Design and Fabrication of an Integrated Bi-layered Scaffold for Osteochondral Tissue Engineering

Anna Galperin[1], Rachael A. Oldinski[1], Stephen J. Florczyk[1], James D. Bryers[1], Miqin Zhang[1], Manner A. Paul[1], Buddy D. Ratner[1]

[1]University of Washington, Seattle, WA 98195, USA

Statement of Purpose: Erosion of articular cartilage results in loss of tissue and subsequent destruction of subchondral bone, generating an osteochondral defect. Osteochondral tissue engineering would benefit from the use of bi-layered scaffolds to promote simultaneous regeneration of articular cartilage and subchondral bone.[1] Here we demonstrate the design and fabrication of a bi-layered scaffold with controlled porosity composed of degradable poly(hydroxyethyl methacrylate) (polyHEMA) with defined molecular weight (Mw). Each layer of the bi-layered scaffold was designed with defined pore sizes and biomaterial compositions selected specifically to support regeneration of the two different tissues: avascular cartilage and vascular subchondral bone. In the preliminary study the bone region of the scaffold was successfully seeded with human mesenchymal stem cells (hMSCs).

Methods: A bi-layered scaffold based on degradable polyHEMA (Mw 20K) was fabricated using a sphere-templating technique.[2] Briefly, poly(methyl methacrylate) (PMMA) beads 188±26 µm in diameter were packed together and sintered. PMMA beads 36±2 µm in diameter were coated with hydroxylapatite (HAp) nanoparticles (1% w/w) and sintered. The two PMMA bead templates were placed together in a glass mold and infiltrated with a reaction mixture of HEMA, bi-functional polycaprolactone (PCL)-based initiator, PCL dimethacrylate (PCLDMA), methacrylated hyaluronic acid (HAc-MA), 2,2'-bipyridyl, and $CuCl_2$ dissolved in DMSO. Atom transfer radical polymerization (ATRP) of HEMA was performed for 24 h at RT.[3] Dissolution of PMMA templates generates the porous, bi-layered degradable scaffold. Scaffold morphology was characterized by SEM. Cylindrical hydrated and dry samples were tested in compression using a load cell of 10N or 500N, respectively, following ASTM D695-02a. The compressive modulus was calculated as the ratio of stress to strain of the initial linear region. Water uptake of the cartilage layer of the scaffold was evaluated by immersion of dry cartilage mono-scaffold with pore diameter of 200 µm in PBS at 37 ^{0}C. Cytotoxicity of the scaffold was measured by an MTT assay where hMSCs were exposed to media eluted from the material. To demonstrate interactions of hMSCs with the material, bone region of the scaffold with and w/o HAp was seeded with hMSCs and cultured for 7 days. The scaffold was then fixed, embedded in paraffin and stained with H&E.

Results: Figure 1 illustrates the bi-layered scaffold. The MTT assay suggested that the scaffold is non-toxic. The compressive moduli of swollen bone mono-scaffold, cartilage mono-scaffold, and bi-layered scaffold are 0.110±0.001, 0.044±0.003 and 0.09±0.01 MPa, respectively The compressive moduli of dry bone and cartilage mono-scaffolds and of bi-layered scaffolds are 62±6, 30±6 and 39±7MPa, respectively. The cartilage layer reaches a swelling equilibrium of 169±9 % after 24 h. Histology shows hMSCs adhesion, efficient distribution and pore infiltration of the bone region of the scaffold containing HAp after 7 days of culture (Fig. 2).

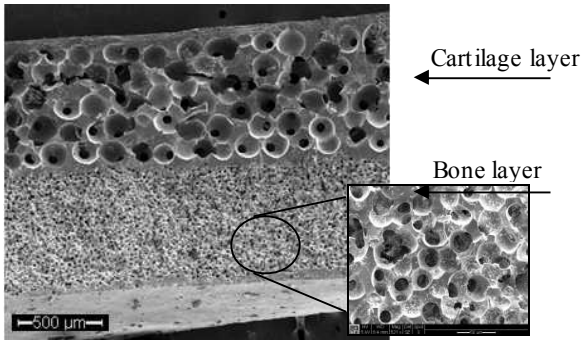

Figure 1. SEM image of the bi-layered scaffold.

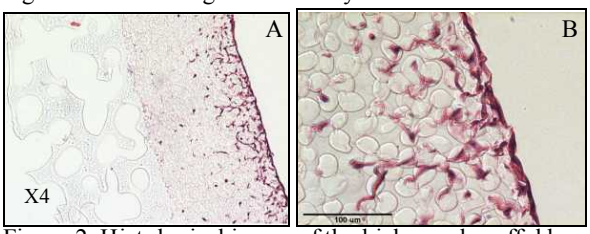

Figure 2. Histological image of the bi-layered scaffold (A) and magnified image of the bone region (B) seeded with hMSCs.

Conclusions: The sphere template fabrication technique results in a bi-layered scaffold with optimized pore sizes. The cartilage region of the scaffold has a pore diameter of 200 µm and covalent HAc. The bone region of the scaffold was designed to have a pore diameter of 38 µm plus incorporated HAp nanoparticles. The bone layer was engineered to have 38 µm pores (in contrast to more typical 200-300 µm pores[1]) since pores of approximately 35-40 µm have been found optimal for vascularized, relatively non-fibrotic integration into tissue.[2] Both layers are composed of degradable polyHEMA.[3] The advantage of the continuous 2-layer structure is that it does not require assembly of two scaffolds during surgical implantation.[1] The compressive modulus of the hydrated scaffold is relevant to this application. Matrix and mineral deposition should increase the mechanical function of the scaffold. The cartilage induction region of the scaffold shows excellent water uptake to mimic hydrated articular cartilage tissue. Presence of HAp in bone region has a great impact on hMSCs interaction with the material. Next, we will seed the bi-layered scaffold with hMSCs and chondrocytes and examine the development of extracellular matrix in each layer.

References: 1. O'Shea MT. *et al*. TISSUE ENG. Part B 2008;14: 447-464; 2. Marshal AJ. *et al*. ACS Polym Prepr. 2004;45:100-101; 3. Atzet S. *et al*. Biomacromolecules 2008; 9: 3370-3377. **Funding:** UWEB21, Coulter Foundation. We thank Rocky Tuan for the gift of the hMSCs.