Ovalbumin-Based Scaffolds reinforced with Cellulose Nanocrystals for Bone Tissue Regeneration

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Statement of Purpose: In the field of tissue engineering, a major area of study is developing bone scaffolds that will provide support for osteoblasts. Despite many advances in recent years there is still a significant need for new bio-based 3-D porous scaffolds that possess sufficient initial mechanical properties to prevent immediate failure upon implantation. Ovalbumin, a protein from chicken egg whites, has been use to fabricate biodegradable, porous hydrogel bone scaffolds that promote osteoblast attachment and proliferation (Farrar G. J of Tissue Eng. 2010;2010:6 pages). Although ovalbumin scaffolds encourage bioactivity and are naturally resorbed into the body after bone regeneration, they are also very fragile. Cellulose nanocrystals, derived from wood pulp, can be utilized to reinforce these scaffolds while sustaining biocompatibility. When chemically modified to incorporate surface amine groups, cellulose nanocrystals become capable of covalently crosslinking with the ovalbumin matrix for improved mechanical strength.

Materials and Methods: A 6.25% ovalbumin solution was mixed and sonicated with salt and aminated cellulose nanocrystals (CNCs) and then crosslinked with glutaraldehyde for 20 hours. Scaffolds had compositions between 0 and 30 wt. % CNC. After being rinsed with a 7.5% glycine solution, the scaffolds underwent salt leeching in DI water for 3 days, freezing at –80 °C, and finally lyophilization for 2 days to prepare dry, porous scaffolds. SEM was used to determine the pore size, structure, and examine the incorporation of the cellulose nanocrystals into the pore walls. Mechanical properties were evaluated using an Instron universal testing machine in compression mode (10% strain/min). Furthermore, the scaffolds were seeded with MC3T3-E1 pre-osteoblasts for cell culture studies. Cell proliferation was determined with a MTT assay, and cell differentiation with OCN and ALP assays.

Results: Pore sizes fell between 50 and 160 μm for all samples (Figure 1A), with the larger pores at the top and bottom portions of each cross section. Under high magnification, the CNCs were shown to have been incorporated into the pore walls, providing a contoured surface (Figure 1B). Regarding mechanical properties, the compressive modulus did not change significantly with the increased CNC loading (Figure 2A), while there was a 40% increase in yield strength from 0 to 10 wt. % (Figure 2B). Also, the yield strain doubled when a 10 wt. % CNC loading was used. With compositions past 10 wt. % there is a sharp decrease in yield strength and yield strain. In addition, the cell studies indicated that the ovalbumin-based scaffolds containing aminated cellulose nanocrystals were able to support cell proliferation and differentiation.

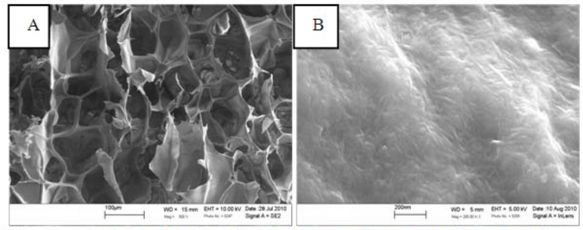

Figure 1: Representatvie scanning electron micrographs of A) the porous ovalbumin-CNC structure and B) the CNCs coating the surface of a scaffold pore

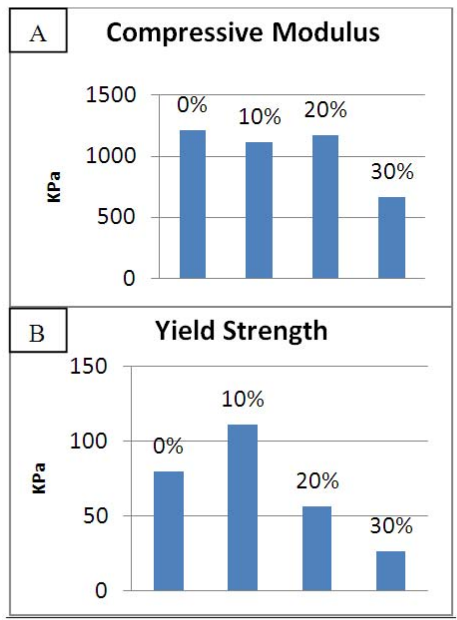

Figure 2. Compressive testing of ovalbumin-CNC nanocomplexes of various compositions A) Compressive modulus and B) Yield Strength

Conclusions: Nanocomposites of ovalbumin and aminated cellulose nanocrystals were successfully fabricated and evaluated for mechanical integrity and bioactivity. At a loading of 10 wt. %, the scaffolds showed a significant increase in yield strength and strain from the control samples. Given resilience is equivalent to one half the yield stress multiplied by the yield strain, it can be concluded the aforementioned composite had substantially improved resilience over the other compositions. So, for the 10 wt. % scaffold, the high resilience proves a greater likelihood of elastic recovery following some form of deformation. Better elasticity should provide a more ideal material to be handled by surgeons for implantation. Cell studies confirmed the viability of ovalbumin-CNC scaffolds as a support structure for osteoblast cell growth.