Nucleation of Hydroxyapatite on Alginate Sponges: Mineral Identification, Biological Characterization and Spatial Regulation

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Statement of Purpose: Current bone tissue engineering strategies aim to grow a tissue similar to native bone by combining cells and biologically active molecules with a scaffold material. In this study, a macroporous scaffold made from the seaweed-derived polymer alginate was synthesized and mineralized for applications such as soluble signal delivery and cell-based tissue engineering. Alginate represents an outstanding template material for mineralization, as it presents a large number of carboxylic acid groups, which provide sites for heterogeneous mineral nucleation. In addition, alginate has become an attractive material for tissue engineering due to its degradability under normal physiological conditions, mild processing, and low toxicity when purified.

Methods: Alginate sponges were fabricated as previously reported by Shapiro et al and mineralized by incubation in modified simulated body fluids (mSBF) at 37 ºC for periods of up to 4 weeks. Modified SBF is a solution similar in ionic composition and temperature to blood plasma but with twice the concentration of calcium and phosphate. In order to assess mineral formation on the material, the change in mass of the sponges during the course of the experiment was calculated. The mineral nucleated on the material was characterized using scanning electron microscopy (SEM), energy dispersive x- ray analysis (EDS) and x-ray diffraction (XRD). The extent of control over the site of nucleation was explored using samples containing millimeter-scale channels. Finally, mineralized alginate sponges were biologically characterized by seeding human mesenchymal stem cells (hMSCs) and investigating the proliferation of these cells on the materials. Cell titer blue, which uses the indicator dye resazuring, measured metabolic capacity of hMSCs which could be correlated to cell proliferation.

Results: Nucleation of a bone-like mineral was achieved by incubating the scaffold in modified simulated body fluids (mSBF) for four weeks. The change in scaffold mass showed an 8-fold increase with mineralization time suggesting the growth of a mineral layer (Fig. 1). Analysis using scanning electron microscopy and energy dispersive x-ray analysis indicated growth of a continuous layer of mineral with a high calcium and phosphorous content with a Ca to P ratio of 1.60 (Fig. 2). X-ray diffraction analysis showed peaks associated with hydroxyapatite, the major inorganic constituent of human bone tissue (Fig. 2). In addition to the mineral characterization, the ability to control nucleation on the surface, into the bulk of the material, or on the inner pore surfaces of scaffolds with controlled pore structure was observed (Fig. 3). Human MSCs attached and proliferated on the mineralized sponges and showed a spindle-shaped morphology (Fig. 4).

Conclusions: This novel alginate- HAp composite material could be used in bone tissue engineering as a scaffold material to deliver cells, and perhaps also biologically active molecules.

Reference:
Shapiro L. Biomaterials. 1997. 18(8):583-90

Figures:

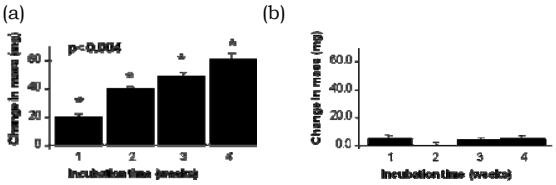

Figure 1: Change in mass of alginate sponges after incubation in A) mSBF and B) mSBF that did not contain KH_2PO_4 (control group)

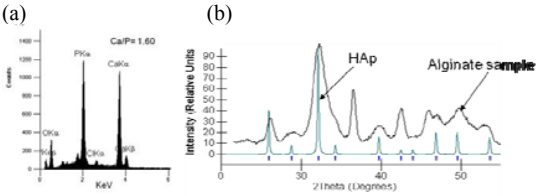

Figure 2: Incubation in mSBF resulted in the nucleation of a hydroxyapatite (HAp) like mineral as confirmed by (a) EDS analysis and (b) x-ray diffraction.

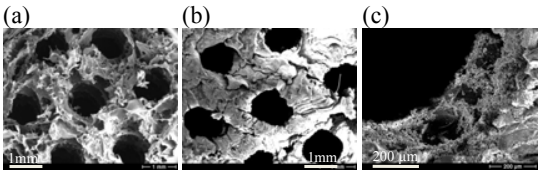

Figure 3: Alginate scaffold containing macroscopic channels (a) before the nucleation of a hydroxyapatite like mineral (b) after 4 weeks incubation in mSBF. (c) Mineral nucleation was observed specifically on the surfaces of the cylindrical channels.

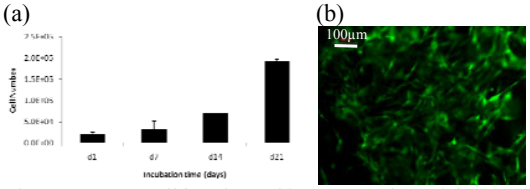

Figure 4: (a) Proliferation of hMSCs cultured for 3 weeks on the mineralized alginate sponges (b) hMSCs exhibit a spindle shaped morphology