Degradation Study of Raw Material Encapsulated Microsphere-Based Scaffolds for Osteochondral Tissue Engineering

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Introduction: Chondroitin sulfate (CS) and tri-calcium phosphate (TCP) provide biological cues that promote ostechondral tissue induction. Enabling release of these 'raw materials' in a time-controlled fashion may facilitate the regeneration of the osteochondral interface. The current degradation project focused on using poly(D, L-lactic acid-co-glycolic acid) (PLGA) with different intrinsic viscosities and measured release rates of CS and TCP, and the dry weight and molecular degradation of PLGA over a period of four weeks.

Materials and Methods: PLGA-CS was prepared by dissolving 4% w/v CS to 16% PLGA(50:50; IV: 0.65dL/G) and PLGS-TCP was similarly prepared by dissolving 1.5% TCP w/v to 18.5% PLGA (75:25; IV: 0.71dL/G) (Evonik Biomaterials,USA). Encapsulated CS or TCP microspheres were fabricated by our Precision Particle Fabrication (PPF) apparatus, lyophilized, and sintered using ethanol-acetone (95:5 v/v) for 45 minutes. Additionally, sodium carbonate buffer was encapsulated with raw materials to help neutralize the acidity of PLGA degradation products. Following ethylene oxide sterilization, scaffolds were placed in 24 well plates and incubated with sterile PBS (Invitrogen, Carlsbad, USA) at 37^{o}C for up to four weeks and collected for different assays. Over different time points, these scaffolds were sputter coated and analyzed for surface topology using Scanning Electron Microscopy (SEM). Further, mechanical integrity was measured by a uniaxial testing apparatus (Instron Microtester 5848, Norwood, MA) under unconfined compression at all of the time points. Biochemical assays quantified the amount of glycosaminoglycans (GAG) and calcium (Blyscan assay, Biocolor life sciences, UK) at weeks 0, 1, 2, 3 & 4.

Results: The chondrogenic microsphere-based scaffolds (PLGA-CS) had interconnected pores throughout the material, while the osteogenic microsphere-based scaffolds (PLGA-TCP) had rough edges and the surface was observed to be more uniform. However, at successive time points (weeks 2 and 3), pores in the chondrogenic scaffolds increased in diameter and by the end of week 3, almost becoming macroporous and completely degraded. Week-4 represents the flat scaffold without any semblance of initial morphology. Osteogenic microspheres on the other hand developed wider cracks with successive weeks but did not degrade macroscopically at the end of week-4 (Fig. 1).

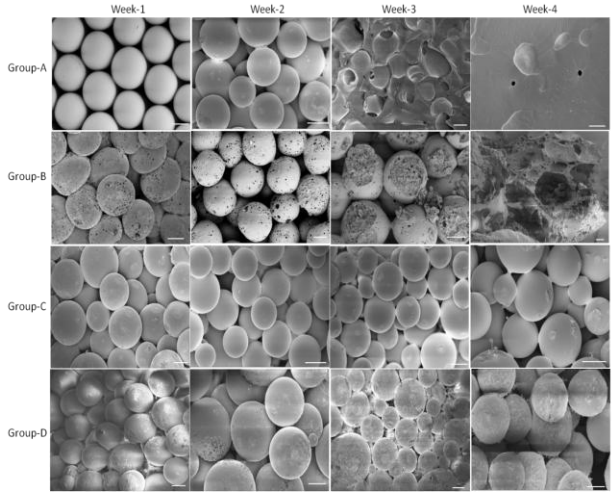

Figure 1. **SEM micrographs of the two types of microspheres with the control groups at different time points. (**A): PLGA (50:50) without CS,(B): PLGA-CS, (C): PLGA (75:25) without TCP, (D): PLGA-TCP. Scale bars: 200μm.

Conclusions: From the SEM images, it can be concluded that PLGA (50:50) degraded at a much faster rate than PLGA (75: 25) and the presence of CS led to increased porosity and altered the surface morphology. By the end of four weeks, we observed complete degradation of the scaffold, which implies that most of the CS was released into the microenvironment. On the other hand, the PLGA (75:25) scaffold increased in pore size but did not macroscopically degrade within four weeks, which can be exploited to release late biochemical cues (TCP) for bone regeneration in a controlled fashion. Additional experiments testing our claim (GAG assay, calcium assay, compressive modulus, molecular degradation measurement, mass loss analysis) are in progress and will reported at the conference.

References:

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