Effect of resorbable calcium-alkali-orthophosphate bone substitute cements on osteogenesis after implantation in the rabbit femur

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Introduction: Although autogenous bone grafts are currently the standard of care for bone reconstruction, bone substitute materials are extensively studied in order to avoid harvesting autogenous bone. To fill bone defects, calcium phosphates are mainly applied as granules. Bone substitutes with improved surgical handling properties include mouldable calcium phosphate cements in paste form that can be either introduced into a bony defect with a spatula or injected with a syringe; they subsequently set in situ, which makes them an intriguing group of materials for bone reconstruction. Over the past decade various bioactive calcium phosphate cements have been developed. In most cases hydroxyapatite is formed during setting, which limits their biodegradability. More recent developments include cements which form calcium-alkali-phosphates during setting, which have been shown to have a stimulatory effect on osteogenesis in vitro,[1] and in vivo.[2] These cements are designed for higher biodegradability. This study evaluates the effect of four calcium-alkali-phosphate-based bone substitute cements as compared to the currently clinically used material β-tricalcium phosphate (β-TCP) on bone regeneration and expression of osteogenic markers after implantation in critical size defects in the rabbit femur. This was in addition to examining the biodegradability.

Methods: Test materials were four resorbable calcium-alkali-phosphate-based bone substitute cements: first, a cement which forms the crystalline phase $Ca_2KNa(PO_4)_2$ and also has a small amorphous portion containing silica phosphate (denominated GB9-Z); second and third, two cements which form the crystalline phase $Ca_2KNa(PO_4)_2$ and have a small or slightly greater amorphous portion containing magnesium potassium phosphate (GB14-Z and GB14/433-Z); fourth, a cement which forms the crystalline phase $Ca_{10}[K/Na](PO_4)_7$ (352i-Z). These cements were implanted in critical size defects in the rabbit femur and were compared to β-TCP particles (Cerasorb®, Curasan Inc., Germany). Animals were sacrificed at 4, 12, 24, and 48 weeks. At implant retrieval the tissue samples were fixed in an alcohol based fixative as described previously.[3] Subsequently, the specimens were embedded in a resin which facilitated performing immunohistochemical analysis on hard tissue sections.[3] 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.[3] 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, the bone-area-fraction as well as the cement-area-fraction in the defects 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-cement-contact in order to characterize the bone-bonding behavior.

Results: Among the various bone substitute cements tested, defects augmented with the GB9-Z cement exhibited a greater bone-area-fraction after 4, 12, 24 and 24 weeks of implantation than all other cements (Fig. 1). This was in addition to a greater bone-cement-contact and a significantly smaller cement-area-fraction (i.e. greater biodegradability), and was accompanied by enhanced expression of ALP, OC and BSP in the cell and matrix components of the surrounding bone tissue. At 12 and 24 weeks the bone-area-fraction, however, was smaller than that in defects augmented with the reference TCP particles. The biodegradability of the TCP particles was higher than that of the various cements examined.

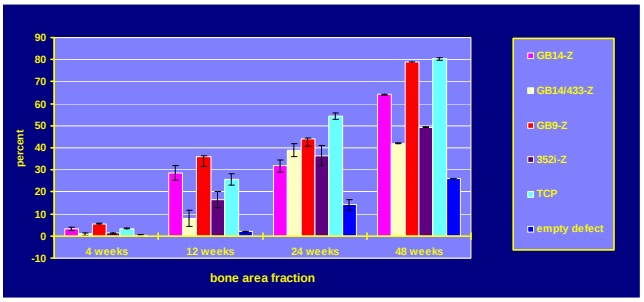

Fig. 1. Bone-area-fraction in rabbit femoral defects augmented with various injectable bone substitute cements.

Discussion / Conclusions: Of the various bone substitute cements studied, the GB9 cement 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. The biodegradability, however, was lower than that of the TCP granules. Thus, the calcium-alkali-phosphate based cement GB9-Z facilitated excellent bone regeneration of critical size defects in the rabbit femur. Current efforts deal with increasing the porosity of the cement during setting in order to accelerate the biodegradation.

References:

[1] Knabe C et al., JBMR A. 2008;85:856-68.
[2] Knabe C et al., Trans 8th WBC 2008; p. 035.
[3] Knabe C et al., Biotech Histochem. 2006;81:31-9.

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