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Sinus floor augmentation with β-tricalciumphosphate (β-TCP): does platelet-rich plasma promote its osseous integration and degradation?

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Abstract: When dental implants are to be inserted, sinus floor augmentation is an effective treatment procedure to improve bone height in the posterior maxilla. In addition to autogenous bone material, allogenic materials, e.g. β-tricalciumphosphate (β-TCP), have been used successfully. The purpose of this study was to investigate whether the combination of β-TCP with platelet-rich plasma (PRP) enhances bony regeneration and resorption of the tricalciumphosphate material. In a randomized prospective trial, 45 sinus floor elevations were performed in 39 patients. In 22 sites, PRP was added to the β-TCP granules, while in 23 sites β-TCP without PRP was used. Six months later, bone specimens were harvested from the augmented region during the implant insertion procedure. The formation of new bone was about 8–10% higher when PRP was applied. A faster degradation of the ceramic bone substitute was not observed. In conclusion, when PRP was added to β-TCP, bone regeneration was supported to a small extent. However, the resorption of β-TCP was not accelerated and foreign-body giant cells and soft tissue surrounding the β-TCP granules were present.

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Sinus floor elevation in combination with dental implants is an accepted treatment procedure in the prosthetic restoration and esthetic rehabilitation of the severely atrophic maxilla (Erbe et al. 1996; Engelke & Deckwer 1997; Wiltfang et al. 2000). In less severe cases, if the remaining alveolar bone has a minimum height of 5 mm, sinus floor elevation and implant insertion can be performed in one session. In cases of severe atrophy of the maxillary alveolar process, sinus floor elevation and implant insertion are usually performed in two stages (Boyne & James 1980; Wood & Moore 1988; Tatum et al. 1993). It is preferred to use autogenous bone from various donor sites for the augmentation procedure (Schenk et al. 2000; Nkenke et al. 2001). It takes approximately 4–6 months following augmentation for the transplanted bone to be integrated and substituted by osteoconduction (creeping substitution). Alternatively, autogenous bone transplants can be replaced by bone substitutes, e.g. tricalciumphosphate ceramics, to avoid donor site morbidity (Leonardis & Pecora 1999; Piattelli et al. 1999). The degradation of these materials may take up to 12 months if used for sinus floor augmentation.

It would be beneficial for the patient to reduce this time interval by accelerating the process of integration of the transplanted bone or the bone substitute.

The application of osteoinductive substances is a promising option as they are constituents of platelet-rich plasma (PRP). Osteoinductive cytokines such as the transforming growth factors TGF-β_1 and TGF-β_2, the platelet-derived growth factor (PDGF) and the insulin-like growth factor

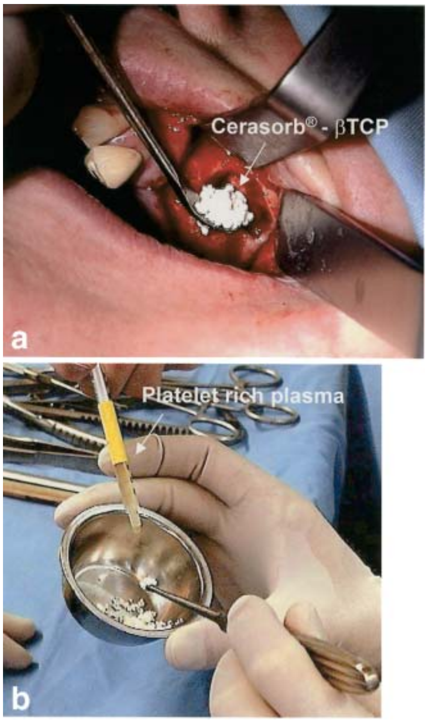

Fig. 1. (a) Access to the sinus by the lateral window technique and sinus augmentation with 1–1.5 g β-TCP ceramic granules of 1000–2000 μm diameter. (b) Careful mixing of 1 ml of PRP with the β-TCP granules.

Fig. 2. Harvesting of the bone biopsies 6 months after sinus floor augmentation with a trephine bur of 3 mm diameter. The cranial 4 mm of the biopsies was used for histological examination.

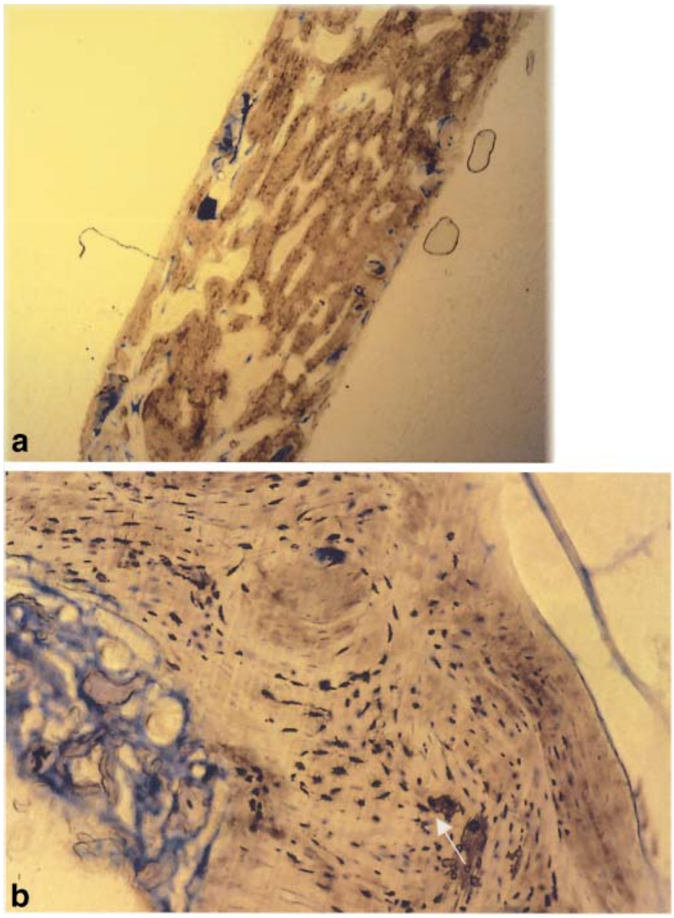

Fig. 3. With PRP, light microscopy revealed that the remaining TCP ceramic bone substitute was surrounded by newly formed bone trabeculae. Direct bone-to-ceramic substitute contact could be observed (toluidine/alkaline fuchsine staining; a, magnification ×2.8; b, magnification ×7).

IGF-I could be used for a faster integration and substitution of autogenous bone transplants and bone substitutes (Canalis 1981; Cho et al. 1995; Giannobile 1996; Marx et al. 1998; Anitua 1999; Lynch et al. 1999).

The aim of this clinical investigation was to determine whether the application of PRP in combination with tricalciumphophate ceramics could accelerate the degradation and bony substitution of this allogenic material in the case of sinus floor elevation (Merten et al. 2000).

Material and methods

Patients and methods

In the year 2000, 35 sinus floor elevations were performed with β-tricalciumphosphate (β-TCP) in healthy subjects. The inclusion criteria were a blood concentration of thrombocytes within the normal range and an absence of a history of maxillary sinus inflammations.

Preoperatively, the residual bone heights were assessed from panoramic radiographs. In the anterior maxillary sinus wall, a bone lid was created using the window technique. Perforation of the maxillary sinus mucosal lining resulted in exclusion from the study. After careful elevation of the mucosal layer, 1–1.5 g Cerasorb® (Curasan AG, Kleinostheim, Germany) containing β-TCP ceramic granules of 1000–2000 μm diameter were instilled for augmentation of the sinus floor. One milliliter of PRP was administered in addition to the ceramic material in 17 sites in a randomized prospective study approved by the ethics commission of the University of Erlangen-Nuremberg (application no. 2075). Care was taken to equally distribute the PRP over the β-TCP granules. In 18 sites no PRP was added (Figs 1a and b). The average age of the patients in the PRP group was 45 years (range 37–54 years; 13 women and four men). In the second group the average age was 47 years (range 32–64 years; 14 women and four men).

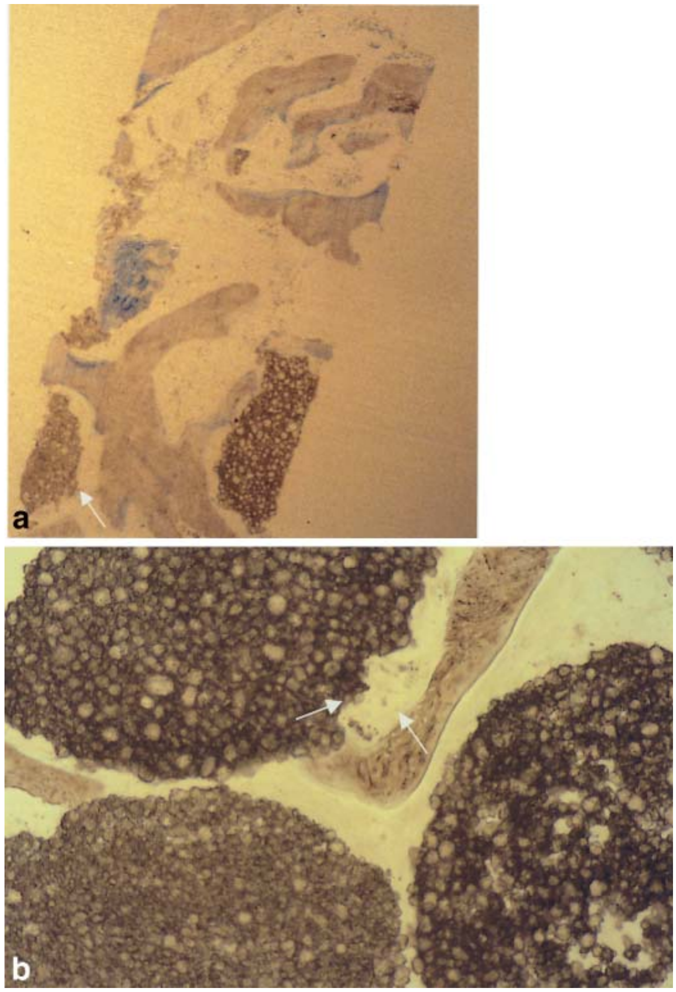

Fig. 4. Direct bone-to-ceramic substitute contact could be observed as well as embedding of the ceramic granules in the soft tissue layer (toluidine/alkaline fuchsine staining; a, magnification ×2.4; b, magnification ×6.8).

PRP preparation

Using the tube technique ad modum Curasan® (Curasan AG, Kleinostheim, Germany), the patient's blood plasma was prepared immediately prior to the operation in the Institute for Transfusion Medicine of the University of Erlangen-Nuremberg. The PRP was required to contain platelet concentrations at least triple the value for normal plasma. Patients were excluded when the concentration factor was below 3. The average platelet concentration was 4.1 times higher. The PRP was then delivered to the surgical theatre so that it could be applied within 2 h or less.

Harvesting of the specimens

Implant insertion was performed 6 months following sinus floor augmentation. During this procedure, a bone biopsy from the augmented site was harvested using a trephine bur of 3 mm diameter. To guarantee that the augmented region of interest was examined, only 4 mm of the cranial end of the bone cylinder was taken for histological examination (Fig. 2).

Histological examination

Undecalcified thin sections of the bone specimen were examined under the light microscope. The specimens were sectioned longitudinally in the axial plane. After fixation in 4% buffered formalin and dehydration through a series of decreasing concentrations of alcohol into de-ionized water, the test material was embedded in LR white hard-grade (Science Service, Munich, Germany) acrylic material. The samples were cut perpendicularly to the longitudinal axis. Utilizing the 'sawing and grinding' technique, the specimens were ground to 30–15 μm (Exakt Apparatebau, Norderstedt, Germany). After toluidine/alkaline fuchsine staining, the thin sections were examined under the light microscope. The purpose of the light-microscope examination was to measure and evaluate the bone regenerate as well as the relation between the ceramic substitute and bone. The images were assessed at magnifications of ×1.25 to ×100 and digitized (Duoscan T 2000 XL, Agfa, Mortsel, Belgium). NIH-Image software was used for image analysis (National Institutes of Health, Bethesda, MD). The bone area ratio was defined as the area of new bone inside an area of 9 mm^2/total area of 9 mm^2 (×100 to give a percentage).

Statistical analysis

Median values were obtained with the interquartile range. For presentation of the data, box-plots were used. To evaluate the influence of PRP on bony regeneration and degradation of the ceramics, the Wilcoxon test was used. A P-value $\leq$ 0.05% was considered statistically significant.

Results

Healing was uneventful in all patients. All operation sites showed primary wound healing so that the sutures could be removed at term. Abnormal swelling or signs of infection were absent.

The preoperative bone height ranged from 2 to 7 mm in the postoperative maxilla in the test and control groups. Directly after augmentation, the radioopacity varied in the augmented sinus floors. The cranial border of the implanted material was cloudy. Radiographs taken 6 months later immediately before implant insertion showed a reduction of the vertical height

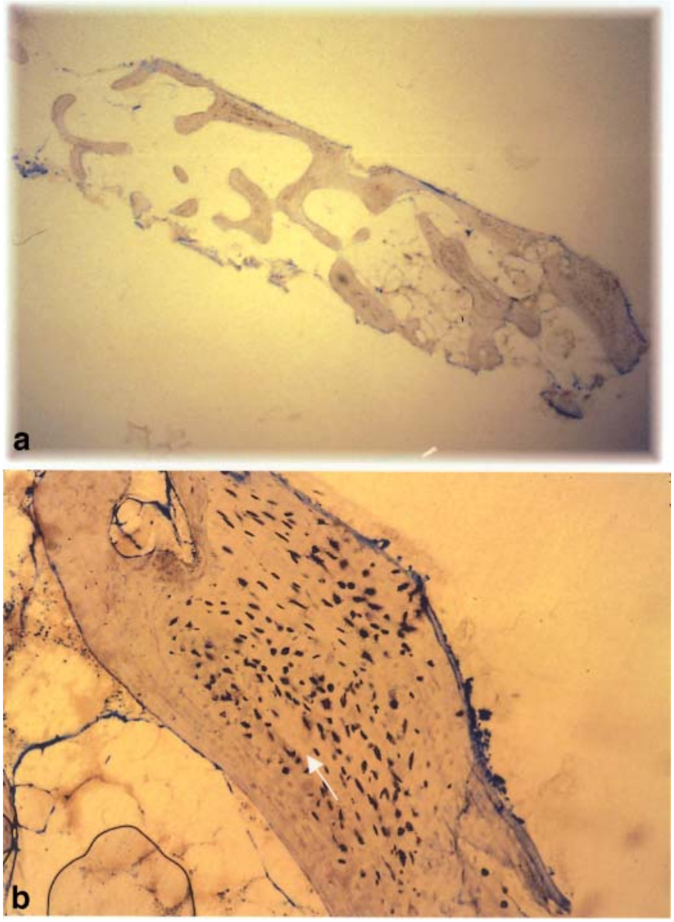

Fig. 5. Without the addition of PRP, TCP ceramic remnants were surrounded by newly formed bone trabeculae. Direct bone-to-ceramic substitute contact could be observed (toluidine/alkaline fuchsine staining; a, magnification ×2.72; b, magnification ×6.8).

by 10–20% in both groups. All implant sites allowed the installation of two to three rough-surfaced implants of different systems with diameters up to 4.8 mm in the posterior maxillae. The structure of the augmented material was now more homogenous. After implant insertion, all implants radiologically displayed full bony integration.

All osseous biopsies of test and control groups could be included in the study. The cranial 4 mm never included bone of the residual alveolar crest. Without PRP instillation osseous regeneration reached an average level of 29% compared to 38% in the PRP group. In the PRP group, bone formation was between 32 and 43%, whereas in the non-PRP group values between 25 and 37% were found. The wide range of scatter was striking in both groups.

Light microscopy revealed that the remaining TCP ceramic bone substitute was surrounded by newly formed bone trabeculae (Kassolis et al. 2000). Direct bone-to-ceramic substitute contact could be observed as well as an embedding of the ceramic granules in a soft tissue layer (Figs 3, 4, 5a and 5b). Foreign-body giant cells could be detected in these soft tissue layers that were more present in the bone specimens of patients that were treated with PRP (Figs 6a and b). A faster degradation of the ceramic material (β-TCP) could not be observed in the group in which PRP had been applied. The relation between β-TCP granules and newly formed bone was 13.8% in the group substituted with PRP compared to 15% in the patient group without PRP (Figs 7 and 8) (Donath 1985).

Discussion

Thrombocytic β-granules contain various growth-stimulating factors, e.g. the platelet-derived growth factor (PDGF) and the transforming growth factors TGF-β_1 and -β_2 (Harrison & Cramer 1993). These cytokines play an important role in osteogenetic and angiogenetic processes. Furthermore, PDGF is involved in almost all wound-healing reactions. Physiological wound healing is characteristically triple-phased and is supported by cellular and humoral factors. First, a catabolic latency period lasting approximately 1 week can be observed. This period is succeeded by a proliferation phase of about 2 weeks. After the proliferation phase a period of reparation and reorganization follows, which may last for several months. The proliferation phase is characterized by a high cellular and vascular density (Hosgood 1993). The intense cell metabolism provides the necessary matrix for cell differentiation, cell migration and the initial steps for tissue repair. Thrombocytic growth factors on the whole have their main effect during the phase of proliferation. They support cell mitosis, neoangiogenesis and the activation and immigration of macrophages. Cytokines of the TGF family in particular attract preosteoblastic cells and fibroblasts by chemotaxis and support their mitotic activity.

The combined application of PRP and bone transplants leads to a higher cellular activity in the bone-transforming cells, resulting in an accelerated integration of the transplanted bone. The aim of this investigation was to determine whether the combination of β-TCP with PRP results in accelerated bone repair based on faster ceramic degradation and transformation. In a randomized prospective study, we tried to find an answer to this question by clinically applying ceramic bone substitutes in combination with PRP during sinus floor augmentation. In the two-stage procedure, there was no problem in harvesting relevant biopsy specimens during the implant insertion 6 months after sinus floor augmentation.

Six months post-augmentation, our results showed a great intra- and interindividual range with respect to the amount of newly formed bone and the degree of ceramic degradation. With regard to the rate of bone regeneration, slightly increased new bone formation of about 8–10% could be observed when PRP was applied. A faster degradation of the ceramic bone substitute Cerasorb® was not found. A striking result in the PRP group was the large number of foreign-body giant cells that

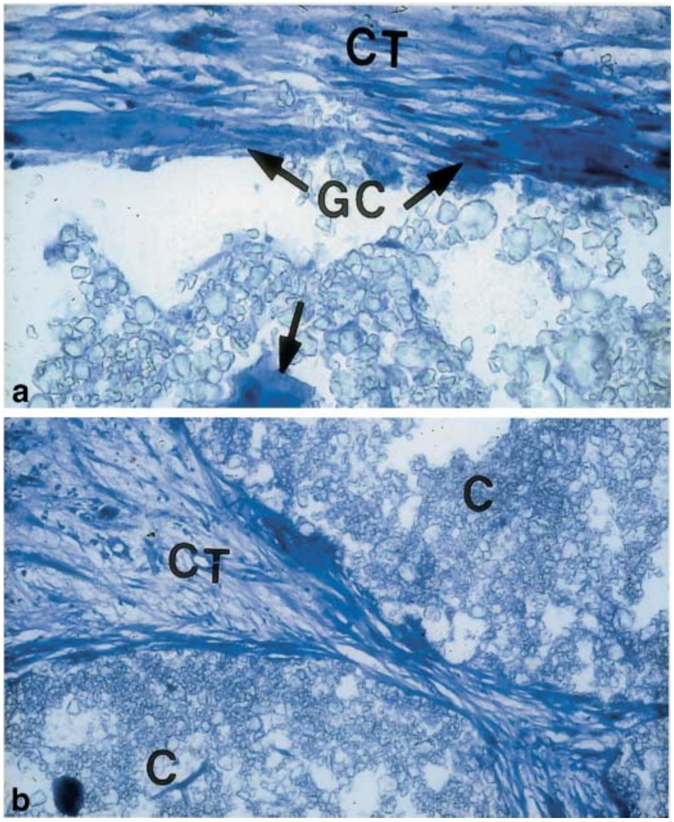

Fig. 6. Foreign-body giant cells could be detected in these soft tissue layers that were more present in the bone specimens of patients that were treated with PRP (a, microtome section, Giemsa staining, magnification ×68; b, microtome section, Giemsa staining, magnification ×27.2).

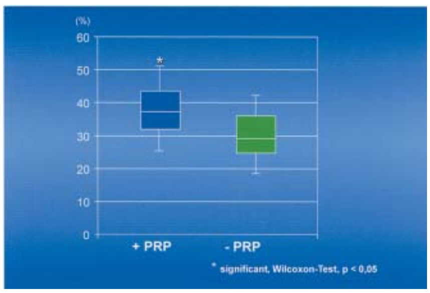

Fig. 7. Box-plot analysis for the bony regeneration.

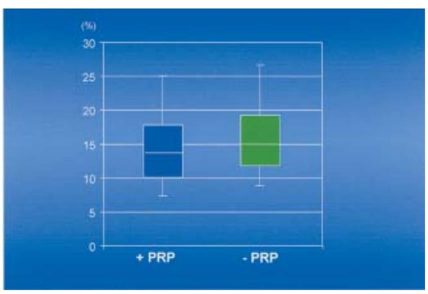

Fig. 8. Box-plot analysis for the degradation of the β-TCP ceramics.

could be observed and the fact that not all ceramic granules had direct bone contact but were partially embedded in a soft tissue shed. The latter can be explained by TGF, which is included in PRP and supports the chemotaxis of fibroblasts.

In summary, this study suggests that the application of PRP will only result in accelerated new bone formation if target cells such as osteoblasts or osteocytes are present. A faster degradation of ceramic bone substitutes cannot be expected. Further studies are necessary to investigate the influence of varying concentrations of PRP on wound-healing processes alone and in combination with other bone substitutes as tested here. Moreover, the patients operated on in the present study will be followed up once a year to obtain long-term data on the success rate of the implants installed in the different sites.

Résumé

L'épaississement du plancher sinusal est un processus efficace d'amélioration de la hauteur à l'arrière du maxillaire si des implants dentaires doivent y être insérés. A côté du matériel osseux autogène, des matériaux allogènes tels que le phosphate tricalcique-β (β-TCP) ont été utilisés avec succès. Le but de cette étude a été de vérifier si l'association du β-TCP et du plasma riche en plaquettes (PRP) pouvait augmenter la régéneration osseuse et la résorption du matériel phosphate tricalcique. Dans un essai prospectif au hasard, 45 épaississements du plancher sinusal ont été effectués chez 39 patients. Au niveau de 22 sites, le PRP a été injecté aux granules β-TCP tandis que 23 sites β-TCP se retrouvaient sans PRP. Six mois plus tard, des échantillons osseux ont été prélevés des régions épaissies durant le processus d'insertion des implants. La formation du nouvel os était de 8 à 10% plus importante losque le PRP avait été utilisé. Une dégradation plus rapide du substitut osseux en céramique n's pas pû être mis en évidence. Lorsque le PRP est ajouté au β-TCP la régénération osseuse est légérement favorisée. Cependant la résorption de β-TCP n'était pas accélérée et des cellules géantes de corps étrangers ainsi que du tissu mou entourant les granules TCP étaient présents en plus grand nombre.

Zusammenfassung

Ziel: Die Augmentation des Sinusbodens stellt eine effiziente Behandlung zur Verbesserung der Knochenhöhe in der posterioren Maxilla dar, wenn Implantate eingesetzt werden sollen. Neben autologem Knochenmaterial wurden auch allogene Materialien wie z.B. β-Trikalziumphosphat (β-TCP) erfolgreich verwendet. Es war das Ziel dieser Studie, zu untersuchen, ob die Kombination von β-TCP mit plättchenreichem Plasma (PRP) die Knochenregeneration und die Resorption des Trikalziumphosphatmaterials fördern wird.

Studienaufbau: Bei 39 Patienten wurden 45 Elevationen des Sinusbodens in einer randomisierten prospektiven Untersuchung durchgeführt. Bei 22 Stellen wurde dem β-TCP-Granulat PRP zugefügt, während bei 23 Stellen β-TCP ohne PRP verwendet wurde. Nach sechs Monaten wurden während des Eingriffs zum Setzen der Implantate Knochenbiopsien von den augmentierten Regionen gewonnen.

Resultate: Die Bildung von neuem Knochen war ca. 8 bis 10% grösser wenn PRP appliziert wurde. Eine schnellere Auflösung des keramischen Knochenersatzmaterials konnte nicht beobachtet werden.

Schlussfolgerung: Wenn PRP dem β-TCP zugefügt wird, wird die Knochenregeneration leicht unterstützt. Jedoch wird die Resorption von β-TCP nicht bescheunigt. Fremdkörperriesenzellen und Weichgewebe um die TCP-Granulate sind mehr vorhanden und auffälliger.

Resumen

Objetivo: La elevación del suelo del seno es un procedimiento de tratamiento efectivo para mejorar la altura de hueso en el maxilar posterior, si hay que insertar implantes dentales. Aparte de material óseo autógeno, se han usado con éxito materiales alogénicos como β-tricalciumphosphate (β-TCP). El propósito de este estudio fue investigar, si la combinación de β-TCP con plasma rico en plaquetas (PRP) incrementaria la regeneración ósea y la reabsorción del material tricalciumphosphate.

Diseño del estudio: En un ensayo aleatorio prospectivo se llevaron a cabo 45 elevaciones del suelo del seno en 39 pacientes. En 22 lugares se añadió PRP a gránulos de β-TCP, mientras que en 23 lugares se usó β-TCP sin PRP. Seis meses mas tarde se recolectaron especimenes de hueso de la zona aumentada durante el procedimiento de inserción del implante.

Resultados: La formación de nuevo hueso fue un 8 a 10 % mas alta cuando se aplicó PRP. No se observó una degradación mas rápida del sustituto óseo cerámico.

Conclusiones: Cuando se añade PRP a β-TCP, la regeneracion ósea es ligeramente ayudada. De todos modos, la reabsorción de β-TCP no se acelera y las células gigantes de cuerpo extraño y el tejido blando rodeando los gránulos de β-TCP fueron mas evidentes y estaban presentes.

要旨

目的：上顎洞底増多術は、上顎臼歯部にインプラントを埋入する場合、骨高径を改善するための効果的な術式である。自家骨材料のみならず、β３燐酸カルシウム（β－ＴＣＰ）などの同種材料が成功裏に用いられている。本研究ではβ－ＴＣＰと血小板濃厚血漿（ＰＲＰ）の併用が骨再生と３燐酸カルシウム材料の吸収を促進するかどうかを検討した。

研究デザイン：無作為前向き試験において、患者３９名、４５箇所の上顎洞底挙上術を行った。２２部位ではβ－ＴＣＰ顆粒にＰＲＰを追加し、２３部位はＰＲＰなしでβ－ＴＣＰのみとした。６ヶ月後のインプラント埋入時に、増多部位から生検標本を採取した。

結果：ＰＲＰを追加した場合、約８％から１０％高い新生骨の形成を示したが、セラミック骨代替材料の分解は促進されなかった。

結論：β－ＴＣＰにＰＲＰが追加する事で、骨再生はやや促進されたが、β－ＴＣＰの吸収は促進されず、ＴＣＰ顆粒周囲の異物巨大細胞と軟組織は、より顕著に存在していた。

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