Preparation and Evaluation of β-Tricalcium Phosphate/Polylactide Microspheres as Bone Repairing Materials

Cheng-Wen Lan, Pei-Hua Tsai, Shwu Jen Chang, Shyh Ming Kuo, Gregory Cheng-Chie Niu
Department of Biomedical Engineering, I-Shou University Kaohsiung County, Taiwan, ROC.

Introduction: Synthetic biodegradable polymers, particularly for polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers, have been used widely in tissue repairing materials and drug delivery systems for their relatively good biocompatibility and ability to be manufactured into a variety of shapes to fit a given defect. However, the release of acidic by-products, during the degradation and the lack of bioactive function, limits their applications on bone tissue repairing. Having the chemical composition close to the mineral composition of natural bone, calcium phosphate ceramics have been extensively employed as a bone substitute and revealed to be an invaluable osteo-integrative material. In this study, novel bioactive and biodegradable microspheres, composed of β-tricalcium phosphate (β-TCP) and PLA, were fabricated by w/o/w emulsion technique. Our results demonstrated that these composite microspheres showed tardy release behavior and remarkable osteoconductive ability for bony tissue repairing.

Methods: The β-TCP/PLA microspheres were prepared by using a water-in-oil-in-water (w/o/w) emulsion system. Briefly, the inner water phase, consisted of β-TCP (0.18 g) in aqueous PVA solution (3%, 0.5 mL), was emulsified into oil phase (12.5% PLA, 4 mL) for 9 min as primary emulsion. The mixture was then poured into the outer aqueous PVA solution (3%, 100 mL) under vigorous stirring for 6 h at room temperature as the second emulsion. The microspheres, collected by centrifugation, were washed three times in distilled water and freeze-dried.

Results/Discussion: From the results of the particle size analysis, it revealed that the higher the concentration of PLA solution, the higher the viscosity of oil phase and thus the lager the particle size. The SEM examination also showed that the composite microspheres exhibited good sphericity and a relatively smooth surface (Fig. 1a). The energy dispersive X-ray (EDX) analysis suggested that TCP was entrapped inside of PLA microspheres by the w/o/w emulsion method (Fig. 1b).

(a) (b)

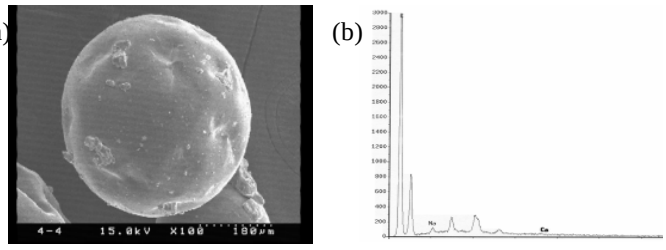

Figure 1. The SEM photograph (a), and the EDS analysis pattern (b) of the β-TCP/PLA microsphere surface.

The results of degradation test show that the microspheres had weight loss ratio about 40% after 30 days of shaking and there was no significantly difference between the composite and PLA-only microspheres (Fig. 2a). The released calcium ion due to the degradation of β-TCP/PLA microsphere was detected by atomic absorption spectrophotometer. At the first 10 days, the concentration of Ca^{2+} rapidly raised with a linear relation to the degradation rate. The other β-TCP inside of microsphere would be released while the middle layer, PLA, degraded much later and that caused the tardy release ration (Fig. 2b).

(a) (b)

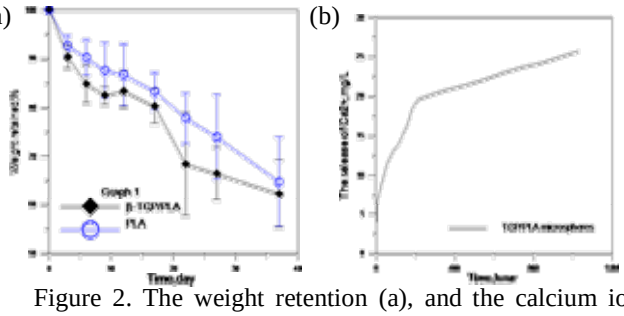

Figure 2. The weight retention (a), and the calcium ion release behavior (b) during degradation of microspheres.

The macroscopic appearance of the histological sections showed that in the unfilled defect, which was created in the interior of the knee joint of New Zealand white rabbits, hardly showed any osteoblast in 4 weeks. And therefore, suggested that no callus would emerged (Fig. 3a). In the β-TCP/PLA group, although a small amount of fibroblasts and mononuclear cells appeared around the implant, and osseous callus also appeared toward the central of the defect (Fig. 3b).

(a) (b)

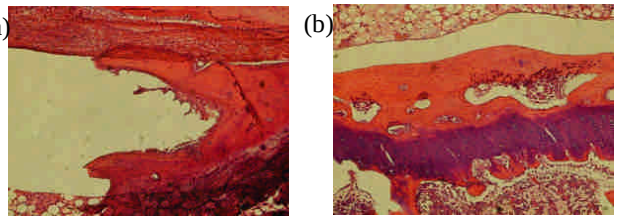

Figure 3. Histological section of condyle defect at 4 weeks for (a) unfilled control group and (b) β-TCP/PLA microspheres implantation.

Conclusions: In this study we have successfully fabricated β-TCP/PLA microspheres by water-in-oil-in-water (w/o/w) emulsion system. The EDX analysis and X-Ray diffractometer results confirm that β-TCP is packed inside of PLA microsphere and the loading capacity is 75%. These composite microspheres also show remarkable osteoconductive ability and thus enable the gradual remodeling of new bone.