Effect of rapidly resorbable calcium-alkali-orthophosphate bone substitute materials on osteogenesis and osteoblastic phenotype expression *in vivo*

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Introduction: Among the various techniques to reconstruct or enlarge a deficient alveolar ridge, the concept of guided bone regeneration (GBR) has become a predictable and well-documented surgical approach.[1] At present, autogenous bone grafts are preferably combined with barrier membranes. Using synthetic biodegradable bone substitute materials, however, is advantageous, since it avoids second-site surgery for autograft harvesting. 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 implantation in the sheep mandible. 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 magnesium potassium phosphate (material denominated GB14) 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 implanted in the sheep mandible for 4 , 12 and 24 weeks to regenerate membrane protected critical size defects[1] and were compared to β-TCP particles of the same grain size (Cerasorb®, Curasan AG, Germany). At implant retrieval the tissue samples were fixed in an alcohol based fixative as described previously.[2] Subsequently the specimens were embedded in a resin which facilitated performing immunohisto-chemical analysis on hard tissue sections.[2] 50 µm-sections were cut in a buccal - lingual direction 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.[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 16 mm^2 in size was defined in the centre of the critical size defects. The bone area fraction as well as the particle area fraction was measured using a light microscope in combination with a digital camera (Colourview III) and SIS Analysis software (Olympus, Germany).
Results: With GB14 the mean particle size decreased from 0.06mm^2 to 0.01mm^2 after 24 weeks, while the mean particle sizes of 352i and TCP were 0.0375 and 0.03 mm^2 (respectively) after 24 weeks of implantation. Moreover, the implantation sites, in which GB14 was used as a grafting material, exhibited the greatest bone area fraction in combination with the smallest particle area fraction after 4, 12 and 24 weeks. (Table I).

Table I - Bone and particle area fraction of critical size defects augmented with various grafting materials

Implantation period	Graft material	Particle size (mean) [mm^2]	Bone area fraction (mean)	Particle area fraction (mean)
4 weeks	GB14	0.053	42.6%	19.33%
	352i	0.070	21.5%	37.10%
	TCP	0.055	40.0%	26.00%
12 weeks	GB14	0.0225	74.67%	1.29%
	352i	0.05	34.7%	18.92%
	TCP	0.04	73.0%	4.00%
24 weeks	GB14	0.01	78.17%	0.04%
	352i	0.0375	58.3%	12.40%
	TCP	0.03	77.2%	3.54%

Furthermore, with GB14 enhanced expression of Col I, OC, BSP and OP was noted in the cell and matrix com-ponents of the surrounding bone tissue, when compared to TCP and 352i after 4 , 12 and 24 weeks of implantation.
Discussion / Conclusions: Ot the various grafting materials studied, GB14 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 GB14 showed a stimulatory effect on osteoblast differentiation *in vitro*.[3] Thus, the calcium- alkali-phosphate material GB14 facilitated excellent bone regeneration of critical size defects in the sheep mandible.
References:
[1] von Arx T et al., Clin Oral Impl Res 2001;12:260-9.
[2] Knabe C et al., Biotech Histochem. 2006;81:31-9.
[3] Knabe C et al., JBMR A. 2004;69:145-54.
Acknowledgements: This work was funded by the German Research Foundation (DFG Grant KN 377/3-1).
The authors wish to thank Dr. L. Fisher, NIDCR, U.S.A.