In Vitro Osteocompatibility Studies of Polysaccharide Scaffolds for Bone Tissue Engineering

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Statement of Purpose: As an alternative to traditional autografts and allografts, three-dimensional (3D) porous scaffolds have been fabricated from polymers of both synthetic and natural origin. However, majority of the scaffolds derived from polymers of synthetic origin are often limited to provide the desirable combination of osteoconductive, osteoinductive, and osteogenic properties that are present in autografts. Furthermore, these scaffolds are required to provide adequate structural, mechanical and biochemical properties during the bone regeneration process. Ceramic scaffolds are often brittle and poorly absorbed. In spite of many desirable scaffold properties acidic degradation products of polyester based scaffolds are known to induce inflammatory responses at the site of implantation. Polysaccharides due to their chemical similarity to the components of natural extracellular matrix are known to be biocompatible. Polysaccharide cellulose and its derivatives inherently possess higher mechanical strength and biocompatibility due to their β-glycosidic linkage. Present study reports the design and development of mechanically competent cellulose based scaffolds for bone tissue engineering.

Methods: 600-710µm sized particles of cellulose acetate (CA) (Sigma-Aldrich, 30kDa) and ethyl cellulose (EC) (Sigma-Aldrich, 30kDa) were prepared by emulsion-solvent evaporation method[1]. Scaffolds were prepared using solvent/non-solvent sintering technique[2]. Collagen-coated scaffolds were created to improve the bioactivity of 3D porous structures. Cylindrical scaffolds (5×10mm) were tested for compressive mechanical properties and degradation behavior up to 6 months. Sterile 3D disc scaffolds (8×2mm) and composite scaffolds functionalized with collagen nanofibers were used to evaluate osteocompatibility. These scaffolds were characterized for surface morphology, degradation and compressive mechanical properties. Osteocompatibility was assessed by culturing human osteoblasts (HOB) on these scaffolds at a density of 50,000 cells /scaffold. Cell viability was monitored for 28 days by live/dead, SEM, and MTS assay. Mature osteoblast phenotype such as ALP and mineralized matrix synthesis were quantified at specified culture time points.

Results/Discussion: Polysaccharide scaffolds showed a 3D interconnected porous structures and rougher surface morphologies than PLAGA scaffolds. Composite scaffolds showed a uniform distribution of collagen nanofibers throughout the scaffold 3D architecture. Nanofibers were in the in the diameter range of 140 ± 40 nm for CA scaffolds and 120 ± 34 nm for EC (Fig. 1). The compressive modulus values were found to be 227 ± 57 and 200 ± 40 MPa for CA and EC scaffolds, respectively. Further compressive strength, maximum load and energy at failure were significantly higher than PLAGA sintered microsphere scaffolds of identical pore properties[3].

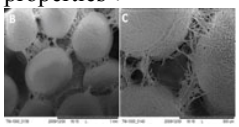
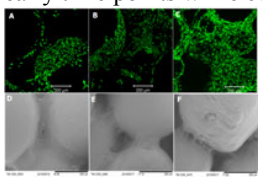


Fig.1 SEM micrographs of collagen coated scaffolds. Collagen coating mimics the extracellular matrix morphology.

HOB seeded on both CA and EC scaffolds showed a progressive growth with time and attained confluency by day 21 indicating the cell survival during *in vitro* culture (**Fig. 2 and 3**). Mineralized matrix synthesis by HOB is quantified using Alizarin Red staining for calcium at various culture time points (Fig.4). Neat CA and EC scaffolds showed higher mineralized matrix synthesis at early time points while composite scaffold mineralization



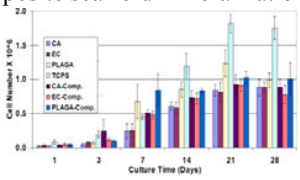


Fig. 2. (left top row) Confocal micrographs taken for (A) CA (B) EC and (C) CA-composite on day 14 and **(left bottom row)** SEM micrographs taken for (D) CA on day 7, (E) CA on day 28 and (F) EC on day 28 illustrate robust cell growth on polysaccharide scaffolds. **Fig 3. (Right)** Proliferation of human osteoblasts seeded on polysaccharide and composite scaffolds in basal media measured using MTS assay shows comparable cell growth to PLAGA control by day 28.

increased with culture time. For example, on day 7 almost 8 and 5 fold increase in calcium deposition on CA and EC scaffolds were observed compared to PLAGA control. ALP expression also followed a similar trend. Significantly higher ALP activity was found on neat and composite polysaccharide scaffolds at early time points compared to PLAGA.

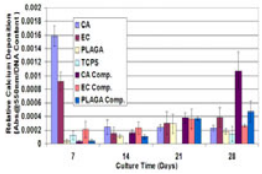


Fig.4. Mineralized calcium deposition on scaffolds normalized with DNA content at various time points.

Conclusions: Polysaccharide scaffolds showed improved mechanical strength than PLAGA scaffolds. Comparable cell viability and greater calcium deposition on both the neat and collagen coated scaffolds compared to PLAGA controls indicates potential use of polysaccharide scaffolds for accelerated bone healing.

References: 1. O'Donnell et. al. Adv. Drug Del. Rev. 1997;28:25-42. 2. Brown et. al. J Biomed Mater Res 2008;86B:396-406. 3. Borden et al. Biomaterials 2003;24:597-609.

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