Tricalcium Phosphate Paste and Foam Bone Grafting Materials enhance Osteogenesis *in vivo*

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Introduction: Over the last two decades, there has been an ever increasing search for adequate synthetic bone grafting materials in order to avoid autograft harvesting as well as the risk of disease transmission associated with human allografts. An ideal bone substitute should be bioactive, thus stimulate osteogenesis and then resorb within the newly formed bone, resulting in bone repair with fully functional bone tissue. The clinical success rates which have been achieved with bioactive tricalcium phosphate (TCP) bone grafts demonstrate that these materials have become an excellent alternative graft choice.[1,2] To fill bone defects, TCPs are mainly applied as granules. In order to improve surgical handling properties TCP granules have been combined with natural polymers in order to create paste- and foam-like materials, which can easily be moulded into any desired shape when grafting a given bony defect or injecting them with a syringe. This study evaluates the effect of a TCP-paste and a TCP-foam bone grafting material as compared to β-TCP granules on bone regeneration and osteogenic marker expression after implantation in the ovine scapula. This was in addition to examining the biodegradability.

Materials & Methods: Test materials were: first: a TCP-paste (TCP-P) made by mixing TCP granules (65% porosity, grain size 7-63 µm with an aqueous polymer solution composed of 0.7 Ma.-% modified methylcellulose and 0.4 Ma.-% sodium hyaluronate; and second: a TCP-foam material (TCP-F) made from 85 wt% β-TCP granules (porosity 65%), composed of two grain size fractions: (1) 150-500 µm and (2) 1000-2000 µm, and 15% porcine collagen. These test materials were implanted in the sheep scapula for 1, 3, 6, 12 and 18 months to regenerate critical-size defects, 8 mm in diameter and length, and were compared to β-TCP (Cerasorb M®, Curasan AG, Germany) granules (grain size 1000-2000 µm) (TCP-G) of the same porosity. Empty defects served as controls. At implant retrieval the tissue blocks were fixed in an alcohol based fixative and then embedded in a resin which facilitated performing immunohistochemical analysis on hard tissue sections.[3] 50 µm-sections were cut parallel to the long axis of the defects 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), and bone sialoprotein (BSP), in combination with the DAKO EnVision+TM 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.[1-3] Furthermore, histomorphometric evaluation of the sections was performed. The bone area fraction as well as the grafting material area fraction of the defect was measured using a light microscope in combination with a digital camera and Cell Sense software (Olympus).[1]

Results: Already at 1 month, defects grafted with the TCP-P and TCP-F test materials displayed excellent woven bone formation, which was advancing from the periphery towards the center of the defect. This was accompanied by enhanced expression of OC and BSP in the cell and matrix components of the surrounding bone tissue. By 3 months, with all three grafting materials woven bone formation had increased and defect regeneration had occurred with beginning remodeling of the woven bone. The remodeling process had further progressed by 6 months and was completed by 12 and 18 months (Fig. 1a). Thus, by 3 and 6 months all bone grafting materials studied facilitated excellent bony regeneration of critical-size defects in the ovine scapula with further bone remodeling at 12 and 18 months, while only marginal bone formation was noted in the empty defects (Fig. 1a). The TCP residues of all three materials exhibited excellent bone-particle contact that is excellent bone-bonding behavior. At 1 and 3 months TCP-F displayed the highest biodegradability, while at 6 and 12 months the biodegradability was greatest with TCP-P (Fig. 1b). At 18 months, all three grafting materials were almost fully resorbed with the original bony architecture being fully restored.

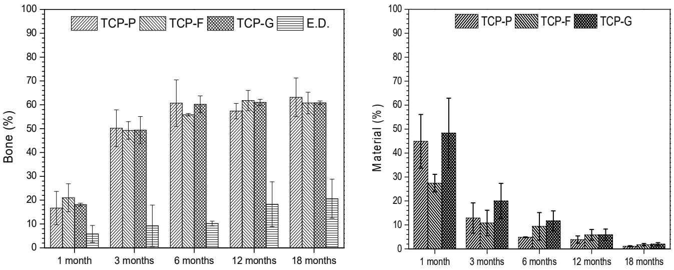

Fig. 1. Histomorphometric results (mean ± SD): (a, left) bone area fraction; (b, right) graft area fraction in the grafted area. The groups are TCP-P (paste), TCP-F (foam), TCP-G (granules), E.D. empty defects.

Discussion/Conclusions: Both, TCP-P and TCP-F facilitated excellent bone regeneration of critical-size defects in the sheep scapula similar to that of the clinically used TCP-G while exhibiting a greater biodegradability. Hence, both TCP-P and TCP-F proved to be excellent bone grafting materials with slightly superior osteogenic capacities than TCP-G, thereby providing TCP-based bone substitutes with advanced surgical handling properties for clinical applications, in which granules are not the most suitable form of application for grafting of a given bony defect.

References:

[1] Knabe C. Biomaterials 2008;29:2249-2258.
[2] Knabe C. Biomaterials 2017;123:48-62.
[3] Knabe C. Biotech Histochem. 2006;81:31-39.