Effect of rapidly resorbable calcium-alkali-phosphate bone grafting materials for alveolar ridge augmentation on bone formation 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. 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 materials β-tricalcium phosphate (β-TCP) and bioactive glass 45S5 (BG) on bone regeneration and expression of osteogenic markers after implantation in the sheep mandible. This was in addition to examining the biodegradability.

Materials and 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 the crystalline phase $Ca_2KNa(PO_4)_2$ and with a small amorphous portion containing silica phosphate (material denominated GB9). These materials (grain size 300-350 µm) were implanted in the sheep mandible for 1, 4, 12 and 24 weeks to regenerate membrane protected critical size defects and were compared to β-TCP and BG particles of the same grain size. Autogenous bone chips and empty defects, which were filled with collagen sponges, served as controls. 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.[1] 50 µm-sections were cut in a buccal - lingual direction using a Leitz 1600 sawing microtom. 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) as described previously.[1] 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: Already after 4 weeks of implantation, defects grafted with GB9 displayed a significantly greater bone area fraction (Fig. 1) and bone-particle-contact than defects grafted with TCP and BG. In this it was followed by GB14, which however at 4 weeks exhibited a significantly lower bone-particle-contact than GB9. This was accompanied by enhanced expression of Col I, OP, OC, ON and BSP in the cell and matrix components of the surrounding bone tissue. By 12 weeks, defects, which were augmented using GB9 and BG, displayed greater bone formation compared to defects, which were augmented with autogenous bone chips (Fig. 1). These findings are clinically very significant, since autogenous bone is considered to be the gold standard. Moreover, already after 4 weeks defects grafted with GB9 and GB14 exhibited a significantly greater decrease in particle area fraction and particle size compared to defects grafted with TCP and BG.

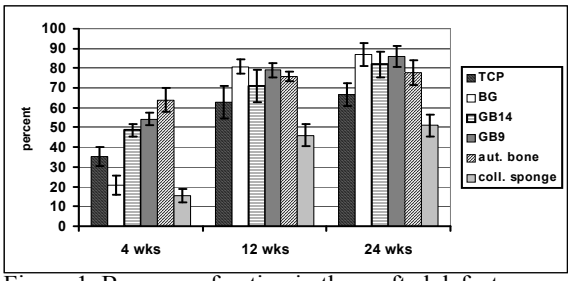

Figure 1. Bone area fraction in the grafted defect area

Discussion/Conclusions: Of the various grafting materials studied, GB9 had the greatest stimulatory effect on bone formation and expression of osteogenic markers, while exhibiting the best bone bonding behavior and highest biodegradability. These findings are consistent with those of a previous study, in which GB9 showed the greatest stimulatory effect on osteoblast differentiation *in vitro*.[2] Thus, the material GB9 facilitated excellent bone regeneration of critical size defects in the sheep mandible.

References:

1. Knabe, C., et al., Biotech Histochem., 81, 31, 2006.
2. Knabe, C., et al., JBMR A., 84, 856-68, 2008.

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