J Clin Periodontol 2006; 33: 500–509 doi: 10.1111/j.1600-051X.2006.00938.x

Journal of Clinical Periodontology

Lack of beneficial effects of platelet-rich plasma on sinus augmentation using a fluorohydroxyapatite or autogenous bone: an explorative study

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Klongnoi B, Rupprecht S, Kessler P, Zimmermann R, Thorwarth M, Pongsiri S, Neukam FW, Wiltfang J, Schlegel KA. Lack of beneficial effects of platelet-rich plasma on sinus augmentation using a fluorohydroxyapatite or autogenous bone: an explorative study. J Clin Periodontol 2006; 33: 500–509. doi: 10.1111/j.1600-051X.2006.00938.x.

Abstract

Background: Maxillary sinus augmentation is frequently necessary before placement of dental implants in the posterior maxilla. Besides autogenous bone graft, various bone substitutes have been used, with favourable results. Although platelet-rich plasma (PRP) has been used in the field of oral and maxillofacial surgery for years, its beneficial effects on osseous regeneration still remain unclear. The aim of this study was to evaluate the short and long time effects of PRP on single-stage sinus augmentation using autogenous bone or a fluorohydroxyapatite (Algipore®) in a randomized prospective animal study.

Methods: After extraction of maxillary premolars of sixteen minipigs, the wounds were allowed to heal for 2 months. Then, sinus augmentations were performed bilaterally using one of the following grafting materials: autogenous bone and Algipore® with or without PRP. Three dental implants (Ankylos®) were installed in each sinus simultaneously. Four animals were euthanized at each period of observation (1, 2, 8 and 12 months). Implant-bearing specimens were sectioned bucco-lingually along the long axis of implants and undecalcified ground specimens were prepared. The bone-implant-contact (BIC) was measured by means of microradiographic examination. For histological evaluation, the specimens were stained with toluidin blue, and the percentage of the newly formed bone and the remaining bone substitute were evaluated.

Results: The grafting materials chosen showed increasing levels of BIC and newly formed bone throughout the period of observation in both PRP and non-PRP groups. Adding PRP resulted in lower BIC and newly formed bone compared with autogenous bone grafts or Algipore® alone. However, *a statistical significance was not found*. The percentages of the remaining bone substitute in both the PRP and non-PRP groups were closely comparable in all observation periods.

Conclusions: The application of PRP could not reveal significant beneficial effects on the BIC, the percentage of the newly formed bone and the remaining bone substitute in this study.

Key words: Algipore®; autogenous bone; bone-implant-contact; growth factor; implant; platelet-rich plasma; sinus augmentation; thrombocyte

Accepted for publication 31 March 2006.

Implant placement in the posterior maxilla can be problematic due to insufficient bone volume in both the vertical and horizontal directions and the proximity to the maxillary sinus (Razavi et al. 1995, Ulm et al. 1995). Additionally, the bone quality is often unfavourable. The cancellous bone is frequently of low density (Misch 1990, Wheeler et al. 1996, Ulm et al. 1999). Maxillary sinus augmentation had been developed (Tatum 1986) in the mid-1970s in order to increase vertical bone height to achieve primary stability of endosseous implants and was proven to be a safe procedure with high predictable outcomes (Adell et al. 1990, Hirsch & Ericsson 1991, Smiler et al. 1992, Raghoebar et al. 1993, Blomqvist et al. 1998).

Intra-oral and extra-oral autogenous bone grafts are considered to be the gold standard as they have no risk of immunological rejection or possible disease transmission. Moreover, they contain osteoinductive and osteoconductive potential, and are also a source of osteoprogenitor cells, leading to satisfactory clinical results (Burchardt 1983, Wood & Moore 1988, Hirsch & Ericsson 1991, Raghoebar et al. 1993, Lundgren et al. 1996). However, their disadvantages are the limited amount of intra-oral graft available, the need for general anaesthesia and hospitalization when extra-oral sites are used, which can also lead to donor site morbidity (Laurie et al. 1984, Younger & Chapman 1989, Nkenke et al. 2001, 2002, 2004). These drawbacks associated with autogenous bone harvesting implicate the need for alternative sources. Various bone substitutes have been extensively studied by several authors (Burchardt 1983, Smiler & Holmes 1987, Hardesty & Marsh 1990, Tidwell et al. 1992, Misch & Dietsh 1993, Moy et al. 1993, Wetzel et al. 1995, Furusawa & Mizunuma 1997, Schepers & Ducheyne 1997, Haas et al. 1998, Tadjoedin et al. 2000, Al Ruhaimi 2001, Cordioli et al. 2001, Yildirim et al. 2001, Artzi et al. 2003, Schlegel et al. 2003, Schopper et al. 2003). They can be used alone, serving as an osteoconductive scaffold (Burchardt 1983, Hardesty & Marsh 1990). However, some authors suggested the use of combination grafts between bone substitutes and autogenous bone in order to obtain osteoinductive capacities and therefore improve the bone quality of the augmented region (Tidwell et al. 1992, Misch & Dietsh 1993, Moy et al. 1993, Yildirim et al. 2001).

To enhance the wound-healing process, an application of topical substances has drawn considerable attention. Matras (1982, 1985) primarily presented the use of fibrin glue in the field of oral and maxillofacial surgery and determined its quality of tissue sealing, haemostasis and promotion of wound healing. This technique was later modified using an autologous fibrin adhesive with cancellous bone graft in mandibular continuity reconstruction (Tayapongsak et al. 1994). The next crucial step had begun after the publication of an article (Whitman et al. 1997), which introduced the use of platelet-rich plasma (PRP) in oral and maxillofacial surgery, as well as one by Marx et al. (1998). The authors presented the use of PRP in the reconstruction of mandibular continuity defects with cancellous marrow grafts and described a higher maturation rate and greater bone density radiographically compared with grafts without PRP (Marx et al. 1998).

PRP is an autologous concentration of platelets, containing a number of important growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), transforming growth factor-β2 (TGF-β2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial-cell growth factor (ECGF) and vascular endothelial cell growth factor (VEGF) (Kiuru et al. 1991, Marx et al. 1998, Marx 2004). Additionally, PRP also contains proteins (i.e. fibrin, fibronectin, vitronectin) known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue and epithelial cell migration (Marx 2004).

The use of PRP is based on the premise that the large number of platelets in PRP release significant quantities of growth factors that promote chemotaxis of precursor cells, cell mitosis, collagen production, initiating vascular in-growth, and inducing cell differentiation (Freymiller & Aghaloo 2004). Currently, the use and benefits of PRP in bone grafting procedures are still generating controversial discussions. Previous studies have shown that addition of PRP to autogenous bone grafts (Whitman et al. 1997, Marx et al. 1998, Anitua 1999, Fennis et al. 2004, Oyama et al. 2004) or bone substitutes (Kassolis et al. 2000) could increase the rate of osteogenesis and enhance bone formation. Nevertheless, other studies could find beneficial effects of PRP only in the initial phase of healing (Schlegel et al. 2003, Schlegel et al. 2003, 2004, Zechner et al. 2003, Wiltfang et al. 2004). On the other hand, certain articles did not reveal significant differences (Danesh-Meyer et al. 2001, Shanaman et al. 2001, Aghaloo et al. 2002, Furst et al. 2003, Jakse et al. 2003, Jensen et al. 2004, Roldan et al. 2004), or even showed a lower level of bone formation and a delay in the remodelling of bone grafts when PRP was added (Choi et al. 2004).

Algipore® (Friadent GmbH, Mannheim, Germany), a fluorohydroxyapatite, is manufactured by calcifying marine algae (*Corallina officinalis*). The particles contain a pore system with a mean diameter of 10 μm (Schopper et al. 2003). This material is biocompatible, osteoconductive and has an additional desirable property of being slowly resorbable and replaced by newly formed bone (Ricci et al. 1992, Schopper et al. 2003, Ewers et al. 2004). In in vitro studies, this material proved to be a suitable carrier for bone morphogenic proteins and growth factors (Herr et al. 1993, Gille et al. 2002), and its use in sinus augmentation demonstrated good clinical results (Ricci et al. 1992, Schopper et al. 2003). In the present study, the short- and long-term effects of PRP on sinus augmentation using autogenous bone and Algipore® were compared.

Material and Methods

Animal

Sixteen adult minipigs (18 months old) were included in this study. The minipig's morphological and anatomical characteristics as well as the bone regeneration rate are closely comparable to humans (minipigs 1.2–1.5 μm/day; humans 1.0–1.5 μm/day) (Laiblin & Jaeschke 1979). The animal study protocol was approved by the local animal committee of the government of Midfrankonia, Ansbach, Germany (approval no.31-05/00).

General procedure

Initially, the three maxillary premolars were removed bilaterally. Following alveolectomy and wound closure, the bone was allowed to heal for 2 months. Then, all animals underwent a bilateral sinus floor elevation procedure and grafting randomly with autogenous

bone or Algipore® on one side as a control group and with the same grafting material plus PRP on another side as a test group. Four groups were formed as followed:

Group A : Autogenous bone;
Group B : Autogenous bone with PRP;
Group C : Algipore®; and
Group D : Algipore® with PRP.

In all augmentation areas, three dental implants (Ankylos®, Dentsply Co., Mannheim, Germany) with a length of 14 mm and a diameter of 3.5 mm were placed simultaneously.

Four animals were killed at 1, 2, 8 or 12 months after augmentation and implant installation; thus, two specimens with six implants were obtained from each group in each period. At 6 months, the implants of the animals in the 8- and 12-month groups were uncovered and loaded by the insertion of healing abutments penetrating the oral mucosa.

Extraction procedure

The animals were anaesthetized by an intra-venous injection of Immobilion® (Pherrovet, Malmö, Sweden). Three maxillary premolar teeth were divided in order to minimize the bone trauma and then removed bilaterally. Following the alveolectomy, the flaps were repositioned and sutured with resorbable sutures (Vicryl® 4.0, Ethicon Co., Norderstedt, Germany). At the end of the procedure, the anaesthesia was terminated by injection of Revivon® (Pherrovet).

Fabrication of platelet-rich plasma (PRP)

Two hundred and fifty millilitre of blood was drawn from the jugular vein from each animal to produce PRP in accordance with the manufacturer's recommendations using the two-tube technique (Curasan AG, Kleinostheim, Germany) during the augmentation procedure. This technique is suitable and easy to perform in clinical use, and a thrombocyte concentration 4.1 times higher than the native whole blood could be achieved (Wiltfang et al. 2004). 5 ml PRP was available for each augmentation site for the grafts in groups B and D.

Sinus augmentation and dental implant installation

All surgical procedures were performed under intra-venous anaesthesia with Immobilion® (Pherrovet) and Ketamin HCL (Ketavet®, Ratiopharm, Ulm, Germany). Standard monitoring was performed during the entire surgical course. The general anaesthesia was supplemented by local administration of 4% Articain® containing Epinephrine (1:100,000) (Ultracain-DS forte, Hoechst GmbH, Frankfurt am Main, Germany) in both maxillary sites and the forehead region for those animals that were selected for the augmentation with autogenous bone grafts with or without PRP.

The bone was harvested from the forehead region following a coronal approach and elevation of the periosteum. Three pieces of bone were obtained using a trephine bur with a diameter of 10 mm and a depth of 10 mm. The flaps were repositioned and sutured in layers with Vicryl® 0 and 3.0 (Ethicon Co.). The bone graft was ground into particles with a bone mill (Quentin® bone mill, Quentin Dental products, Leimen, Germany) and then stored in physiologic saline solution.

The floor elevation procedure was performed identically on both sides of each animal. From an intra-oral incision in the area of the previously extracted teeth and a mucoperiosteum elevation, the facial antral wall was exposed. A standardized bone window (35 mm long and 10 mm high) was created with a bur. The sinus membrane was gently elevated from the basal bone with bent dissectors (Frios® sinus Set, Friatec, Friedrichsfeld, Germany). In case of membrane perforation ($n = 2$), a bovine collagen sponge (Lyostypt®, Braun Co., Braun-Melsungen, Germany) was applied to cover the perforation. The prepared PRP was then mixed well with the graft materials in groups B and D. The created cavity was carefully packed with randomly selected materials. Three dental implants (Ankylos®) with a length of 14 mm and a diameter of 3.5 mm were placed before the cavity was completely filled with grafting materials (Fig. 1a and b). Primary stability of all implants was achieved in the residual bone. Consequently, the flap was readapted and sutured with Vicryl® 4.0 (Ethicon Co.). The anaesthesia was terminated by injection of Revivon® (Pherrovet). All animals were given 0.5 g/kg/day of Streptomycin (Grünenthal GmbH, Stolberg, Germany) and an analgesic treatment with Temgesic® for the first three days postoperatively. They were kept on a liquid diet during the first week after the surgical procedures.

Healing abutment insertion

After 6 months, the animals were generally anaesthetized in the same manner as described above in conjunction with local anaesthesia with 4% Articain® containing epinephrine (1:1,00,000) (Ultracain-DS forte, Hoechst GmbH, Frankfurt am Main, Germany) in the surgical fields. A mid-alveolar incision was performed to expose all previously placed implants. The cover screws were then removed and healing abutments were inserted. Antibiotic and analgesic treatments were administrated as described above.

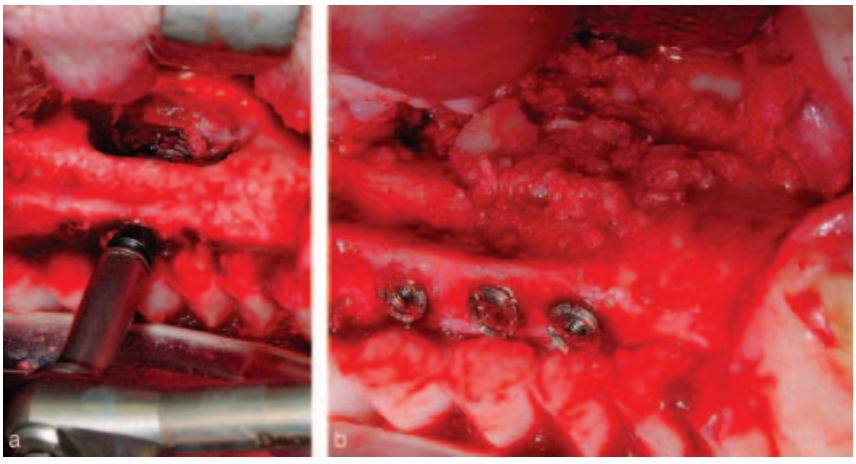

Fig. 1. (a, b) Three implants are installed after preparation of a standardized bone window (35 mm long and 10 mm high) and sinus floor elevation. Bone graft is then completely packed in the created cavity.

Sacrification procedure

Four animals were killed at each of the four observation periods, i.e. at 1, 2, 8 and 12 months. Sedation was carried out by an intra-muscular injection with 1 mg/kg of Azaperone and Midazolam. Then, the animals were euthanized by an intra-vascular injection of 20% Pentobarbital solution into the ear vein.

Specimen preparation

The relevant parts of the maxillas were retrieved and fixed in 1.4% paraformadehyde solution at 4 degrees Celsius. They were separated into several implant-bearing blocks by saw cutting parallel to each implant. The blocks were later dehydrated in ascending grades of alcohol at room temperature in a dehydration unit (Shandon Citadel 1000®, Shandon GmbH, Frankfurt am Main, Germany). Xylol was used as an intermediary fixation. Then, they were embedded in a resin; Technovit 9100® (Heraeus Kulzer, Kulzer Division, Werheim, Germany). The blocks were divided bucco-lingually along the long axis of each implant; thus, six implant-bearing specimens were obtained from each sinus for further microradiographic and light-microscopic studies. Subsequently, the specimens were cut and ground with the Exact Cutting and Grinding Equipment (Exact Apparatebau, Norderstedt, Germany) to a thickness of 180 μm, according to the technique described by Donath & Breuner (1982). For histological study, the specimens were further ground to a thickness of 30 μm and dyed with toluidin blue.

Microradiography

For microradiographic analysis (Freitag et al. 1980), 12 implant-bearing specimens from each group in each observation period were X-rayed in a Faxitron® cabinet (Faxitron, Rohde and Schwarz GmbH and CoKG, Köln, Germany) using a tube voltage of 11 kV and 0.25 mA for 6 h. The developed films (Agfa ZF, AGFA, Köln, Germany) were scanned with an AGFA A scanner at 1200 dpi and 12-bit grey scale and stored in jpeg format. The bone implant contact was evaluated by Osiris image analysis software (Digital Imaging unit, Center for Medical Informatics, University of Geneva, Switzerland).

Light microscopy

After microradiographic evaluation, all specimens were further ground to a thickness of 30 μm and dyed with toluidin blue. A percentage of newly formed bone from all grafts (Group A–D) and a percentage of remaining bone substitute from grafting with Algipore® and Algipore® plus PRP (Groups C and D) were evaluated by Bioquant (Image Analysis Corporation, Nashville, TN, USA).

Statistical analysis

All parameters were evaluated by two test persons. As the sample size in our study was small, we compared the control and study groups in each observation period by means of a randomized block design. The difference between the means of BIC, newly formed bone and the remaining bone substitute from PRP and non-PRP groups was calculated by a pair-difference *t*-test using SPSS 11.0 software. A *p*-value <0.05 was considered to be significant.

Results

Clinical observation after surgical procedures showed uneventful healing patterns in all animals. The peri-implant tissues around the loaded implants appeared clinically healthy. The heights of the augmented area were closely comparable in all groups (7.2 ± 1.23, 7.4 ± 0.97, 7.7 ± 1.02 and 7.1 ± 1.14 mm in groups A, B, C and D, respectively).

Microradiographic findings

The BIC of all grafting materials increased during the observation time (Table 1). Adding PRP resulted in lower mean values than grafts with autogenous bone or Algipore® solely through the whole observation period, *but a statistically significant difference was not found*. At 1 month, the graft with autogenous bone plus PRP produced a slightly higher BIC than that with autogenous bone alone ($28.4 \pm 4.64\%$ and $25.1 \pm 9.96\%$, respectively), but no statistically significant difference. At 1, 2 and 8 months, in both the PRP and non-PRP groups, grafting with autogenous bone led to higher BIC levels than Algipore®. At 12 months, the BIC of both materials were closely comparable (autogenous bone with and without PRP $55.1 \pm 13.10\%$ rsp. $52.5 \pm 17.06\%$; Algipore® with and without PRP were $55.9 \pm 11.30\%$ rsp. $52.7 \pm 12.30\%$) (Table 1, Figs 8–10).

Light microscopic findings (Tables 2 and 3)

One month

Group A: Remnants of the transplanted bone could still be identified. Most of these were engaged in woven bone. The percentage of newly formed bone amounted to $21.5 \pm 2.61\%$.

Group B: Adding PRP, islets of resorption could be located and more

Table 1. Percentage of the bone-implant-contact of the sinus augmentation using autogenous bone and Algipore® with or without PRP

Observation period (months)	Grafting materials	Bone-implant-contact (%)	*p*-value
1	Autogenous bone	25.1 ± 9.96	0.156
	Autogenous bone+PRP	28.4 ± 4.64	
	Algipore®	20.1 ± 7.25	0.657
	Algipore®+PRP	19.33 ± 5.03	
2	Autogenous bone	40.1 ± 5.25	0.306
	Autogenous bone+PRP	34.1 ± 8.92	
	Algipore®	28.6 ± 8.91	0.208
	Algipore®+PRP	24.4 ± 6.07	
8	Autogenous bone	51.7 ± 9.96	0.051
	Autogenous bone+PRP	47.7 ± 6.71	
	Algipore®	41.0 ± 15.27	0.468
	Algipore®+PRP	37.1 ± 9.52	
12	Autogenous bone	55.1 ± 13.10	0.665
	Autogenous bone+PRP	52.5 ± 17.06	
	Algipore®	55.9 ± 11.30	0.386
	Algipore®+PRP	52.7 ± 12.30	

PRP, platelet rich plasma.

Table 2. Percentage of the newly formed bone of the sinus augmentation using autogenous bone and Algipore® with or without PRP

Observation period (months)	Grafting materials	Percentage of newly formed bone	*p*-value
1	Autogenous bone	21.5 ± 2.61	0.367
	Autogenous bone+PRP	21.2 ± 2.46	
	Algipore®	18.3 ± 2.75	0.465
	Algipore®+PRP	18.1 ± 2.79	
2	Autogenous bone	38.5 ± 2.94	0.173
	Autogenous bone+PRP	36.8 ± 3.44	
	Algipore®	22.5 ± 3.93	0.101
	Algipore®+PRP	22.0 ± 3.79	
8	Autogenous bone	50.1 ± 4.46	0.112
	Autogenous bone+PRP	48.5 ± 4.80	
	Algipore®	30.5 ± 2.99	0.189
	Algipore®+PRP	30.1 ± 2.91	
12	Autogenous bone	56.0 ± 5.19	0.064
	Autogenous bone+PRP	55.2 ± 5.83	
	Algipore®	36.2 ± 4.01	0.200
	Algipore®+PRP	35.3 ± 3.83	

PRP, platelet rich plasma.

Table 3. Percentage of the remaining bone substitute of the sinus augmentation using autogenous bone and Algipore®

Observation period (months)	Grafting materials	Percentage of remaining bone substitute	*p*-value
1	Algipore®	62.0 ± 3.47	0.361
	Algipore®+PRP	61.3 ± 3.94	
2	Algipore®	59.7 ± 3.79	0.147
	Algipore®+PRP	60.2 ± 3.60	
8	Algipore®	45.1 ± 5.20	0.752
	Algipore®+PRP	45.0 ± 4.78	
12	Algipore®	41.4 ± 7.27	0.051
	Algipore®+PRP	40.7 ± 7.13	

PRP, platelet rich plasma.

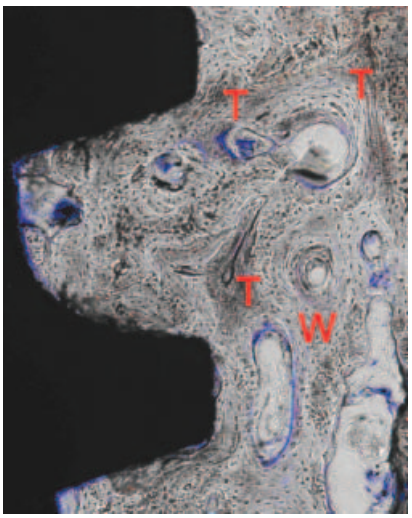

Fig. 2. Histologic specimen of autogenous bone graft with platelet rich plasma (PRP) at 1 month (× 10). Remnants of the transplanted bone (T) can be identified. The newly formed bone is mostly woven bone (W) with a large quantity of osteogenic connective tissue and immature bone trabeculae.

mature bone formations with a large quantity of osteogenic connective tissue as well as a few immature bone trabeculae were observed. The percentage of newly formed bone was 21.2 ± 2.46% (Fig. 2).

Group C: The majority of the augmented regions was filled with Algipore® particles (62.0 ± 3.47%). Some of them showed superficial resorptive signs. The area of newly formed bone was 18.3 ± 2.75%. De novo bone formation with spongiosa structures in between the particles besides capillaries and collagen could be seen.

Group D: Algipore® with PRP showed findings similar to those in group C, with a percentage of newly formed bone of 18.1 ± 2.79%, and the remaining particles amounted to 61.3 ± 3.94%.

Two months

Group A: The autogenous bone group showed remodelling in 2/3 of the augmented region. Harversian canals and osteoclasts were seen in newly formed bone. A mean value of newly formed bone of 38.5 ± 2.94% was determined.

Group B: Addition of PRP led to more immature woven bone with broad margins of osteoblasts. The percentage of newly formed bone amounted to 36.8 ± 3.44%.

Group C: Algipore® without PRP did not show significant differences from the 1-month group, only a higher amount of collagen fibres. In the central portions, some fibrous encapsulated particles could be seen. The remaining particles had a mean value of 59.7 ± 3.79% and the resorptive signs were not obviously different compared with the 1-month findings. A mean value of 22.5 ± 3.93% for newly formed bone was found.

Group D: In the PRP group, the findings were similar to those of group C. The remaining Algipore® particles *versus* the newly formed bone were 60.2 ± 3.60% rsp. 22.0 ± 3.79%.

Eight months

Group A: De novo bone formation presented mostly a lamellar structure with a mean percentage of 50.1 ± 4.46%. The distinction between the augmented area and the residual ridge was difficult to determine.

Group B: Autogenous bone graft with PRP exhibited findings similar to those of group A. The mean value of newly formed bone was 48.5 ± 4.80%.

Group C: Algipore® particles were mostly osseointegrated with surrounding bony tissue. The majority of the newly formed bone had lamellar structures. Small amounts of woven bone were also detectable. The remaining Algipore® was found at a level of 45.1 ± 5.20%. Various degrees of particle resorption with morphologic changes could be commonly observed. The percentage of newly formed bone amounted to 30.5 ± 2.99% (Fig. 3).

Group D: Addition of PRP led to similar findings. The remaining Algipore® particles and the newly formed bone were 45.0 ± 4.78%, rsp. 30.1 ± 3.91% (Fig. 7a and b).

Twelve months

Group A: At final observation, the newly formed bone achieved a mean value of 56.0 ± 5.19%. In contact with implant surfaces, compact cortical bone with extensive trabeculae formation was observed (Fig. 4).

Group B: Addition of PRP led findings similar to those in group A, with a percentage of newly formed bone of 55.2 ± 5.83% (Fig. 5).

Group C: The findings in the Algipore® group did not show remarkable changes compared with the 8-month group, except for more resorption signs of the particles. Ingrowth of newly formed bone or marrow stroma was somewhat visible in the degraded pores of the resorbed particles. The percentage of remaining bone substitute and the newly formed bone was 41.4 ± 7.27%, rsp. 56.0 ± 5.19% (Fig. 6).

Group D: Algipore® plus PRP led to similar findings as in grafts with Algipore® solely. The mean value of the remaining bone substitute was 40.7 ± 7.13%.

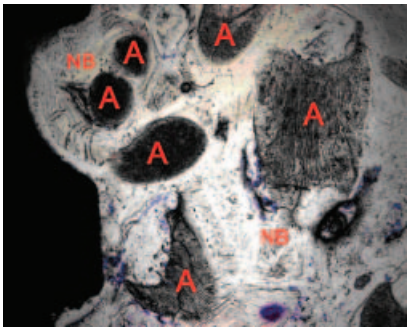

Fig. 3. Histologic specimen of augmentation using Algipore® at 8 months (× 10). *Various degrees of Algipore® (A) particle resorption with morphologic changes can be commonly observed*. Apposition of newly formed bone to resorbed particles can be seen. The majority of newly formed bone *(NB)* had a lamellar structure.

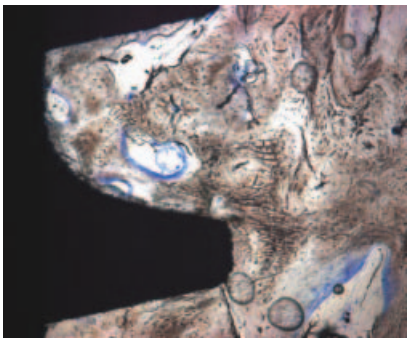

Fig. 4. Histologic specimen of an autogenous bone graft at 12 months (× 5). The majority of newly formed bone had a lamellar structure. No differentiation between graft and local bone is detectable.

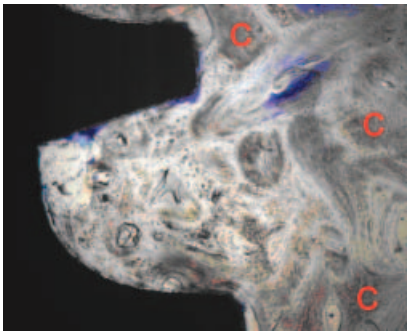

Fig. 5. Histologic specimen of an autogenous bone graft with platelet rich plasma (PRP) at 12 months (× 10). *Compact cortical bone (C) can be commonly observed.*

Discussion

In the present study, the effects of PRP on autogenous bone graft and a bone substitute, Algipore®, in terms of percentages of the bone-implant-contact, newly formed bone and the remaining bone substitute after sinus floor augmentation and simultaneous implant placement were evaluated. The microradiographic findings indicated that the BIC of both materials increased throughout the observation time in both PRP and non-PRP groups. At 12 months, the BIC of the autogenous bone and Algipore® were closely comparable (autogenous bone without PRP = 55.1 ± 13.10%, with PRP = 52.5 ± 17.06%; Algipore® without PRP = 55.9 ± 11.30%, with PRP = 52.7 ± 12.30%).

Platelets are a natural source of several growth factors. Platelet-derived growth factor (PDGF) is a polypeptide, that comprises a dimeric structured form. PDGF stimulates angiogenesis and differentiation of undifferentiated mesenchymal cells. Chemotaxis of osteoblast precursors can be enhanced by this growth factor. PDGF also activates macrophages, which play an important role in the secondary wound-healing phase (Pierce et al. 1989, Hock & Canalis 1994, Marx et al. 1998, Anitua 1999, Weibrich et al. 2003). Application of PDGF enhanced soft tissue healing in an experimental wound model (Pierce et al. 1989) and osteogenic differentiation and bone repair in osteotomy models (Nash et al. 1994).

TGF-β plays a role in bone formation by enhancing chemotaxis and the rate of stem cell proliferation. Another suggested effect is the inhibition of osteoclast formation (Anitua 1999, Weibrich et al. 2003).

With respect to the biological effects of PRP on bone regeneration, the microradiographic and light-microscopic results from this experiment could not support previous studies, which showed beneficial effects of PRP (Whitman et al. 1997, Marx et al. 1998, Anitua 1999, Kassolis et al. 2000, Fennis et al. 2004, Oyama et al. 2004). One of the probable explanations lies in the method of PRP preparation. Different techniques of preparation have been known to lead to substantially different amounts of cells, i.e. platelets and leucocytes, as well as different levels of growth factors (Zimmermann et al. 2001, 2003, Weibrich et al. 2002, 2003, Schlegel et al. 2004, Wiltfang et al. 2004). The two-tube technique of PRP preparation from Curasan used in our experiment was found to lead to a higher leucocyte count and PDGF in comparison with the preparations using the PCCS kit and the discontinuous cell separation method from a blood bank, whereas the two later techniques could lead to a higher thrombocyte count and TGF-β (Zimmermann et al. 2001, Weibrich et al. 2003, 2002). In an in-vitro study, TGF-β showed more potent than equimolar concentrations of PDGF or IGF-I in stimulating bone formation (Pfeilschifter et al. 1990). Schlegel et al. (2004) compared the beneficial effects of PRP on bone regeneration by means of grafting with autogenous bone or a bovine collagen (Colloss™) in critical-sized defects. The authors found a higher effect of PRP from the PCCS kit

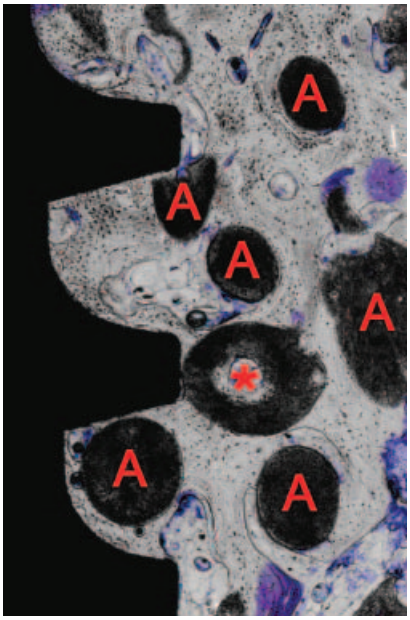

Fig. 6. Histologic specimen of augmentation using Algipore® at 12 months (× 10). Various degrees of particle *(A)* resorption with morphologic changes can be commonly found. Ingrowth of newly formed bone or marrow stroma is also seen in the degraded pores of some resorbed particles (*).

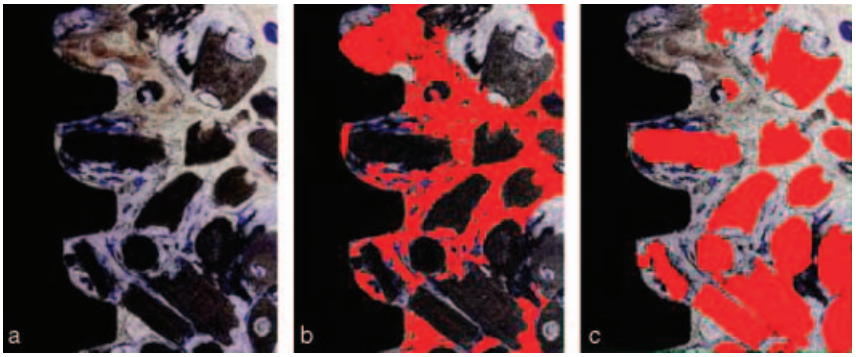

Fig. 7. (a) Histologic specimen of augmentation using Algipore® with platelet rich plasma (PRP) at 8 months (× 10). (b) Newly formed bone in the same area as in Fig. 7a is analysed and labelled with red colour using Bioquant software program (Image Analysis Corporation, Nashville, TN, USA) to evaluate the percentage of the newly formed bone. (c) Algipore® particles in the same area are analysed in the same manner to evaluate the percentage of the remaining bone substitute.

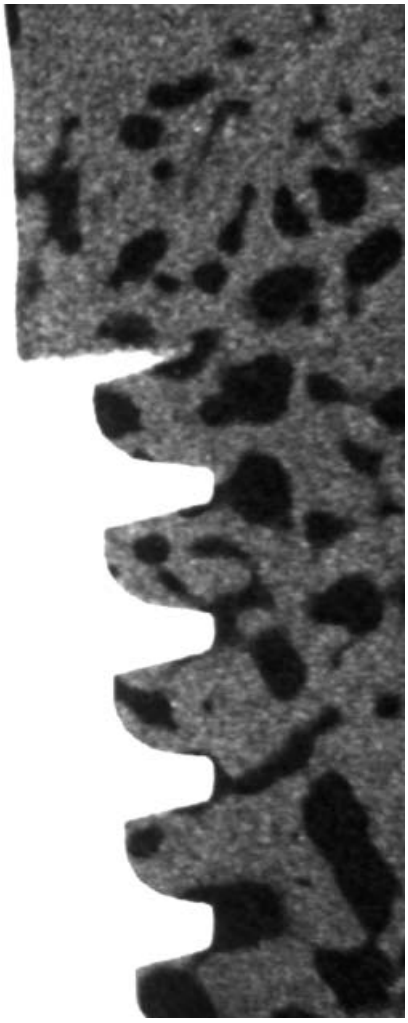

Fig. 8. Microradiographic specimen of an autogenous bone graft at 12 months (× 1.25).

on de novo bone formation at 2 weeks compared with the two-tube technique. Weibrich et al. (2004) studied the

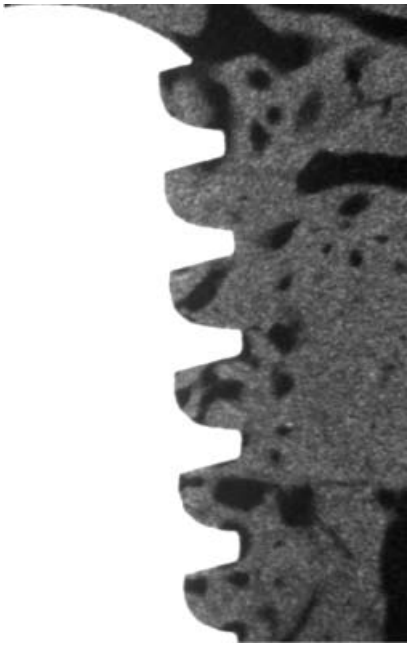

Fig. 9. Microradiographic specimen of an autogenous bone graft with PRP at 12 months (× 1.25).

effects of PRP in different concentrations on peri-implant bone regeneration and showed significant accelerated results only in PRP with an intermediate concentration of platelets (2–6 fold from native donor blood), whereas in lower (0.5–1.5 folds) or higher (9–11 folds) concentrations, a beneficial effect could not be demonstrated. They also suggested PRP with a platelet count of approximately 1,000,000/μl to be the optimal concentration. However, an experiment by Aghaloo et al.(2002) could not show significant differences in rabbit cranial defect model using autogenous bone graft with or without

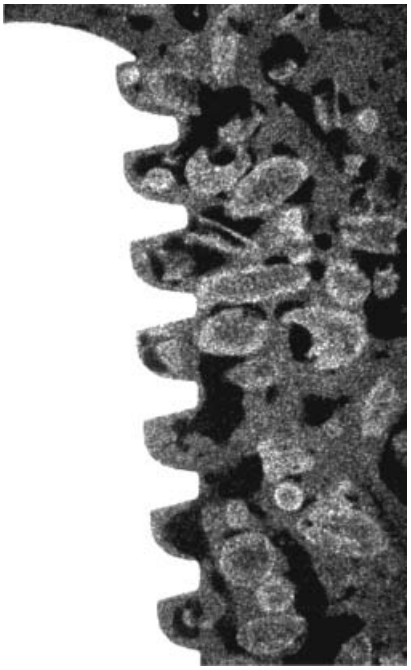

Fig. 10. Microradiographic specimen of augmentation using Algipore® at 12 months (× 1.25).

PRP with a mean platelet concentration of 1,050,000/μl. In critical-sized defects in a canine mandible model, Choi et al. (2004) even revealed significantly lower levels of bone regeneration and a delay in the remodelling of grafts on adding PRP with a mean platelet concentration of 1 112 000/μl to autogenous bone. Moreover, precise predictions of growth factor levels based on the platelet count of whole blood or PRP are limited Jakse et al. 2003, Weibrich et al. 2003, 2002). Also, bleeding in the surgical field could more or less dilute the concentration of PRP (Jensen et al. 2004).

The other possibility, which seemly directs to the point, but has been infrequently discussed in previously published articles, is an inhibitory effect of PRP and its growth factors in some concentrations. Certain studies revealed the dose-dependent effect of PRP and its growth factors (Nash et al. 1994, Schlegel et al. 2003, 2004, Wiltfang et al. 2004). In an in-vitro study (Arpornmaeklong et al. 2004), PRP showed a dose-dependent stimulation of proliferation of marrow-derived bone-forming cells but inhibited osteogenic differentiation, leading to a lower mineralization in cell culture compared with platelet-poor plasma. Gruber et al. (2004) found that platelet-released supernatants could accelerate migration and proliferation of marrow-derived mesenchymal cells, but

decreased osteogenic differentiation in dose-dependent manner. The supernatant corresponding to platelet numbers of 2×10^8/ml significantly decreased alkaline phosphatase activity, whereas the supernatants at lower concentrations (4×10^7 and 8×10^6/ml) did not lead to significant differences. In a mandibular defect model in minipigs, Fuerst et al. (2004) showed a significantly lower amount of newly formed bone in defects filled with collagen type I plus platelet-released growth factors, compared with that with collagen type I solely. In addition, PDGF, in some concentrations, could also inhibit bone regeneration (Marden et al. 1993, Hock & Canalis 1994, Gruber et al. 2004). Hock & Canalis (1994) demonstrated a significant inhibition of bone matrix formation of foetal rat calvarial cultures by exposure for 24 h of PDGF-AA at 0.3–3.0 nM and PDGF-BB at 0.03–3.0 nM. Accordingly, it should be noted that PDGF is the major growth factor in PRP used in our study (Zimmermann et al. 2001, Weibrich et al. 2003, 2002, Schlegel et al. 2004), so the inhibitory potential of this growth factor might have to be taken into account.

The next considerable factor could be time. It is known that after a few days, the effects of platelets fade (Pierce et al. 1989, Marx et al. 1998). Zechner et al. (2003) showed that application of PRP in implant beds before implant placement could lead to more bone-implant-contact in the early healing phase (6 weeks), but at 12 weeks the results were comparable. These findings correlate to those of (Schlegel et al. 2003), in which a significant effect of PRP on bone-implant-contact was only exhibited in the initial healing phase (2 weeks). Wiltfang et al. (2004) demonstrated a significant enhancement of reossification following autogenous bone graft with PRP by the PCCS kit in a critical-sized defect model only in the 2-week period, but the benefit of PRP beyond 2 weeks could not be found. However, the authors could not determine the positive effects of PRP on tricalcium-phosphate (Cerasorb™), bovine spongious blocks (BioOss™) and bovine collagenous sponge (Colloss™) in terms of de novo bone formation and ceramic degradation, which correlated to the findings in our study.

One of the aspects discussed is the need to add autogenous bone in bone substitutes when PRP is applied. As in the study by Roldan et al., where 15%-volume of autogenous bone chips was added to 3 ml anorganic bovine bone (BioOss®) to supply osteolasts in order to support the effect of PRP on sinus floor augmentation in minipigs, they found a higher percentage of newly mineralized bone from this grafting combination compared with anorganic bovine bone plus rhBMP-7, but no statistical significance. The grafts with PRP application in their study showed a significantly lower BIC and height of the newly formed bone than those in the rhBMP-7 group. Moreover, in spite of adding autogenous bone in the PRP group, vital bone could only be seen in the proximity to the sinus walls. This may indicate that the target cells of PRP are mainly recruited from the surrounding tissue. In our study, augmentation with Algipore yields a favourable result and by 12 months, the BIC is also closely comparable to that in the autogenous bone group.

In summary, our study could not reveal significant accelerating effects of PRP on the BIC, de novo bone formation and degradation of bone substitute following sinus augmentation using autogenous bone and Algipore®. Moreover, appropriate conditions according to its use, namely the technique of PRP preparation, the optimal concentration and type of bone substitutes, must also be determined.

Acknowledgements

The authors gratefully acknowledge Dr. Patcharee Wongkasem PhD (Statistic), National Institute of Development Administration, Bangkok, Thailand, for advice on the statistical analysis.

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Clinical Relevance

To improve the outcome of bony regeneration, application of PRP is one of the well-known procedures and has been used for years. However, the beneficial effects associated with its use are still unclear with various results from to date articles. This study was carried out to evaluate the influence of PRP on sinus augmentation using autogenous bone or Algipore®. The *beneficial* effects of PRP on both materials could not be determined in terms of the bone-implant-contact, the newly formed bone and the degradation of bone substitute. The differences in the technique of PRP preparation, observation time as well as the inhibitory potential of PRP were discussed. Concerning the results from this study, routine clinical use of PRP in sinus augmentation using both materials could not be recommended.