Layered, multicompartment scaffolds with continuous interfaces for osteochondral tissue engineering

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Statement of Purpose: There is a pressing need to improve upon current treatments for injuries to articular cartilage and the underlying subchondral bone. Articular-joint surfaces contain two distinct tissue types, bone and articular cartilage, meeting at a smooth, stable interface. An emerging trend in the field of biomaterials research is the production of layered osteochondral scaffolds for the simultaneous treatment of compromised cartilage and subchondral bone in articular joint defects. In nearly all layered scaffolds reported to date the transition between the osseous and cartilaginous compartments occurs suddenly, at an abrupt "hard" or "discrete" interface created by joining of two materials via suturing or gluing. Few scaffolds incorporate the characteristics of the *interface* between bone and cartilage as a fundamental design consideration. The objective of this study was to build upon previous technologies to fabricate uniform collagen-GAG (CG) [1] and mineralized CG (CGCaP) [2] scaffolds to create multi-compartment scaffolds that incorporated a continuous interface between the mineralized ('osseous') and non-mineralized ('cartilagenous') regions.

Methods: Multicompartment scaffolds were fabricated via lyophilization from CG and CGCaP suspensions. The CGCaP suspension was produced by combining type I collagen, GAG, and calcium salts ($Ca(NO_3)_2{\cdot}4H_2O$, $Ca(OH)_2$) in a solution of phosphoric acid (pH 3.2) [2]. Reactant ratios were selected to produce a CGCaP suspension with a 50% CaP mass yield via simultaneous, titrant-free control of pH and brushite mass yield [2]. The unmineralized type II collagen-GAG (CG) suspension was obtained from Geistlich Biomaterials (Wolhusen, Switzerland). A novel 'liquid-phase co-synthesis' method was used to form both scaffold compartments from separate liquid precursors. The CGCaP and CG suspensions were layered and allowed to interdiffuse before lyophilization. The layered, interdiffused suspensions were solidified using a previously described constant cooling rate technique [1] to produce a uniform pore microstructure with regular, polyhedral pores. The ice phase was sublimated to produce a porous osteochondral scaffold that comprised a mineralized, (type I) CGCaP scaffold (osseous) compartment, an unmineralized, (type II) CG scaffold (cartilagenous) compartment, and a continuous interface between the two regions. Results of microstructural, compositional, and mechanical tests on the interfacial scaffolds are reported.

Results: MicroCT analysis of the osteochondral scaffolds showed clearly distinguishable mineralized and unmineralized scaffold layers. A homogeneous pore microstructure with interconnected pores is observed throughout the scaffold; pore volume fractions of 84 ± 3% and 98.3 ± 0.2% (Mean ± SD) with mean pore sizes of 419 ± 67 μm and 653 ± 52 μm (Mean ± SD) were observed in the mineralized and unmineralized compartments, respectively. Pores in the mineralized and unmineralized compartments were polyhedral and interconnected. No voids are observed at the interface, and collagen fibers that extend across the interface between osseous and cartilagenous compartments appear mineralized in the osseous compartment and unmineralized in the cartilagenous compartment. Quantification of mineral (Ca and P) content via EDX analysis through the thickness of the osteochondral scaffold revealed a high concentration of mineral in the osseous compartment that was almost two orders of magnitude greater than that in the cartilagenous compartment. The more compliant unmineralized layer (E = 30.0 ± 3.9 kPa, dry) experiences large deformations under compressive loading, resulting in near-complete densification of the cartilaginous compartment at stresses insufficient to induce observable deformation in the mineralized, (E = 762 ± 188 kPa dry) osseous compartment. Despite repeated application of a strong compressive force resulting in greater than 75% strain in the cartilagenous compartment, the scaffold maintains its structural integrity and shows no signs of delamination.

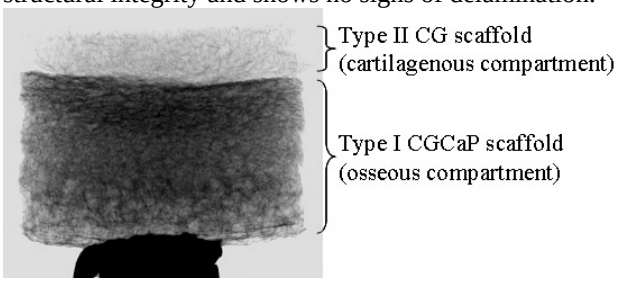

Figure 1. MicroCT image of the osteochondral scaffold (8mm diameter) showing distinct CG and CGCaP regions.

Conclusions: A current emerging trend for the simultaneous treatment of compromised cartilage and subchondral bone in articular-joint defects in the field of biomaterials research is the production of layered, osteochondral scaffolds. Microstructural, chemical, and mechanical analysis of the osteochondral scaffold suggests that the liquid-phase co-synthesis method allows fabrication of a scaffold with distinct regions characterized by differential pore microstructure, mechanical properties, and chemical composition but with a continuous interface between the regions. These layered, osteochondral scaffolds provide a platform for developing *in vivo* cartilage regeneration strategies as well as a framework for developing additional tissue engineering constructs for other orthopedic applications where an interface between hard and soft tissue exists.

References:

[1] Harley BA. Acta Biomat. 3(4):463-474, 2007.
[2] Harley BA. J Biomed Mater Res Pt A, In press 2008.