Microparticle Amount in CalciumPhosphate/PLGA Composites affects Material Degradation and Bone Formation In Vivo

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Introduction: Calcium phosphate (CaP) cements are promising candidates for bone substitution in dentistry and orthopedics and reconstructive surgery, due to their biocompatibility and osteoconductive properties, as well as the potential to use minimally invasive surgery. The main disadvantage of the cement is its poor degradation. Degradation speed can be modulated by introducing biodegradable polymeric microparticles to increase the porosity. A previous study with poly(DL-lactic-co-glycolic acid) (PLGA) microparticles, the addition of 20 wt.% PLGA (low molecular weight; LMW) in CaP cement demonstrated the capability of closing a rat cranial critical-sized defect in 12 weeks[1]. However, that particular study provided no temporal information on degradation, bone formation and the effect of different amounts of polymeric microparticles. Consequently, the current study aimed to evaluate temporal degradation and bone formation related to amount of PLGA microparticles incorporated in CaP cement using a rat critical-sized cranial defect model. It was hypothesized that a higher amount of PLGA microparticles will accelerate degradation and hence allow faster bone formation.

Methods: *Preparation of PLGA/CaP cement composites* LMW PLGA (Purac biomaterials, The Netherlands) (4.54 ± 0.02 kg/mol) was used for microparticle preparation. PLGA-microparticles were prepared by a double-emulsion-solvent-extraction technique. PLGA microparticles were mixed with the CaP cement powder at 20 or 30 wt.% resulting in two experimental groups, i.e. 20PLGA and 30PLGA. The cement was created by adding 2 wt. % Na_2HPO_4 to the PLGA/CaP powder in a liquid/powder ratio of 0.35. After mixing, the cements were injected in Teflon molds (diameter 7.8 mm, height 1.8 mm).
Surgical procedure In 48 adult male Wistar rats (295 ± 29 g), the outline of the cranial critical-sized defect was created with a dental trephine drill (OD:8.0 mm, ACE dental implant system, USA). Subsequently, a full thickness bone defect was created by removing cortical bone with an ultrasonic device (Piezosurgery, Mectron, Carasco, Italy) using constant cooling with sterile saline. Pre-set implants were placed in the created defects for 4, 8 and 12 weeks.
Histology and histomorphometrical procedures After sacrifice, implants and surrounding tissue were retrieved and embedded in methylmethacrylate. The sections (n=3 per specimen) were stained with methylene blue and basic fuchsin. From digitalized images, the amounts of implant material and newly formed bone was determined within two regions of interest (ROI 1, within the regions of the implant; ROI 2, situated directly under implant) using computer-based image analysis techniques (Leica Qwin Proimage analysis system, Wetzlar, Germany).

Results: Implantation of the composite discs did not induce any macro- or microscopical signs of inflammation or adverse tissue responses. All animals remained in good health during the experiment. Although only limited implant degradation was observed during the entire implantation period, implant degradation was significantly higher for 30PLGA compared to 20PLGA. Within the initial implant region (ROI-1), no significant differences in bone formation were observed between the experimental groups. However, at 4 and 12 weeks more bone ingrowth was observed in 30PLGA compared to 20PLGA. Bone formation underneath the initial implant region (ROI-2) showed a temporal increase for 20PLGA and 30PLGA. The total bone formation (ROI1 + ROI2) was comparable for 20PLGA and 30PLGA at all times. However, a significant temporal increase of total bone formation was only observed for 30PLGA (4wks vs. 12 wks p=0.029; 8wks vs 12 wks p=0.036). After 12 weeks, the total amount of newly-formed bone was ~20% of the native amount for both groups. A significant temporal increase of total bone formation related to the native initial bone was observed for 30PLGA. The percentage of defect bridging was comparable for 20PLGA and 30PLGA at all times.

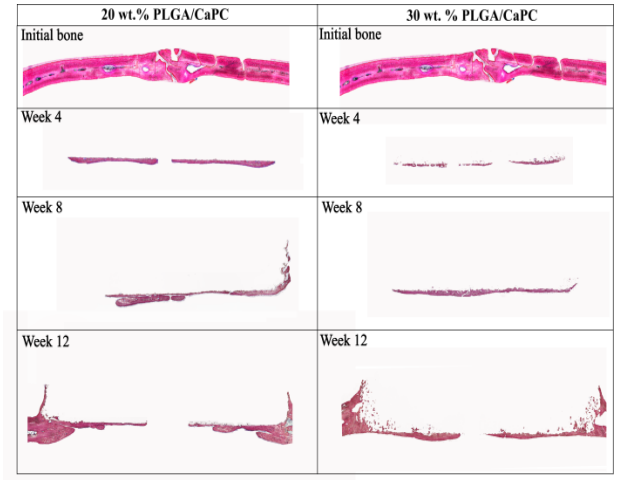

Figure 1: Overview of the temporal total bone formation for 20PLGA and 30PLGA compared to the initial native bone.

Conclusion: A higher amount of PLGA-microparticles in CaP cement beneficially affects bone formation. This higher bone formation can be explained by the accelerated degradation of the composite material due to the increased porosity of the composite material after hydrolytical microparticle degradation. Further optimization of the composite material is necessary to increase control over degradation and tissue ingrowth.

References:
(1) Bodde EWH. Tissue Eng. Part A. 2009;15: 3183-3191