Effect of rapidly resorbable calcium-alkali-orthophosphate bone grafting materials on osteogenesis after sinus floor augmentation in sheep

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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. Although autogenous bone grafts are currently the standard of care, bone substitute materials are extensively studied in order to avoid harvesting autogenous bone. A bone substitute for alveolar ridge augmentation must be rapidly resorbable and should undergo complete substitution by newly formed functional bone tissue in view of placing dental implants in such augmented sites. Compared to the bone substitutes which are currently clinically available, there is a significant need for bone substitutes which degrade more rapidly, but still stimulate osteogenesis at the same time. This has led to the development of novel, bioactive, rapidly resorbable glassy crystalline calcium-alkali-orthophosphate materials.

This study evaluates the effect of two particulate calcium-alkali-phosphate graft materials as compared to the currently clinically used material β-tricalcium phosphate (β-TCP) on bone regeneration and expression of osteogenic markers after sinus floor augmentation in sheep. This was in addition to examining the biodegradability.

Methods: Test materials were two glassy crystalline calcium-alkali-orthophosphates: first, a material with a crystalline phase $Ca_2KNa(PO_4)_2$ and with a small amorphous portion containing silica phosphate and diphosphates ($Ca_2P_2O_7$) (material denominated GB9/25) and second, a material with a novel crystalline phase $Ca_{10}[K/Na](PO_4)_7$ (material denominated 352i). These materials (grain size 300-350 µm) were used for sinus floor augmentation in sheep and were compared to β-TCP particles of the same grain size (Cerasorb®, Curasan AG, Germany). Animals were sacrificed at 4 , 12 and 24 weeks. At implant retrieval the tissue samples were fixed in an alcohol based fixative as described previously.[1] Subsequently the specimens were embedded in a resin which facilitated performing immunohisto-chemical analysis on hard tissue sections.[2] 50 µm-sections were cut using a Leitz 1600 sawing microtome. Sections were then deacrylized and immunohistochemical staining was performed using primary antibodies specific to collagen type I (Col I), alkaline phosphatase (ALP), osteocalcin (OC), bone sialoprotein (BSP), osteopontin (OP) and osteonectin (ON) in combination with the DAKO EnVision+™ Dual link System Peroxidase.[1] Mayer's haematoxylin was used as a counterstain. Semi-quantitative analysis of the sections was performed. 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, the bone area fraction as well as the particle area fraction in the augmented sinus was measured using a light microscope in combination with a digital camera (Colourview III) and SIS Analysis software (Olympus, Germany). This was in addition to determining the bone-particle contact in order to characterize the bone-bonding behavior.

Results: With GB9/25 the mean particle size decreased from 0.060mm^2 to 0.014mm^2 after 24 weeks, while the mean particle sizes of 352i and TCP were 0.042 and 0.022mm^2 (respectively) after 24 weeks of implantation. Furthermore, implantation sites, in which GB9/25 was used as a grafting material, exhibited the smallest particle area fraction after 4, 12 and 24 weeks (Table I) and a greater bone area fraction than 352i and TCP after 12 and 24 weeks. Moreover, GB9/25 particles exhibited the greatest bone-particle-contact. This was accompanied by enhanced expression of OP, OC, ON and BSP in the cell and matrix components of the surrounding bone tissue.

Table I – Bone-particle-contact and particle area fraction in the ovian sinus floor augmented with various grafting materials

Implantation period	Graft material	Particle size (mean) [mm^2]	Bone-particle contact (mean)	Particle area fraction (mean)
4 weeks	GB9/25	0.060	14.23%	59.31%
	352i	0.083	2.01%	67.37%
	TCP	0.063	13.61%	69.74%
12 weeks	GB9/25	0.026	29.29%	10.10%
	352i	0.067	5.63%	50.24%
	TCP	0.032	25.26%	24.50%
24 weeks	GB9/25	0.014	78.31%	0.88%
	352i	0.042	10.53%	31.67%
	TCP	0.022	69.83%	18.58.%

Discussion / Conclusions: Of the various grafting materials studied, GB9/25 showed the best bone-bonding behavior and had the greatest stimulatory effect on bone formation and expression of osteogenic markers, while exhibiting the highest biodegradability. These findings are in accordance with those of a previous study, in which GB9/25 showed a stimulatory effect on osteoblast differentiation *in vitro*.[2] Thus, the calcium- alkali-phosphate material GB9/25 facilitated excellent bone formation in the augmented sinus floor in sheep.

References:

[1] Knabe C et al., Biotech Histochem. 2006;81:31-9.
[2] Knabe C et al., JBMR A. 2008;85:856-68.

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