

Size Matters: Effects of PLGA-Microsphere Size in Injectable CPC/PLGA on Bone Formation

Jeroen JJP van den Beucken¹, Rosa Felix Lanao¹, Hongbing Liao¹, Nuo Zhou², Sanne K Both¹, Joop GC Wolke¹, John A Jansen¹

¹Department of Biomaterials, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

²College of Stomatology, Guangxi Medical University, Nanning, People's Republic of China

Statement of Purpose: Bone grafting has become a substantial surgical intervention in medical and dental healthcare. In view of availability and quality, off-the-shelf available synthetic bone substitute materials are becoming increasingly important over autograft. Especially injectable bone substitutes are appealing owing to minimally invasive surgery and optimal bone defect filling. Injectable calcium phosphate cement (CPC) holds promise for its osteophilic properties, but remains limited regarding slow degradation properties¹.

In an approach to increase degradation of CPC, we have developed a porogen platform based on poly(lactic-co-glycolic acid) (PLGA) microspheres, which can be mixed with the CPC ceramic platform. Modulation of this porogen platform has shown effects of PLGA molecular weight, end-functionalization and morphology on CPC/PLGA degradation^{2,3}. So far, effects of PLGA-microsphere size have not been explored.

The aim of this study was to elucidate the effect of pore dimensions within CPC on bone formation in a guinea pig tibial intramedullary model after injection of CPC/PLGA formulations with either small (S; ~25 μ m) or large (L; ~100 μ m) microspheres.

Methods: CPC consisted of 85% (wt/wt) α -TCP, 10% (wt/wt) DCPA and 5% (wt/wt) precipitated HA. PLGA-microspheres were made from Purasorb[®] PLGA (MW=17kDa, acid-terminated, L:G=50:50) according to an established double emulsion solvent evaporation technique with variation in emulsification speed to obtain size differences. CPC/PLGA formulations were generated by combining CPC (0.8 g) and PLGA-microspheres (0.2 g) in a 2-ml plastic syringe with subsequent sterilization via gamma irradiation. A filter-sterilized 2% Na₂HPO₄ solution was used as the liquid component (0.38 ml/syringe). The size distribution of PLGA-microspheres was assessed and pre-set scaffolds were physicochemically characterized (SEM and porosity).

A total of 20 female guinea pigs (age: 7 months) were used after approval of the Animal Ethics Committee and according to national guidelines for the care and use of laboratory animals. General inhalation anesthesia was used, during which the animal was placed on its back while 2 incisions were along the distal and proximal tibial epiphysis. After exposure of the tibia, full-thickness cortical defects (2 mm) were made both distally and proximally, after which the content of the marrow space was evacuated by curettage with a dental file and saline irrigation. CPC/PLGA formulations were prepared and injected into the ablated tibial medullary cavity, allowed to set for 10 minutes, and then the soft tissues were closed in 2 layers. After a 12-week implantation period, the tibias were retrieved and histologically and histomorphometrically evaluated following MMA-embedding and sectioning.

Results: Both CPC/PLGA formulations showed bone ingrowth throughout the entire scaffold material upon PLGA-microsphere degradation. Specifically, the pattern of bone and marrow formation showed distinct differences related to PLGA-microsphere dimension, with large round bone structures containing marrow-like tissue for CPC/PLGA-L and small round bone structures with marrow-like tissue in between fused bone structures for CPC/PLGA-S (Figure 1). Additionally, the amount of bone formation for CPC/PLGA-S was 2-fold higher compared to CPC/PLGA-L (Figure 2).

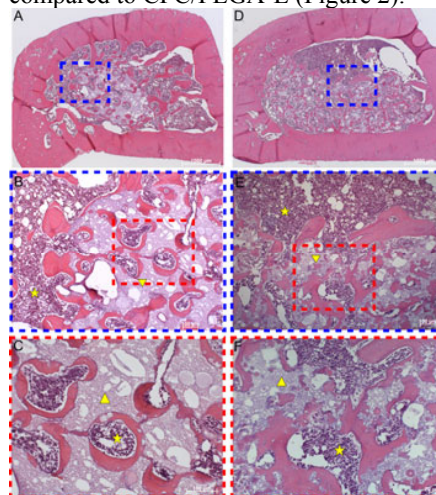


Figure 1: Histological sections of CPC/PLGA-L (left panels) and CPC/PLGA-S (right panels) within the tibial medullary cavity at different magnifications. Asterisks indicate bone-marrow-like tissue, arrows indicate CPC remnants.

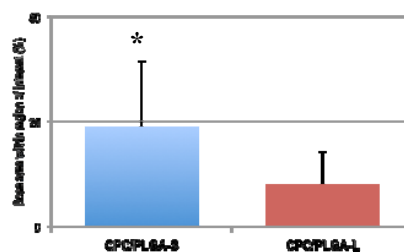


Figure 2: Bone amount within region of interest. (* $p < 0.05$)

Conclusions: This study demonstrates that PLGA-microsphere dimensions of ~25 μ m are sufficient for bone ingrowth and allow significantly more bone formation compared to ~100 μ m PLGA-microspheres. Further, the histological data demonstrate that PLGA-microsphere dimensions provide a tool to control bone formation for injectable CPC/PLGA bone substitute materials.

References:

- Liao H, Walboomers XF, Habraken WJ, Zhang Z, Li Y, Grijpma DW, Mikos AG, Wolke JG, Jansen JA. Injectable calcium phosphate cement with PLGA, gelatin and PTMC microspheres in a rabbit femoral defect. *Acta Biomater.* 2011 Apr;7(4):1752-9.
- Félix Lanao RP, Leeuwenburgh SC, Wolke JG, Jansen JA. In vitro degradation rate of apatitic calcium phosphate cement with incorporated PLGA microspheres. *Acta Biomater.* 2011 Sep;7(9):3459-68.