Effect of Tricalcium Phosphate-based Paste- and Foam-like Bone Grafting Materials on Bone Regeneration and Osteogenic Marker Expression *in vivo*

C Knabe, M Lopez-Heredia, D Barnewitz*, A Genzel*, F Peters**, A Kuhr, B Stang, W-D Huebner**
Department of Experimental and Orofacial Medicine, Philipps University Marburg, Marburg, Germany; * Veterinary Research Center, Bad Langensalza, Germany; ** Curasan AG, Frankfurt, Germany

Introduction: Although autogenous bone grafts are currently the gold standard for bone reconstruction in orthopaedics and maxillofacial repair, biodegradable synthetic bone substitutes are extensively studied in order to avoid harvesting of autogenous bone. Over the last decade, the use of tricalcium phosphate (TCP) granules as alloplastic bone graft has received increasing attention in implant dentistry and orthopaedics.[1,2] More, recently, the combination of TCP ceramics with collagen scaffolds or carriers including natural polymers such as hyaluronic acid has been proposed in order to improve surgical handling properties,[2] and to create TCP-based foam and paste materials, which can easily be moulded into any desired shape when inserting them in a given bony defect or injecting them with a syringe. This study evaluates the effect of a TCP-paste and a TCP-foam-like bone grafting material as compared to the currently clinically used β-TCP granules on bone regeneration and expression of osteogenic markers after implantation in the sheep scapula, 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 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 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, alkaline phosphatase, osteocalcin, and bone sialoprotein, 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] Furthermore, histomorphometrical 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 (Colourview III) and SIS Analysis 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 marginal defect areas towards the center of the defect. This was accompanied by enhanced expression of osteocalcin and bone sialoprotein 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 sheep 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.

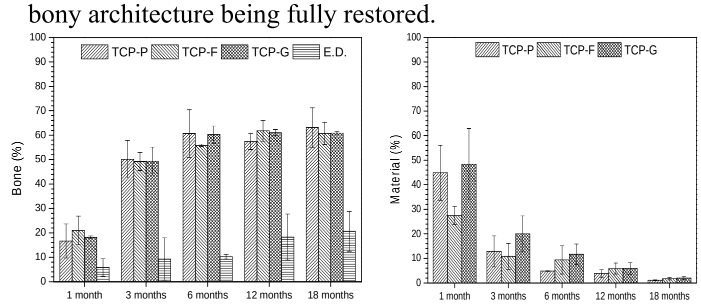

Fig. 1. Histomorphometric results (mean ± SD): (a, left) bone area fraction; (b, right) graft area fraction in the grafted area. The groups are TCP-P (paste), TCP-F (foam), TCP-G (granules), E.D. empty defects.

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 established TCP granules while exhibiting a greater biodegradability. Hence, both TCP-P and TCP-F proved to be excellent bone grafting materials with slightly superior osteogenic capacities compared to the TCP-G, thereby providing TCP-based bone substitutes with advanced surgical handling properties for applications, in which granules are not a suitable form of application for grafting and reconstruction of a given bony defect.

References:

[1] Knabe C. Biomaterials 2008;29:2249-2258.
[2] Bohner M. Mater Today 2010;13:24-30.
[3] Knabe C. Biotech Histochem. 2006;81:31-39.