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THE HISTOMORPHOMETRIC ANALYSIS OF MAXILLARY SINUS LIFT ELEVATION USING THE SYNTHETIC HYDROXYAPATITE AND TRICALCIUM PHOSPHATE GRAFTING MATERIALS

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The aim of the study was to conduct an evaluation of the success of implants inserted into the distal maxilla augmented via the lateral sinus lift method using a combination of synthetic alloplastic hydroxyapatite and β-tricalcium phosphate materials. The solubility of PORESORB-TCP in body fluids leads (due to TCP dissolution) to their hyper-saturation with Ca^{2+} and P ions, which gradually reprecipitate as CaP. OssaBase-HA has been shown to evince osteoconductive properties. The aim was to take advantage of the synergistic osteoconduction potential of the hydroxylapatite OssaBase-HA and β-tricalcium phosphate PORESORB-TCP materials. We selected a ratio of the two materials of 3:2 for experimentation purposes. The histological findings clearly indicated that, concerning the histological sample, the area of the original bone was 21.4 % and the area of augmented tissue occupied 78.6 %. The proportion of bone of the patients' hard tissue was 68.3 % compared to 43.9 % of bone with the new augmented tissue. The volume of the augmentation material in the augmented tissue was determined at 45.6 %. The amount of PORESORB-TCP was 34.1 % of the augmentation material, and the amount of OssaBase-HA was 11.6 % of the augmentation material. Our study thus confirmed the osteoconductive properties of the hydroxylapatite OssaBase-HA and β-tricalcium phosphate PORESORB-TCP materials and demonstrated the osteoconduction of a 3:2 mixture of the two materials, thus proving the synergy of their respective properties.

INTRODUCTION

The maxillary sinus comprises a bilateral air-filled cavity located in the maxillary complex [1]. The distance between the bottom of the sinus and the alveolus peak consists of the vertical height of the bone intended for implant insertion. The length of the implant is essential in terms of successful osseointegration and long-term implant stability, hence the importance of the vertical height of the alveolar bone in the distal maxilla. The floor is formed via the alveolar process of the maxilla. Maxillary sinus pneumatization, sinus expansion and crestal atrophy following the extraction of premolars and molars may lead to the limitation of the volume of the vertical residual bone in terms of the placement of implants [2]; hence, in this respect the posterior maxilla represents a risk area [3]. Moreover, anatomical variability due to membrane thickness and atrophy, a previous operation, scars and septa may lead to the perforation of the sinus membrane and the subsequent failure of the implant [4].

The surgical treatment of the base of the maxillary sinus by means of the sinus lift method is frequently applied in order to adjust adverse conditions. This process involves the vertical addition of augmentation material at the bottom of the inside of the sinus cavity.

Two sinus lift techniques have been introduced in recent years, i.e. the transalveolar (crestal) and lateral window techniques [5]. The lateral window technique is used for cases with residual bone of less than 5 mm. The augmentation material is applied through a window in the lateral wall of the maxilla and the increase in vertical bone is 8 - 20 mm. The crestal osteotomy technique is used for patients with their own residual bone of a vertical distance of at least 5 mm. The augmentate is applied through a drilled hole for fixture purposes. The gain in vertical bone is 2 - 3 mm.

Effective sinus lift bone regeneration techniques present a range of possibilities. Autogenous bone and bone substitutes can be used as the augmentation material, and it has been reported in the literature that there is no significant difference between the two materials. The gold standard is based on autogenous bone grafts, the advantage of which lies in their osteogenic capacity and the disadvantage in their high degree of resorption [4]. Allogenic bone grafts are taken from cadavers of the same species and their osteoinductive capability leads to the creation of a material with a higher

concentration of bone morphogenetic proteins and/or bone-specific proteins than that of alloplastic and xenograft materials. Xenografts are taken from different species of animals, and function as slowly resorbing, osteoconductive grafts. Alloplastic grafting materials with osteoconductive potential consist of synthetic materials – polymers, calcium sulphates, hydroxyapatite, calcium phosphates and coral and algae-derived hydroxyapatite. Osteoconductive materials serve as a scaffold that provides support for, and which is replaced by, newly-formed bone substances. Autogenous bone is osteoinductive for surrounding tissues.

The aim of the study was to analyze the histomorphometrical data regarding the grafting of synthetic alloplastic materials in the external sinus floor.

EXPERIMENTAL

Material and methods

The selected patients were treated at the Department of Stomatology, Charles University, 2nd Medical Faculty and at the Motol University Hospital from May 2008 to February 2018. The patients were requested to provide their consent to involvement in the experiment. Ethical approval for the study was obtained from both the Motol University Hospital and the 2nd Medical Faculty of the Charles University Ethics Committees (EK-973IGA 1.12/ 11) in accordance with the Declaration of Helsinki. Anonymity was strictly respected with respect to the data collection process.

Inclusion/exclusion criteria

The patients included in this study evinced good general health. After receiving initial dental therapy, including oral hygiene instructions, the sinus lift and implantation were performed only after the patients had demonstrated a high standard of self-performed plaque control. The implant surgery patients required sinus lift involving the insertion of single or two implants into the distal maxilla sextant. The research involved a total of 93 BioniQ implants and 71 patients (37 men and 34 women; the average age was 59 years (SD 9).

The BioniQ implants (LASAK, Prague, CZ) were applied with osteoconductive (sand-blasted, acid- and alkalic-etched) surfaces. Following the manufacturer's instructions, the fixtures were inserted at the edentulous posterior part of the maxilla of the patients.

Bone substitutes

OssaBase-HA (LASAK, Prague, CZ) comprises a synthetic, macro- and nano-porous bone regeneration material based on hydroxyapatite with a low substitution rate. It is used for the bone regeneration of missing or lost bone tissue independently or in combination with autologous bone tissue, blood or PRP and PRFG. OssaBase-HA features up to 83 % interconnected porosity for the support of vascularized bone formation. Its low substitution rate helps to provide long-term graft stability and the maintenance of volume in cases where longer healing times are required or if re-entry to the site is expected to be delayed. OssaBase-HA bone regeneration material offers a chemically and structurally similar, synthetic alternative to bovine-derived bone substitutes.

PORESORB-TCP (LASAK, Prague, CZ) consists of a synthetic product based on β-tricalcium phosphate with a mean porosity 40 %, a macro-pore size of 100 - 200 μm and a micropore size of 1 - 5 μm. The micropores provide a convenient surface for cellular attachment by enabling the rapid penetration of blood vessels into the material. The gross structure of PORESORB-TCP serves to conduct the newly-generated blood vessels required to supply blood to the newly-formed bone tissue, and the spaces between the granules are filled with biochemical components. The resorption of calcium phosphate ceramics is based on the amount of bone tissue that replaces the material. In general, PORESORB-TCP is totally replaced by newly-formed bone tissue within 6 - 12 months of resorption [6] (Tab. 1).

Table 5. Bone substitutes characteristics.

Characteristics	OssaBase-HA	PORESORB-TCP
Macro-pore diameter (μm)	100 – 500	100 – 200
Average micro-pore diameter (μm)	–	1 – 5
Average nano-pore diameter (nm)	10 – 20	–
Specific surface area ($m^2 \cdot g^{-1}$)	ca 75	ca 0.25
Porosity (%)	ca 80	ca 40
Ca/P molar ratio	1.65	1.5
Rel. CO_3^{2-} "A" (I1545/I1041)*	1.10 – 6	–
Rel. CO_3^{2-}– "B" (I1420/I1041)**	0.023	–
Bulk density ($kg \cdot m^{-3}$)	750 – 950	1 800 – 2 200
Apparent bulk density ($kg \cdot m^{-3}$)	2 750 – 3 140	2 900 – 3 100
Compressive strength (MPa)	> 0.35	> 6.6

* *OH^- anion substituted by CO_3^{2-}*
** *PO_4^{3-} anion substituted by CO_3^{2-}*

The solubility of PORESORB-TCP in body fluids (due to TCP dissolution) leads to their hyper-saturation with Ca^{2+} and P ions, which gradually reprecipitate as CaP. OssaBase-HA has been shown to evince osteoconductive properties. The aim was to take advantage of the synergistic osteoconduction potential of the hydroxylapatite OssaBase-HA and β-tricalcium phosphate PORESORB-TCP materials. A ratio of 3:2 of the two materials was selected.

Methods

The treatment plan was prepared, including augmentation with the OssaBase-HA and PORESORB-TCP materials (ratio 3:2) based on cone-beam computed tomography (CBCT) (KaVo Dental GmbH, Bismarckring, Germany). The sinus lift surgical protocol was performed by two surgeons by means of the lateral window technique. All the implants were inserted during the augmentation phase.

After a healing period of an average of 5 months, a second surgical procedure was performed which, after three weeks, was followed by the delivery of the prostheses. This second stage included the harvesting of histological specimens at the alveolus peak in the vertical direction by means of a 4.0/4.5 mm bone trepan while cooling with saline. The trepanation depth was 20 - 25 mm. Collection was performed at the site of the future intermediate member or distally behind the last implant (Figure 1).

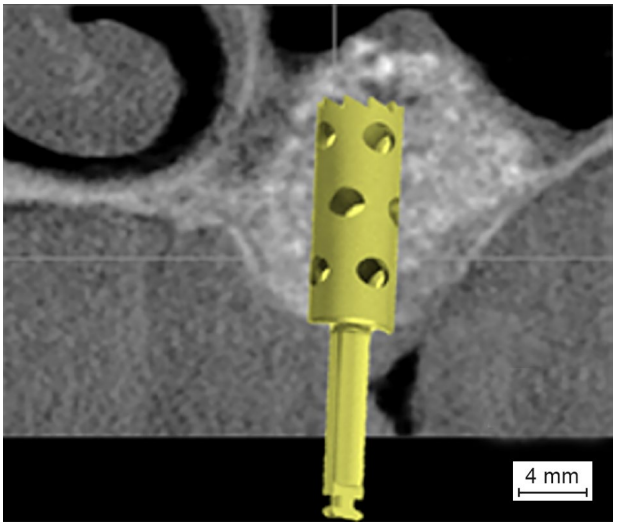

Figure 1. Harvesting of the histological specimens.

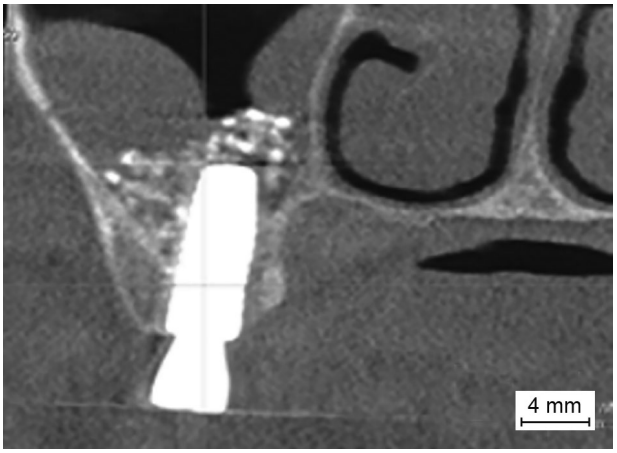

Figure 2. An implant after the 5-month healing period.

The verification of osseointegration by means of cone-beam computed tomography (CBCT) was performed before prosthetic treatment (Figure 2).

The CAD–CAM Zircon Zahn technique (Prettau® Zirconia, ZirconZahn Gmbhc) and a BioCam (LASAK) were employed to establish the supraconstruction. The patients were recalled every 6 months for a thorough professional plaque inspection and continuous oral hygiene training. Intra-oral digital radiographs (Gendex EXPERT® DC with a VistaScan Mini image plate scanner) were taken for each patient following therapy.

Sample preparation

All the tissue samples extracted from the sinus lift samples were of a similar size. The samples were stored in a fixation medium (Formaldehyde solution 4 %, buffered, pH 6.9 – approx. 10 % Formalin solution) and they were subsequently dehydrated by means of an alcohol series using the EXAKT 510 device: 60 % EtOH, 80 % EtOH, 96 % EtOH, EtOH abs. (2×). The retention time was determined for each step according to the relationship: 1 mm sample thickness → 1 day exposure in the medium. The dehydrated samples were infiltrated by exposure to Technovit 7200 VLC acrylic resin using the EXAKT 510 device via the following stages: EtOH/Technovit 70/30, EtOH/Technovit 50/50, EtOH/Technovit 30/70 and Technovit 100 % (3×).

The retention time in each stage was again determined according to the relationship: 1 mm sample thickness → 1 day exposure in the medium. The final stage of infiltration in 100 % Technovit was conducted under vacuum conditions. The infiltrated samples were inserted into plastic molds, embedded in the Technovit 7200 VLC resin and polymerized by means of light (yellow and blue) using the EXAKT 520 device. The samples embedded in the polymer block were glued to a plastic microscope slide (using Technovit 7210 VLC adhesive) and thin sections were cut using the EXAKT 300 CL/CP device with a diamond strip.

The thin sections were ground via the use of the EXAKT 400 CS device using sand paper (SiC) with grain roughnesses of 320, 500, 800, 1000, 1200, 2500 μm and subsequently polished using 4000 μm paper. The thicknesses of the final thin sections ranged between 30 and 120 μm. The resulting histological thin sections were stained via the standard toluidine blue procedure. An Olympus BX51TF (Olympus Corporation, Japan) microscope and Olympus E-410 (Olympus Imaging Corp., China) digital camera were used for the preparation of the microscopic and photographic documentation.

Statistics

The images were analyzed by means of programs written in MatLab (Mathworks Inc, U.S.A.). The values were presented as the mean ± standard deviation (SD). The Mann-Whitney U test was used in order to determine differences between the implanted materials. A P value of less than 0.05 was considered significant.

RESULTS

Samples were taken (as a part of the second stage) following the conclusion of the 5-month healing period, and were subsequently histologically evaluated (Figure 3). The blue vertical line in the Figure serves to separate the original (left) and augmented bone (right). The red outlined areas (a) indicate the original (b) (left) and the newly-created bone (c) (right). The green outlined areas indicate the PORESORB-TCP (d), and the purple outlined area the OssaBase-HA (e). All the samples featured areas of soft tissues that had been replaced with resin (thus, the percentage of bone + PORESORB-TCP + OssaBase-HA did not amount to a full 100 %).

We compared the areas of original bone tissue on the samples – 5.7 mm^2 (SD 5.9) and tissue with the augmented material – 20.8 mm^2 (SD 1.9). The original bone was homogeneous and contained a native bone area of 3.9 mm^2 (68.4 %). An area of 9.1 mm^2 (43.8 %) of new native bone remained in the enlarged portion of the samples, while the remainder was replaced by augmentation materials making up an area of 9.5 mm^2 (45.7 %) of the resulting tissue with a predominance of PORESORB-TCP – 7.1 mm^2 (74.3 %). The surface of the OssaBase-HA – 2.4 mm^2 (25.3 %) was statistically significantly lower than the surface of the PORESORB-TCP. In addition to the new native bone and bone substitute, the remaining space was observed to be filled with soft tissue – 2.2 mm^2 (10.6 %) (Table 2).

Table 2. Comparison of the original and augmented bone tissues.

Area	Mean (mm^2)	SD (mm^2)
area of the original tissue	5.7	5.9
area of bone in the original tissue	3.9	3.9
area of the augmented tissue	20.8	1.9
area of bone in the augmented tissue	9.1	1.2
total area of augmentation	9.5	1.6
OssaBase-HA only	2.4	0.7
PORESORB-TCP only	7.1	1.9

DISCUSSION

Several clinical studies and reviews [7-11] have previously discussed maxillary sinus augmentation and related materials. No significant difference was observed between the use of bone and substitutes in the study of the success of roughened implants in lateral window technique of sinus lift. The annual loss ratio of roughened implants using bone and substituent was 1.10/1.13. The success rate of implants with polished and roughened surfaces after 3 years using particle bone alone is 84.3 %, that of bones combined with a substitute, 95.7 % and that of substitutes alone is 92.5 %. When using a slow resorption bone substitute, no difference is evident between autologous bone and the substitute.

The OssaBase-HA and PORESORB-TCP materials have been used separately [12-14] and as mixtures of HA with β-TCP [15-16] in previous histomorphometric studies.

The comparison of the performance of the separate hydroxyapatite-based OssaBase-HA material with deproteinized bovine bone 6 months following implantation revealed that the newly-formed bone tissue around the bone graft granules amounted to approx. 42 % and approx. 22 % respectively of the total bone defect (bone area ingrowth; BAI) in the cortical and cancellous bone of beagle dogs [17]. These results are in agreement with the amount of newly-formed bone determined in the augmented tissue considered in this study.

Furthermore, Kucera et al. [18] have proved via the conducting of several case studies that PORESORB-TCP is a suitable bone substitution material; new bone formation was observed around the biomaterial as it was with concern to the tissue specimen studied herein.

The histological findings (Figure 3) indicated that the area of the original bone in the histological sample amounted to 21.4 % and the area of the augmented tissue 78.6 %. The bone proportion of the patients' own hard tissue was 68.3 % compared to a bone proportion of 43.9 % in the new augmented tissue. The volume of augmentation material in the augmented tissue was determined at 45.6 %. The proportion of PORESORB-TCP of the augmentation material was 34.1 % and that of the OssaBase-HA 11.6 %.

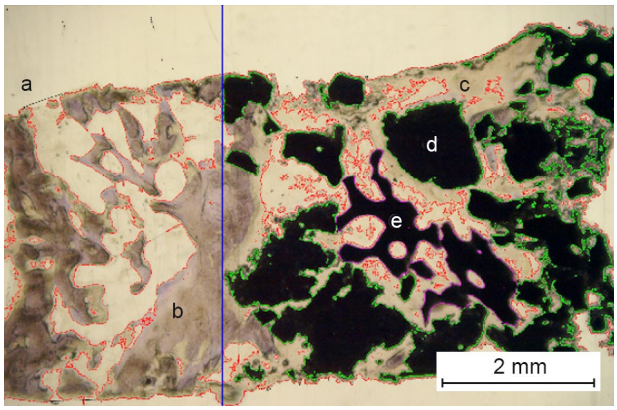

Figure 3. Tissue specimens taken from the sinus lift samples: red outlined areas (a) indicate the original (b) (left) and the newly-created bone (c) (right); green outlined areas indicate the PORESORB-TCP (d), and the purple outlined area the OssaBase-HA (e).

Our study confirmed the osteoconductive properties of the hydroxylapatite OssaBase-HA and β-tricalcium phosphate PORESORB-TCP materials and demonstrated the osteoconduction of a 3:2 mixture of the two materials, thus further demonstrating the synergy of their properties. With concern to procedures using bone substitutes or mixtures of bone and substitutes for lateral window technique sinus lift purposes, it has been demonstrated by means of histomorphometry that the amount of newly-formed bone is comparable to that suggested by our method. Further, it was also demonstrated that the structure of the augmented volume depends not only on the chemical structure of the augmenting material but also on the specific surface area and the size of the (macro) pores thereof.

It was confirmed that bone substitute materials can be used as the effective alternative to autologous bone to simplify the grafting procedure [19]. There is no significant difference between autogenous bone alone, mixture of autogenous bone with a bone substitute and substitute alone in terms of the long-term success of the sinus lift method with implants which were inserted into this augmented site [20]. Our results revealed that the OssaBase-HA and PORESORB-TCP grafting materials are suitable and, indeed, beneficial with concern to bone regeneration applying the sinus lift method. The outcome of our study thus allows for the minimization of the trauma associated with sinus lift since, due to the use of synthetic augmentation materials produced under laboratory conditions, the requirement no longer exists for the collection of autogenous bone.

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