Histological and Immunohistochemical Study of Biopsies Sampled after Sinus Floor Augmentation Using Tricalcium Phosphate Particles with Varying Porosity

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Introduction: Among the various techniques to reconstruct or enlarge a deficient alveolar ridge, augmentation of the maxillary sinus floor with autogenous bone grafts has become a well-established pre-implantology procedure for alveolar ridge augmentation of the posterior maxilla. Using synthetic biodegradable bone substitutes, however, is advantageous, since it avoids second-site surgery for autograft harvesting. In recent years, the use of tricalcium phosphate (TCP) particles as alloplastic bone graft materials for sinus floor elevation procedures has received increasing attention in implant dentistry.[1] More, recently, the use of TCP particles with increased porosity has been promoted in order to increase the biodegradability. Ideally, a bone substitute material should resorb rapidly, but still stimulate osteogenesis at the same time. In the current study the effect of two particulate TCP graft materials on bone regeneration and expression of osteogenic markers was evaluated in biopsies sampled 6 months after augmentation of the sinus floor. This was in addition to examining the biodegradability.

Methods: The study consisted of 23 patients (12 women and 11 man) ranging in age from 25-75 years. In all patients augmentation of the sinus floor was required in order to facilitate dental implant placement in the posterior maxilla. Since the residual alveolus was 1-3 mm in height, a staged approach was used. Sinus floor augmentation was performed using a combination (2:1 ratio) of β-TCP particles and autogenous bone chips. The TCP particles used were globular particles of 1000-2000 µm grain size with 35% porosity (Cerasorb®, Curasan AG, Germany) or polygonal particles of 1000-2000 µm grain size with 65% porosity (Cerasorb M®, Curasan AG). Dental implants were placed 6 months after sinus floor augmentation. At implant surgery when preparing the implant bed, cylindrical biopsies, 2.5 mm in diameter and 10 mm long, were sampled using a trephine drill. The tissue samples were fixed in an alcohol based fixative Neofix® (Merck AG, Germany). Subsequently the specimens were embedding in a resin composed of polymethyl-methacrylate (PMMA) and polybutyl-methacrylate (PBMA) as described previously.[2] This resin facilitated performing immunohistochemical analysis on hard tissue sections. 50 µm-sections were cut longitudinally using a Leitz 1600 sawing microtome (Leitz, Wetzlar, Germany). Sections were then deacrylized by immersion in toluene, xylene and acetone. Subsequently, immunohistochemical staining was performed using primary antibodies specific to collagen type I (Col I), alkaline phosphatase (ALP) (Sigma, USA), osteocalcin (OC) and bone sialoprotein (BSP) in combination with the DAKO EnVision+™ Dual link System Peroxidase, AEC+ (DAKO, Denmark).[2] Mayer's haematoxylin was used as a counterstain. Semi-quantitative analysis of the sections was performed. A scoring system quantified the amount of staining observed using light microscopy. A score of (+++), (++) and (+) corresponded to strong, moderate or mild, whereas a score of (0) correlated with no staining. Furthermore, histomorphometrical evaluation of the sections was performed. To this end, a square area 1 mm^2 in size was defined in three areas of each sections: First crestally, second apically close to the Schneiderian membrane and third in the center of the cylindrical biopsy. The bone area fraction as well as the particle area fraction was measured in these three areas using a light microscope in combination with a digital camera (Colourview III) and SIS Analysis software (Olympus, Germany).

Results: In the apical area close to the Schneiderian membrane the mean bone area fraction was 16.7% and the mean particle area fraction was 42.8% in the patient group, in which Cerasorb was used as grafting material, whereas in the patients, in which Cerasorb M was used, a mean bone area fraction of 24.9% and a mean particle area fraction of 37.1% was observed. In the other areas the differences between Cerasorb and Cerasorb M were less pronounced. Furthermore, with both materials slight expression of ALP and moderate expression of Col I, OC and BSP was noted in the bone matrix and the matrix components of the bone marrow. Good bone bonding behavior was observed with both materials as well as bone formation within the degrading particles. This was accompanied by expression of Col I, BSP and OC in the newly formed bone in contact with the TCP particles.

Discussion / Conclusions: In case of the Cerasorb M particles, which exhibited a greater porosity compared to the Cerasorb particles, a larger amount of bone formation and particle degradation was observed in the area close to the Schneiderian membrane and thus at the largest distance from the crestal bone compared to the more dense Cerasorb particles. Furthermore, six month after implantation of both kind of β-TCP particles bone formation and matrix mineralization was still actively progressing in the tissue surrounding the TCP particles. Consequently, both TCP materials supported bone formation in the augmented sinus floor. A greater porosity of the particles appears to be advantageous for increasing the biodegradability of such particles.

References:

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