Sinus lift augmentation and β-TCP: A microCT and histologic analysis on human bone biopsies

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ABSTRACT

Sinus lift elevation is an interesting method to restore bone mass at the maxilla in edentulated patients. We have investigated the histological effects of beta tricalcium phosphate (β-TCP) combined with autograft bone for sinus lift elevation. A series of 14 patients who were candidate for dental implantation were grafted with β-TCP granules and morcellized autograft bone harvested at the chin. β-TCP was characterized by scanning electron microscopy and optical profilometry. Before implant placement, a small bone biopsy (2 mm in diameter) was done. The amount of residual material and newly formed bone were determined by microcomputed tomography. Histological analysis was done on undecalcified sections stained by Goldner’s trichrome and osteoclast identification (TRAcP). β-TCP served as a template for bone apposition by osteoblasts onto the granules’ surface. The material was simultaneously resorbed by TRACP positive osteoclasts and macrophages. Fragments of the material remained buried in bone trabeculae as long as 12 months post-graft but the formed bone onto the granules surface had a lamellar texture. β-TCP combined with autograft bone appears a suitable biomaterial for sinus lift augmentation before the placement of bone implants. The material favors the apposition of lamellar bone by osteoblasts and is simultaneous resorbed by two types of cells.

1. Introduction

Correction of teeth loss in the posterior zones of the jawbones can be obtained by a removable prosthesis. Nevertheless, for several decades the rehabilitation by implants and prostheses has allowed to rebuild a fixed dental arch to avoid the discomfort of a removable denture. However, initial stabilization of implants is difficult to achieve in the posterior maxillary regions where cortical bone is thin and immediately under the sinus (Marquez, 2008). Posterior loss of teeth at the maxilla is often associated with prolonged painful troubles (Goulet et al., 2007). Autograft was the only possibility during decades but the biomaterials available has been recently proposed (Browaeys et al., 2007). Autograft was the only possibility during decades but the volume of harvested bone is limited and the need for an additional surgery is often associated with prolonged painful troubles (Goulet et al., 1997). Xenogenic bone has proved to have satisfactory osteoconductive properties in dental practice but has suffered from ethical controversies, thereby reducing drastically its use (Butler et al., 1998). Synthetic biomaterials (hydroxyapatite, or other calcium phosphates, polymers, bioglasses...) have been proposed (Giannoudis et al., 2005). However, there is no consensus in the literature if a biomaterial should be used alone or in combination with a limited volume of autograft (Wheeler et al., 1992). After having opened the lateral side of the maxillary sinus, the Schneiderian membrane is pushed away from the bone and the void space is filled with particles of a bone substitute. In this way, several millimeters in thickness of bone can be obtained after healing of the maxillar bone. Several types of biomaterials have been proposed in the literature and an extensive review on the biomaterials available has been recently proposed (Browaeys et al., 2007). When the thickness of the sinus floor is less than 8–10 mm, short implants cannot be used and it is recommended to increase the bone volume by doing a preliminary sinus augmentation (or sinus lift) using a bone graft material (Smiler et al., 1992). Autograft was the only possibility during decades but the volume of harvested bone is limited and the need for an additional surgery is often associated with prolonged painful troubles (Goulet et al., 1997). Xenogenic bone has proved to have satisfactory osteoconductive properties in dental practice but has suffered from ethical controversies, thereby reducing drastically its use (Butler et al., 1998). Synthetic biomaterials (hydroxyapatite, or other calcium phosphates, polymers, bioglasses...) have been proposed (Giannoudis et al., 2005). However, there is no consensus in the literature if a biomaterial should be used alone or in combination with a limited volume of autograft (Wheeler et al., 1992). In the present study, partially edentulated patients have
developed a bone atrophy at the maxilla that necessitated a sinus lift elevation. The bone loss was filled with a mixture of β-TCP (beta tricalcium phosphate) and morcellized autograft bone harvested at the chin. The bone changes were analyzed histologically on a small bone biopsy performed at distance from the graft, immediately before placing the implants.

2. Materials and methods

2.1. The calcium phosphate granules

The β-TCP granules were obtained from Kasios (Launaguet – France). Granules were produced by using the foam technology as previously reported (Filmon et al., 2009). Briefly, 25 g of a β-TCP slurry were used to infiltrate under vacuum 1 g of polyurethane foam. After drying and sintering at high temperature (>1100 °C), granules (1 mm in diameter) were prepared by crushing the blocks and filtering.

2.2. Surface topography of β-TCP

Blocks of β-TCP were examined by SEM after having been carbon-coated (10 nm thick) with a MED 020 (Bal-Tec, Balzers, Liechtenstein). SEM was performed on a JEOL 6301F (Jeol France Paris) field emission microscope. Grain size was determined on SEM images with the ImageJ software (NIH).

Surface topography measurement of β-TCP was obtained in a Wyco N9100 vertical optical profilometer (Veeco, Instruments SAS, Dourdan, France). The microscope is based on light interferometry. A white-light beam passes through a Mirau’s type objective to the sample surface. A beam splitter reflects half of the incident beam to the interferometer, which is aligned with the objective. The reflected beam is recombined with the unreflected beam, and the interference pattern is recorded by a CCD camera. The interferogram is then processed to obtain the surface topography. The surface roughness profile was obtained on a series of 3 images (each of 48×48 mm, pixel size 0.1 μm).

2.3. Patients and surgical protocol

Fourteen patients who presented a partial edentulation at the maxilla and who were candidate for implant placement after a bone graft were enrolled in the study. Each has given her/his informed consent to participate in the present study. The surgical protocol aimed at increasing the thickness of the sinus floor by using a combination of autograft (usually 20–30%, v/v) and β-TCP granules (70–80%). The thickness of the sinus floor was measured on CT scans before sinus lift elevation. An online CT scan was performed before the placement of dental implants of standard diameter.

The bone graft was harvested in first at the chin as previously reported (Guillaume et al., 2009). Briefly, cortico trabecular samples were removed at the mandibular symphysis. The limits of the harvesting graft areas were done with a thin bur and the graft was separated with a chisel by a progressive cleavage. Chips of cortico trabecular bone were obtained and mixed with the patient’s blood together with β-TCP granules to obtain a kind of paste that could be handled more easily.

The grafted area was prepared as follows: the mucoperiosteal flap was removed after a lateral osteotomy on the external face of the maxillary sinus (usually the opening is 10 mm in length and 8 mm in height). The Schneiderian membrane was gently pushed through the window, away from the bone to avoid perforation. The mixture containing autograft particles, β-TCP and blood was inserted into the void space, under the membrane. The amount of material used depended on the patient but usually, several millimeters of the grafted “paste” were added to reconstitute a suitable volume that will support the placement of implants after healing. The full thickness flap was then closed to the primary incisions and sutured with 5/0 vicryl.

2.4. Bone biopsy harvesting

Six to twelve months after the bone graft, the implants were placed in the maxilla. A small bone core was removed with a dental trephine (Dexter, H. Zepf, Ref 08910 02/CE 01202/05, Argenteuil, France) with a 2.3 mm internal diameter and 2.8 mm external diameter, mounted on a contra-angle attachment. Bone biopsies were immediately transferred into an ethanol-formalin fixative at 4 °C in a refrigerator. This procedure was found suitable to preserve bone cell enzymes and retains the staining properties of collagen (Chappard, 2009). Samples were received in the laboratory and analyzed by microcomputed tomography (microCT) while in the fixative prior to histologic embedding. MicroCT allows a 3D imaging of calcified tissues and biomaterials and can be done before histologic analysis. Analyses were performed using a Skyscan 1072 X-ray computed microtomograph (Skyscan, Kontich, Belgium) equipped with an X-ray tube working at 80 kV/100 μA. Bone biopsies were placed in Eppendorf tubes, filled with fixative to prevent desiccation. The tubes were fixed on brass tubes with plasticine and analyzed at a magnification of ~40× (pixel size corresponding to 5.12 μm), the rotation step was fixed at 0.45° and exposure was done with a 1 mm aluminum filter. For each sample, a stack of 2D sections was obtained. The CTA scanner software (Skyscan, release 1.3.0.5) was used for measuring the bone mass and residual material mass. A threshold was determined to eliminate background noise and to select the biomaterial (which was the most X-ray opaque) or both biomaterial and bone. The following parameters were measured in a volume of interest defined by the margins of the bone core (TV, in mm³):

- Calibrated tissue volume (Cal/TV, in %) represented the percentage of all X-ray opaque materials (β-TCP and trabecular bone in the volume of the sample),
- The material volume (Mat/TV, in %), represented the percentage of the residual β-TCP which exhibited the highest X-ray opacity,
- The bone volume (BV/TV, in %), was derived from Cal/TV–Mat/TV/TV and represented the amount of bone that had proliferated inside the β-TCP granules.

The 3D reconstruction of the specimens was obtained with the ANT software (release 2.2.5.0 – Skyscan). The program allows reconstruction of objects from the stacks of 2D sections, after interactive threshold. The reconstructed 3D models were obtained by a surface-rendering algorithm. A very interesting facility for the study of porous structures was the possibility to make the virtual models semi-transparent. Because different 3D models can be reconstructed and made visible simultaneously (thus offering the possibility to combine several images), this possibility was used to...
visualize the β-TCP remnants together with bone which was made semi-transparent.

2.5. Histologic analysis

Fixed specimens were dehydrated and embedded undecalcified in polymethylmethacrylate. Sections (7 μm in thickness) were cut dry on a heavy duty microtome equipped with tungsten carbide knives (Leica Polycut S with 50° knives). They were stained with Goldner’s trichrome for the identification of osteoid, and toluidine blue for cell analysis. Osteoclasts were detected by identification of their tartrate resistant acid phosphatase content (TRAcP) as previously described (Chappard et al., 1983).

2.6. Statistical analysis

Statistical analysis was done using the Systat statistical software, release 11 (SPSS inc., Chicago, IL). All results are expressed as mean ± standard deviation (SD).

3. Results

SEM imaging revealed the surface of β-TCP to be composed of small polyhedric grains (2.73 ± 1.0 μm in width) tightly packed together with occasional micropores corresponding to holes between 4 and 6 grains (Fig. 1). Micropores were 1.01 ± 0.16 μm in width. The macroporosity, controlled by the mesh of the polyurethane foam used for preparing the granules was in the order of 400 μm. Microroughness was clearly evidenced by optical profilometry which evidenced the fused grains at the surface of the material together with the small holes that constituted microporosity. The mean Ra was 0.8 μm.

MicroCT was available in 13 patients. In all bone biopsy samples, β-TCP granules were identified by microCT due to their higher calcium content when compared to bone (Fig. 2). Granules were often covered by bone anchored at the surface. In some areas, the biomaterial appeared completely surrounded by bone. 3D reconstructions of cylinders containing β-TCP and bone appear in Fig. 2. The amount of residual biomaterial was in the order of 5–10% (Table 1); however, differences exist in the heterogeneity of repartition of the granules which were more abundant in the deepest areas of the biopsies. There is a net tendency for bone volume to increase upon time but the correlation did not reach significance in this limited series of patients.

Histological analysis revealed the presence of a non-inflammatory loose connective tissue between the β-TCP granules, the newly formed bone trabeculae and the autograft particles. The inflammatory fibrotic tissue was always highly vascular with numerous sinusoid capillaries. The center of the connective tissue areas was always filled with numerous macrophages containing minute grains of β-TCP (Fig. 3). In some patients, mastocytes were evidenced on toluidine-blue stained sections in the connective tissue. Histological findings confirmed the microCT data with osteoid tissue (or fully mineralized bone) directly laid on the surface of the β-TCP granules. A number of pseudo-epithelial alignments of osteoblasts were observed at surface of newly formed bone on the biomaterial granules or the autograft particles. In some areas, the bone completely filled the concavity of the granules’ macroporosity (Fig. 4). TRAcP-stained sections evidenced true osteoclasts at the surface of the bone material (either for remodeling the newly formed bone or the autograft particles). Bone formed at the surface of the granules had a lamellar texture. Some macrophages in the connective tissue appeared lightly TRAcP positive when their cytoplasm contained β-TCP grains. Some multinucleated cells exhibited a marked TRAcP positivity when in direct contact with the surface of β-TCP granules (Fig. 5). The autograft particles could be observed in several patients due to their different tinctorial affinity and the absence of osteocytes in the osteoplastic lacunae.

4. Discussion

β-TCP, a calcium-phosphate bioceramic, is one of the most frequently used in current periodontal therapy for filling dental alveolar after tooth extraction. It has also been found suitable to repair bone defects (Galois et al., 2002; Saito et al., 2000). Mixtures

Fig. 1. (A) SEM analysis of β-TCP granules at low magnification. Note the concavities of the granules created by the foam technology used for preparing the biomaterial. (B) SEM analysis of the surface of β-TCP granules. The arrows indicate the micropores observed between the polyhedric grains obtained after sintering. (C) Microtopography obtained on a granule with optical profilometry.
of β-TCP and hydroxyapatite (HA) have been proposed (and are often referred as BCP – biphasic calcium phosphate). Controversies exist concerning as to how long HA persist in the body and reports from the orthopedic literature indicate that β-TCP provide better results (Ogose et al., 2005). However, the material, when prepared in granules, has poor biomechanical properties that preclude its use in weight-bearing sites. Numerous reports have confirmed that a direct bone apposition can be observed at the surface of β-TCP without interposition of a HA layer (Kotani et al., 1991). Direct bone matrix anchorage has been shown with collagen fibers deposited in the micropores (Chazono et al., 2008). Recently, the surface chemistry and roughness of β-TCP has been found to favor the attachment and spreading of osteoblast-like cells in vitro (Dos Santos et al., 2009). An ideal biomaterial usable as a bone substitute should be a temporary 3D-scaffold serving for osteoconduction. Macroporosity (pore size >100 μm) is necessary to permit vascular and mesenchymal stem cells invasion. The ideal biomaterial should then be removed after complete replacement by newly formed bone. β-TCP is easily resorbable when in the macroporous form (Chazono et al., 2004). The association with autograft bone was found to increase osteoconduction in animal studies and in human surgical practice (Hossain et al., 1996). Disintegration of β-TCP by giant cells has been recently reported in a rabbit model with cells having or not a ruffled border (a characteristic of osteoclasts) (Chazono et al., 2008). In the present study, TRAcP positive multinucleated cells were observed in direct contact with the exposed surfaces β-TCP particles in several patients. Similar findings were reported by Zerbo et al., in a series of 6 patients with sinus floor elevation (Zerbo et al., 2004). They observed that mononucleated and binucleated TRAcP cells were resorbing the material after 6 months. Controversies exist in the literature concerning the role of TRAcP positive/TRAcP negative cells in the resorption of Ca/P ceramics. We previously reported that multinucleated TRAcP cells first appeared in contact with Ca/P ceramics and were followed by TRAcP positive cells in a second time (Baslé et al., 1993). When BCP particles were injected in the diaphysis of osteopenic rats, giant TRAcP positive cells were observed (Blouin et al., 2006). An association of true osteoclasts (identified by TRAcP staining and the presence of a ruffled border in transmission election microscopy) has also been confirmed with other types of Ca–P biomaterials (Wada et al., 1989; Wensisch et al., 2003). It was also found that fragmentation of the grafted material could release minute grains that accumulate of β-TCP and hydroxyapatite (HA) have been proposed (and are often referred as BCP – biphasic calcium phosphate). Controversies exist concerning as to how long HA persist in the body and reports from the orthopedic literature indicate that β-TCP provide better results (Ogose et al., 2005). However, the material, when prepared in granules, has poor biomechanical properties that preclude its use in weight-bearing sites. Numerous reports have confirmed that a direct bone apposition can be observed at the surface of β-TCP without interposition of a HA layer (Kotani et al., 1991). Direct bone matrix anchorage has been shown with collagen fibers deposited in the micropores (Chazono et al., 2008). Recently, the surface chemistry and roughness of β-TCP has been found to favor the attachment and spreading of osteoblast-like cells in vitro (Dos Santos et al., 2009). An ideal biomaterial usable as a bone substitute should be a temporary 3D-scaffold serving for osteoconduction. Macroporosity (pore size >100 μm) is necessary to permit vascular and mesenchymal stem cells invasion. The ideal biomaterial should then be removed after complete replacement by newly formed bone. β-TCP is easily resorbable when in the macroporous form (Chazono et al., 2004). The association with autograft bone was found to increase osteoconduction in animal studies and in human surgical practice (Hossain et al., 1996).

Table 1
Histomorphometric measurements obtained on the bone biopsies in patients with sinus floor elevation with β-TCP and morcellized autograft.

<table>
<thead>
<tr>
<th>Duration of the graft (in months)</th>
<th>TV (in mm³)</th>
<th>MatV/TV (in %)</th>
<th>BV/TV (in %)</th>
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<tr>
<td>Standard deviation</td>
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<td>2.48</td>
<td>7.81</td>
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Fig. 2. MicroCT analysis of a bone biopsy performed after 12 months in a patient with sinus lift elevation performed with β-TCP granules. (A) 2D transverse section along the axis of the bone core, β-TCP granules (in black) are more calcified than bone trabeculae (in grey); (B) 3D reconstructed model of all calcified structures in the biopsy core (i.e., bone in and β-TCP granules appear in a blue pseudocolor due to the use of a single threshold); (C) combined model with β-TCP granules made visible (in a yellow pseudocolor) inside a semi-transparent model of the bone (in Q1 blue). The granules appear covered by bone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)
in the cytoplasm of macrophages after phagocytosis. Similar findings were observed in the present study performed on human bone. Macrophages containing β-TCP grains were also encountered in the connective tissue and sometimes expressed a discrete TRAcP positivity (a classical sign of macrophage activation). The presence of mast cells, easily identified in the connective tissue, reflects the enhanced bone remodeling and not an "allergic" or an inflammatory reaction. Although the role of mast cells in bone remodeling is not clearly understood at the present time, these cells are frequently associated with osteoblasts in bone forming areas (Chiappetta and Gruber, 2006; Safar and Klapisz-Wolikow, 1990).

In the bone biopsies of this series, mast cells were frequently observed in the vicinity or in direct contact with the osteoblasts. Direct apposition of lamellar bone onto the particles can often lead to a complete incorporation of pieces of the granules inside the bone trabeculae (Chazono et al., 2004). This could be responsible, in part, for the incomplete degradation of the biomaterial. It was reported that β-TCP was incompletely degraded even after 9.5 months in bone biopsies performed in similar conditions than in this series (Knabe et al., 2008; Zerbo et al., 2004). Because the material is fragile in undecalcified bone sections (some areas are lost during sectioning), morphometry was done by microCT, a highly precise method for the analysis of bone and biomaterials (Chappard et al., 2005a,b). In addition, the method is non-destructive and can be performed before histology, it also allows a 3D visualization of the bone sample and the β-TCP could be evidenced inside the newly formed bone due to its higher calcium content.

Particles of β-TCP appear a very interesting biomaterial for sinus lift elevation since it does not induce inflammation, favor osteoconduction and is highly degraded by macrophages and osteoclasts. The morphology of the granules (with macroporosity) ensures a suitable space for vascular sprouts and for osteoprogenitor cells invasion. The 3D arrangement of granules is also a factor that has received little attention and that could favor osteoconduction (Hsu et al., 2007); more porous granules are under consideration in our laboratory.

Acknowledgments

Authors are greatly indebted to Kasios, ZI La Croix, 31140 Launaguette – France for providing the biomaterial. Mrs Guénaëlle Brossard and Nadine Gaborit are thanked for their skilful technical assistance with microCT and histotechnology. SEM analyses were done at SCIAM (service commun d’imagerie et analyse microscopiques, Université d’Angers).

References


