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Alveolar Ridge Augmentation Using Various Bone Substitutes -A Web Form of Titanium Fibers Promotes Rapid Bone Development-

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Alveolar ridge augmentation with β-tricalcium phosphate (β-TCP) granules, calcium phosphate cement (CPC) powder and web form of titanium fibers (TW) added to Platelet-Rich-Plasma (PRP) was histologically observed for 5 months by the experiments using the maxilla of rabbits. As a result, TW was thus found to promote the rapid bone development within a period of less than 5 months, because TW had an appropriate gap between the titanium fibers for new bone progression. However, titanium fibers remained intact within the maxilla. The CPC powder was transformed into dense cement and also remained intact in the bone. These residual materials must be obstacles to perform the placement of dental implants.

On the other hand, β-TCP granulations were gradually replaced by the new bone, but this process took about 5 months to be completed.

Consequently, for an ideal alveolar bone substitute, there needs to be a suitable gap formation within the substitute, such as TW, and properties of total bone replacement, such as that seen in β-TCP granules.

Alveolar bone defects of varying sizes occur as a result of oral surgery for jawbone cysts, tumors and facial traumas, and it is one of the most difficult tasks to regenerate new bone in the extensive defective area. In such cases, autogenous bone transplantation is clinically considered to be the most effective method. However, a surgical invasion to the donor site and the quantitative limitations of the extracted bone may sometimes cause clinical problems.

If an autogenous bone transplant is too difficult to perform, other treatments such as distraction osteogenesis or fillings with various bone substitutes are thus generally performed. For bone regeneration, three conditions of proper scaffolds, efficient growth factors, and stem cells are needed (1). Bone substitutes are thought to be useful as proper scaffoldings. Lately, Platelet-Rich-Plasma (PRP) has been demonstrated to be a beneficial growth factor for bone regeneration (2, 3). Some bone marrow cells have been identified to have a potential similar to that of stem cells, thereby growing out from the surrounding bones via proper scaffoldings and thereafter differentiating into osteoblast cells (4). However, the method to precisely decide which bone substitutes is the most effective for the alveolar ridge

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augmentation for use in autogenous bone transplantation remains to be elucidated. Accordingly, in the present study, β-tricalcium phosphate (β-TCP) granules, calcium phosphate cement (CPC) powder and web form of titanium fibers (TW) were implanted into an artificial hole of the rabbit maxilla with PRP, and the findings were histologically assessed by light microscopy. Moreover, CPC powder mixed with PRP was examined to determine whether it could change into hydroxyapatite (HA) based on the findings of X-ray diffraction.

MATERIALS AND METHODS

1. Experimental animals

We used fifteen male New Zealand albino rabbits weighing 2.5 to 3 kg. We conducted all of the experiments according to the guidelines for animal experimentations established by Kobe University (Registration number: P-041213R).

2. Bone substitutes

The three materials described below were used for bone supplements. β-TCP granules (Osferion®), 500–1000 μm in diameter, were purchased from Olympus Biomaterial (Japan). One hundred mg weight of these granules were mixed with 160 μl of PRP just before filling. CPC powder (Biopex®) to consist of α-tricalcium phosphate (75%), tetracalcium phosphate (18%), calcium hydrogen phosphate (5%), and HA (2%) was obtained from Mitsubishi Pharmaceutical Corporation (Japan). Five hundred mg of CPC powder were mixed with 160 μl of PRP directly before the experiments. A web form of titanium fibers was obtained from Hi-Lex Corporation (Japan). The fibers, coated with HA, are 80 μm in diameter and 500 g/m^2 in density, which consisted of a web measuring 4mm diameter and 1.3mm in thickness whose gap rate was 89%. Three web were mixed with 160 μl of PRP just before implantation.

3. Platelet-rich-plasma (PRP)

Blood samples of 10 ml from the femoral vein of the rabbits added by EDTA-2K were centrifuged at 20°C for 15 minutes at 800 r.p.m （himac,CF7D/HITACHI/Japan), and the plasma components were further centrifuged for 15 minutes at 1300r.p.m. Thereafter, the lower stratum of 1 ml was used as PRP for this experiment. The platelet count level of this stratum was confirmed to be concentrated three- to ten-fold in comparison to the former blood.

4. Animal experiments and histological assessment

The rabbit was put in a fixator and then was given under intravenous anesthesia (injected via the auricle cutaneous vein) with pentobarbital (Nembutal®) (0.5 ml/kg). After giving local anesthesia to the upper gingival with 0.2 ml of 0.5% lidocaine (including a hundred thousand-fold epinephrine), the maxillary gum was dissected using approximately 1.5 cm incisions to expose the maxilla. The maxillary bone was scraped into a round shape (4mm in diameter) under rinsing water with a round bar of dental engine, and it was confirmed that the bone scraping did not intersect the nasal cavity. After stopping bleeding, each bone substitutes mixed with PRP as explained above was implanted into five rabbits (Fig.1a, b, and c), and then the gum was sutured with silk thread. Although no antibiotics were given after surgery, no infections were found. The rabbits, were all able to ingest solid food and water on the day after the surgery, and thereafter they were sacrificed by a megadose of pentobarbital on 1, 2 and 5 months after surgery.

The upper jaw bone was removed and was soaked in Karnovsky fixing fluid. After fixing for over a week, the jaw bone was then made into decalcified specimens and was observed by light microscopy after being stained with toluidine blue.

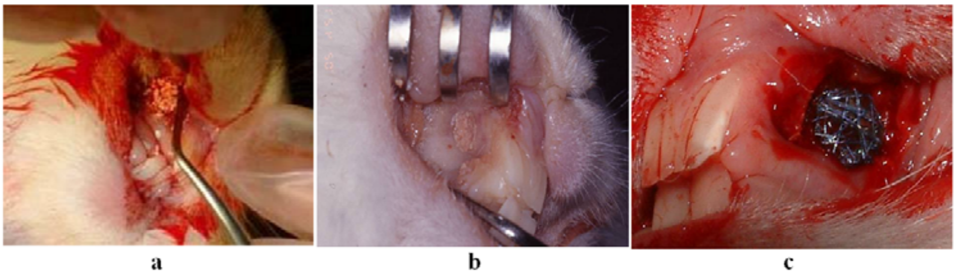

Fig.1 The site of the bone experiment.
Filled various bone supplemental material mixed with PRP to bone defect (maxilla of rabbits).
a; β-tricalcium phosphate (β-TCP) granules. **b**; calcium phosphate cement (CPC) powder.
c; TW implantation

5. X-ray Diffraction of CPC Powder mixed with PRP

Three lumps of CPC powder which hardened mixture with after being mixed with PRP were triturated with freeze-drying methods at 3, 7, 14, and 28 days after mixing, and then were analyzed by an X-ray diffractometer (RINT2400 model by Rigaku Corporation) with the measuring range was 2θ 20-40^{o} and the scanning speed was 4^{o}/min. The CPC powder alone and HA were also analyzed by X-ray diffraction as controls.

RESULTS

Histological assessment

β-TCP granules mixed with PRP

One month after filling, the β-TCP granules remained in various sizes. No new viable bone or soft tissue was thereafter found around the β-TCP granules (Fig.2a). Two months after filling, the absorption of β-TCP granules developed and some areas were replaced with new viable bone (Fig.2b). Five months after filling, the β-TCP granules were completely absorbed and replaced with the new viable bone in almost all of the treated areas (Fig.2c and 2d).

CPC powder mixed with PRP

On a month after filling, an aggregate of crystals of various sizes was observed (Fig.3a). No soft tissues could be found between the original bone and crystals of CPC, which meant to be in direct contact with each other. Two months later, regenerating bone was found to begin to develop into a gap of crystals (Fig.3b), however most of the crystallized substances still remained up until 5 months after surgery (Fig.3c and 3d).

TW with PRP added

One month after implantation, bone regeneration was found in a part of the space of TW (Fig.4a). Two months later a large amount of new bone was found to have developed into most of the space of TW (Fig.4b), and five months later new viable bone was observed throughout the entire space of TW (Fig.4c and 4d).

X-ray Diffraction of CPC Powder mixed with PRP (Fig.5)

By X-ray diffraction, the compound after three days from the mix of CPC powder with PRP showed almost the same waveforms of CPC powder alone, while also demonstrating the distinctive peaks of α-tricalcium phosphate (α-TCP) and slight peaks of HA. On the other hand, the α-TCP peaks became unclear as time passed and the HA peaks also gradually became higher. The HA peaks were clearer after 28 days than after 7 days. These findings were the same for all samples.

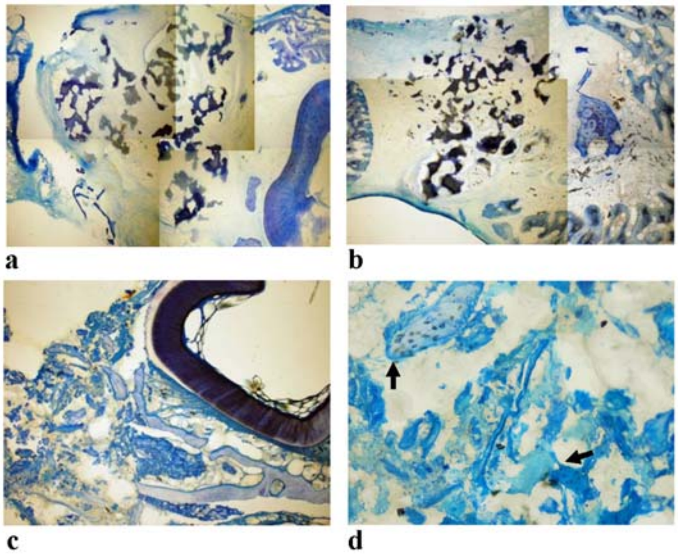

Fig.2 Light microscopical findings of the bone experiment with β-TCP (stained with toluidine blue). **a**; One month after filling(×40). The triturated substitutes remained as β-TCP granules of various sizes. No showing new viable bone or soft tissue in the space. **b**; Two months after filling (×40) Absorption of β-TCP granules had developed. Showing some areas which were replaced with new viable bone. **c and d**; Five months after filling(c:×40, d:×100) β-TCP granules were completely absorbed and almost the entire area had been replaced with new viable bone. Osseous tissue (arrows)

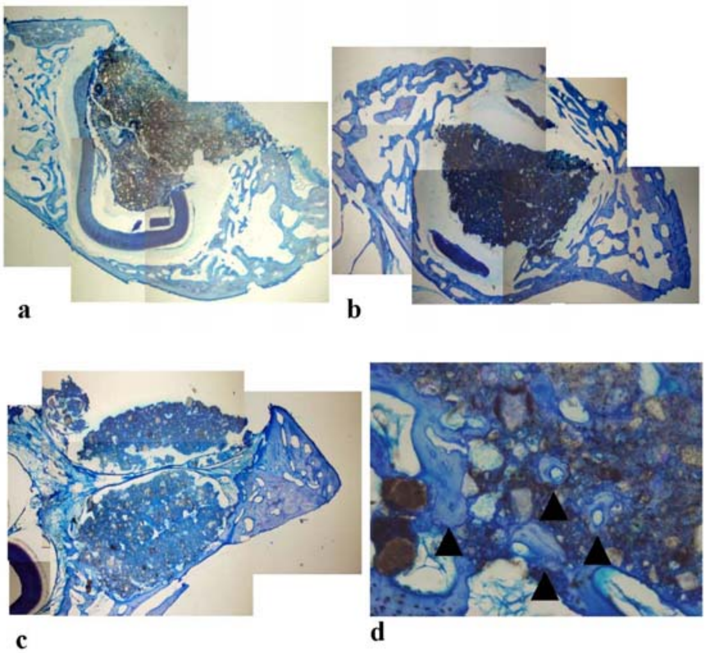

Fig.3 Light microscopical findings of the bone with CPC (stained with toluidine blue).
a; One month after filling (×40). The triturated substances were observed consisting of an aggregate of crystals in various sizes in the space around them. **b;** Two months after filling (×40). The regenerated bone begins to develop into a gap of crystals. **c and d;** Five months after filling (c:×40, d:×100). Showing new viable bone (arrowheads) in a gap of the crystal substances. Most of the crystal substances still remained.

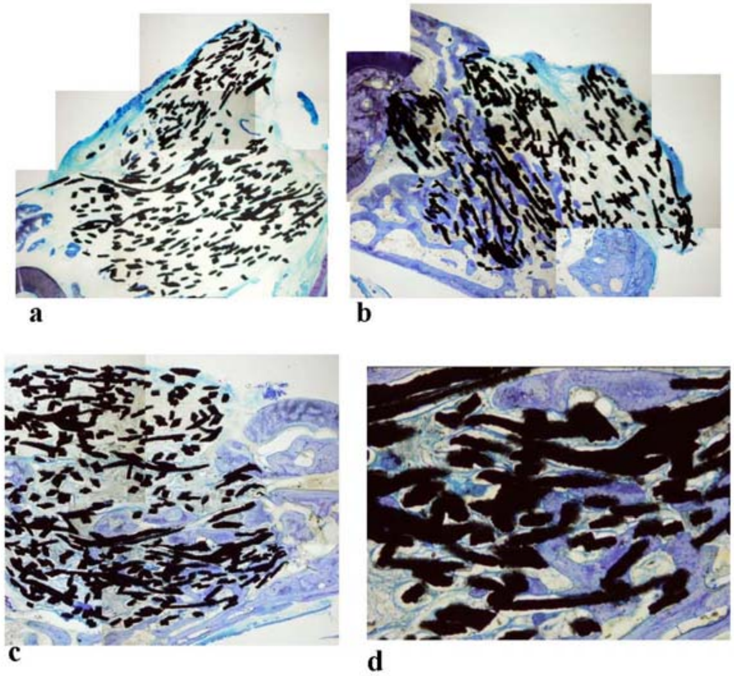

Fig.4 Light microscopical findings of the bone experiment with TW (stained with toluidine blue).
a; One month after filling (×40). Showing new viable bones in a part of the space of TW.
b; Two months after filling (×40). A large amount of bone appeared in the space of TW.
c and d; Five months after filling (c:×40, d:×100). Showing new viable bone in the entire space of TW.

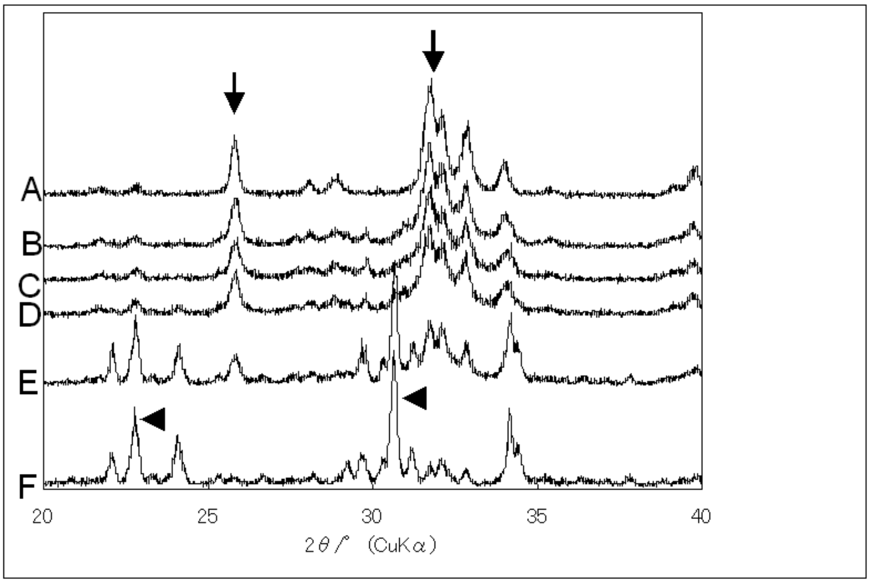

Fig.5 X-ray diffraction of CPC powder mixed with PRP.
A: Control (HA). **B**: On 28 days after mixing the CPC powder with PRP. **C**: On 14 days after mixing the CPC powder with PRP. **D**: On 7 days after mixing the CPC powder with PRP. **E**: On 3 days after mixing the CPC powder with PRP. **F**: CPC powder only. (arrows; HA peak, arrowheads; α-TCP peak)

DISCUSSION

In this experiment, we used β-TCP granules, CPC powder and TW as bone substitutes, and examined any differences in bone regeneration with each bone substitutes. As for the site of the bone experiment, we used the alveolus where bone augmentation is frequently clinically required prior to performing the placement of dental implants.

The PRP, used in this study as a growth factor, has been variously reported to be beneficial to bone regeneration (2,3,5,6). Platelets are cells derived from blood megakaryocytes of bone marrow and then they are divided into inactive cells and active cells. During the inactive periods, they demonstrate a disk appearance measuring 2-5μm in diameter, however they change their forms into a globular shape with one or several cytoplasmic processes and each platelet agglutinates during the active period. Moreover, after attaching to foreign substances, platelets release organ growth factors such as PDGF or TGF-β. PDGF, which is an organ growth factor released from the α-granules of platelets, and it is known to play a role in promoting wound healing and the proliferation of capillary vessels. TGF-β has also been reported to increase the number of osteoblast progenitor cells while also controlling the activation of osteoclasts (5,6,7,8). These growth factors are thus considered to contribute to bone regeneration.

The ideal of bone substitutes are as follows; they should have biocompatibility, excellent osteoconductive properties and appropriate strength, and they should be able to form a suitable shape easily and to ultimately replace the bone completely within a short period (1). Our results showed the availability of the three materials in the biocompatibility and the osteoconductive properties, and these conclusions are also supported by other reports (9,10,11). As for the period until the bone substitutes, we concluded that β-TCP took 5 months, while CPC required more than 5 months, to have all spaces measuring 4 mm in diameter rearranged by the new bones. The reason why CPC showed a delayed bone replacement was that there was no space for new viable bone to progress because CPC developed into densely aggregated crystals. In addition, the crystals of CPC were considered to dissolve with difficulty from the findings of X-ray diffraction; namely, at 7 days after mixing the CPC powder with PRP, the compound was replaced with HA for the most part, and the purity of HA increased gradually after that. In fact, it is known that when CPC powder is mixed with the original hardening solution instead of PRP, CPC also changes into HA for a week, and the HA crystals within the bone have been reported to remain for more than 18 months in the clinical case (9). Consequently, to our disappointment, there was nothing to be identified in the present study about the effectiveness of PRP with CPC powder, because this compound also showed a delay in the bone replacement.

Although TW was remaining intact in the maxilla, the new bone filled up the entire space among titanium fibers within less than 5 months, therefore TW promoted the most rapid bone development among the three materials. TW had a gap between each titanium fiber which averaged 200 - 400μm, which is considered to be appropriate for the new bone progression because the ideal space for the bone development is thought to be 300 - 400μm (12,13). On the other hand, according to the light microscopic findings, the spaces in β-TCP granules were 1.5 to 2.0 times larger than those of TW, which might be inappropriate for proper bone growth and thus might result in a delay in proper bone development.

Bone substitutes must be able to make a suitable shape for the bone defect. β-TCP is in a granular form and CPC hardens gradually into cement, therefore they can fill in the damaged areas in any form. On the other hand, because TW has a web form, it needs to be cut and bent to fit into the damaged area beforehand, and this procedure is therefore considered to be a disadvantage.

Consequently, it is necessary to exploit other new materials to reliably replace bones.

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