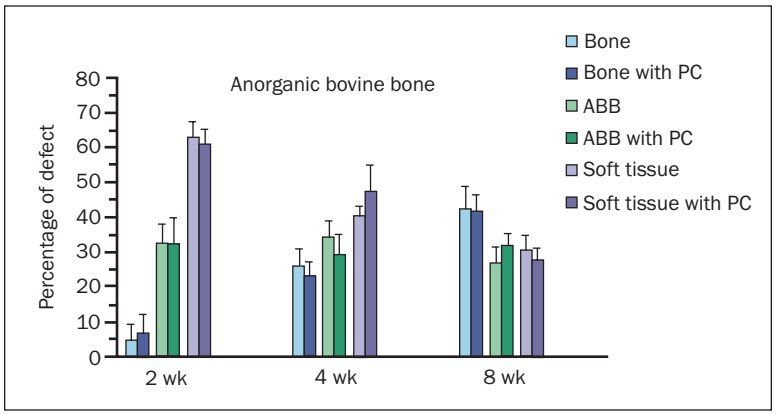

Fig 12 Percentage of newly formed bone, residual grafting material, and soft tissue occupying the defects grafted with anorganic bovine bone with and without PC (SD indicated with vertical bars). ABB= anorganic bovine bone.

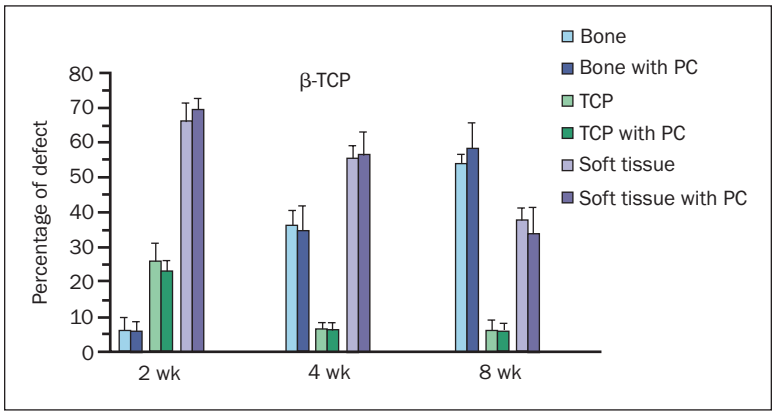

Fig 13 Percentage of newly formed bone, residual grafting material, and soft tissue occupying the defects grafted with β-TCP with and without PC (SD indicated with vertical bars).

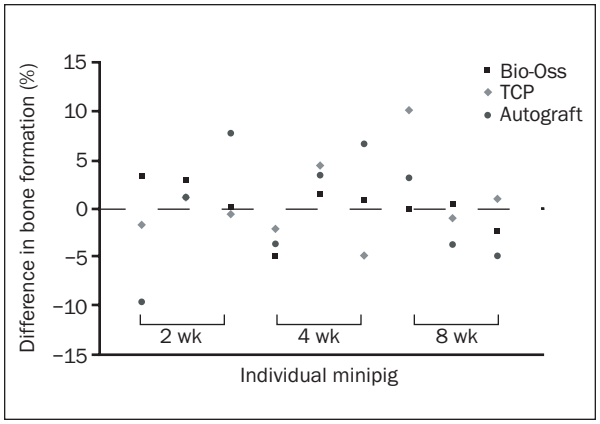

Fig 14 The difference in bone formation was calculated for each animal by subtracting the percentage of bone formation on the PC side from the percentage of bone formation on the non-PC side. If a mark is situated above 0, more bone formation was seen on the PC side than on the non-PC side in that animal.

DISCUSSION

In the present study, PC was analyzed for its possible stimulating effect on bone regeneration in combination with particulated autografts or 2 commercially available bone substitutes of equal particle size. Selection of the minipig for the present investigation

was based on its close similarity to humans in terms of platelet count, platelet size, clotting parameters, and bone structure.[38] The created defects were non–critical-size defects.[39] The purpose of choosing this experimental design was to be able to visualize the healing process from the stage of early ingrowth of new vessels to maturation of the newly formed bone and to evaluate its dependence on PC addition. Each minipig served as its own control.

For preparation of PC, the PCCS was chosen since it is a well documented "point of care" PC system with a high collection efficiency, platelet count, and growth factor concentration.[23,35,40] The concentration of platelets in PC was on average 4.4 times higher than in whole blood. This increased concentration thus fulfills the definition of PRP by Marx.[6] The observed lack of correlation between initial platelet concentration in whole blood and the final concentration in PC may be the result of a low number of specimens. However, a previous study in a larger population of humans also concluded that no reliable prediction of platelet concentration in PC could be made from the platelet concentration in whole blood and that only limited predictions could be made of the growth factor concentration based on platelet count in PC.[35] In contradiction hereto, another comparative laboratory study of 3 different PC preparation methods demonstrated for a limited number of volunteers (n = 12) a strong correlation between platelet count in PC and the corresponding concentration of growth factors using the same immunoassay technique used in the present study.[41] A possible explanation for these differences might be differences in handling of the involved volunteers, as it has been shown that different levels of mental and physiologic stress influence both platelet aggregability and degranulation.[42,43] The concentration of PDGF-AB and TGF-β in whole blood was unfortunately not evaluated in the present study. It is therefore not known whether the collection efficiency of the individual growth factors was as unpredictable as that of the platelets.

The concentration of PDGF-AB in the present material is remarkably low when compared with earlier reports in which human PC was prepared by the PCCS.[35,41] This finding may be the result of the use of a new line of test kits from the manufacturer (the kits used in previously mentioned publications are no longer available), or minipig platelets might contain lower amounts of PDGF-AB. However, mitogenic activity was demonstrated, since the addition of activated PC from minipigs caused proliferation of gingival fibroblasts in a dose-dependent manner.[31]

The impact of PC on bone healing in pigs has earlier been evaluated in relation to the placement of dental implants,[26,33] in sinus grafting procedures,[27,31] and in standardized critical-size defects in the frontal bone.[23,30] The implant studies demonstrated increased bone-to-implant contact (BIC) and bone density in the early healing phase. After 8 to 12 weeks, the effect of PC was no longer evident. Analyses of the applied PC were not performed, which makes a discussion of the large variances in the results of the 2 studies difficult.[26,33] In the 2 sinus grafting studies, the addition of PC to pure anorganic bovine bone[31] or to combinations of anorganic bovine bone and cancellous bone from the iliac crest[27] was investigated. PC had no stimulating effect on BIC or bone density when bovine bone was used alone or in combination with autografts (85% bovine bone and 15% autograft).

These findings correspond well with the present study. However, increased bone density was obtained when PC was added to a combination of 50% anorganic bovine bone and 50% autograft, although no influence was observed on BIC. A possible explanation for the increased bone density might be that pure spongiosa from the iliac crest was used. This type of autogenous bone graft contains the highest amount of osteogenic cells and the least protection against resorption.[44] When combined with the very slowly substituted anorganic bovine bone particles, the autografts might be protected against resorption, leaving the chemotactic and mitogenic factors in PC to act on the many osteogenic target cells. In most dento-alveolar augmentation procedures, bone is harvested from intraoral donor sites. Depending on the specific site, a different ratio of cortical and cancellous bone can be obtained. In the present study, a 1:1 mix of cortical and cancellous chips simulated such an intraoral bone harvesting. As seen in humans,[45] bone from the minipig mandible probably contains less osteogenic cells than bone from the iliac crest, which might explain the increased bone density observed by Terheyden and colleagues[27] as compared to the present data. Unfortunately, the concentrations of platelets and growth factors in PC were not reported in that study,[27] which excludes a comparison of this parameter. In sheep, the addition of PC to cancellous bone grafts from the iliac crest in sinus grafting procedures caused no increase in bone formation or bone-to-graft contact after 4 or 12 weeks of healing.[32]

Standardized critical-size defects in the frontal bone of minipigs may be expected to demonstrate a similar healing pattern compared to the defects in the mandibular angle, despite a different embryonic origin.[23,30] Schlegel and coworkers[30] compared the effect of 2 different PC preparations in combination with autografts alone, autografts combined with an algae-derived bone substitute(1:3 ratio), and autografts combined with a bioactive glass (1:1 ratio). PC

prepared by the PCCS method yielded a concentration of platelets that was 6.5 times the concentration found in whole blood, whereas the corresponding value for PC derived from the Curasan method was 4.1. As shown in the present material, none of the PC concentrations had a relevant stimulating effect in combination with any of the bone substitutes. However, they did demonstrate an accelerated mineralization in the very early healing phase (2 weeks) in defects grafted with autografts. This effect was most pronounced in the defects with the most concentrated PC. After 4 weeks, this effect seemed to be reversed. Unfortunately, it was not clear from the article whether these early tendencies were statistically significant.

Both in clinical and experimental PC studies, it has been assumed that higher concentrations of platelets would result in higher concentrations of growth factors and thus would increase the stimulation of the osteogenic cells.[46] On the other hand, in vitro studies on human osteoblast cell lines have indicated that a linear relationship between concentration and effect does not exist, and that PC with more than a 5-fold platelet concentration actually might have an inhibitory effect.[47,48] Studies with recombinant growth factors have indicated that increasing doses of TGF-β may actually inhibit bone healing compared with lower doses,[49] and that combinations of bone stimulating growth factors can act antagonistically.[50] In vivo data from a rabbit model support the hypothesis of a concentration-dependent biologic effect of autogenous platelet growth factors and their inhibitory effect at very high platelet concentrations (≥ 1,750,000 platelets/μL PC).[34]

CONCLUSION

In summary, no correlations were found in the present study between platelet count in whole blood and in PC. In addition, no correlation could be demonstrated between the platelet count in PC and the concentrations of PDGF-AB and TGF-β. There were no signs of a stimulating effect of PC on new bone formation in combination with autografts or bone substitutes, nor could an altered pattern of graft degradation be demonstrated. The presented results and a review of the literature suggest that no methods are currently available to produce standardized PC in which a certain whole blood platelet count will result in PC with a predictable amount of platelets and combination of growth factors. Use of autologous growth factors is simple and safe compared to allogenic and xenogenic preparation methods. Consistent results, however, cannot be expected until the ideal concentration of platelet growth factors has been identified and reliable PC preparation methods have been developed.

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