Synthesis and Characterization of a Biomimetic Calcium-Deficient Hydroxyapatite – Hydrogel Composite

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Statement of Purpose: A novel composite material consisting of calcium-deficient hydroxyapatite (CdHAP) biomimetically deposited in a bacterial cellulose (BC) hydrogel was developed for potential use as an orthopedic biomaterial. In this study, the composite was characterized using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM).

Methods: Bacterial cellulose was produced from the bacterial strain *Gluconacetobacter hansenii* (ATCC 10821) by the method of Schramm and Hestrin.[1] Twenty-four cellulose pellicles were grown statically under identical conditions in 6cm culture dishes for 31 days at 23°C. The pellicles were initially heated to 90°C in deionized water for 1-2 hours to kill the bacteria. The cellulose was purified by incubation in several changes of 1% NaOH. The BC was then washed with several changes of deionized water to neutralize the NaOH.

CdHAP was produced by incubating the cellulose pellicle in 100mM $CaCl_2$ (pH 4.83) under agitation in an orbital shaker for 24h (23°C), rinsing the cellulose briefly in deionized water, then transferring the pellicle to 60mM Na_2HPO_4 (pH 8.36) under agitation for another 24h (23°C)[2]. To obtain composites with varying amounts of hydroxyapatite, sets of bacterial cellulose pellicles were subjected to increasing numbers of alternating incubation cycles in aqueous $CaCl_2$ and Na_2HPO_4. Six sample groups each consisting of 4 bacterial cellulose pellicles were deposited with varying amounts of CdHAP.

XRD was performed on dried samples with a Scintag PADV instrument operated at 45kV and 40mA with CuKα radiation and a Si(Li) Peltier-cooled solid state detector. Data were collected between 10 and 70° *2θ* at a scan rate of 1° *2θ*/min. Jade® 6.0 Software (Materials Data Incorporated: Livermore, CA) was used to identify the crystal structures of the BC and CdHAP and to calculate the CdHAP crystallite sizes. FTIR analysis of powdered samples was performed in transmission mode on a Biorad FTS-6000e instrument (BioRad: Randolph, MA) at $4cm^{-1}$ resolution with 256 scans in the 4000-400 cm^{-1} range. Peak deconvolution was performed with PeakFit v.4.6 software (SeaSolve Software: Framingham, MA). Lyophilized samples were imaged with a LEO 1525 Scanning Electron Microscope (Zeiss: Oberkochen, Germany) after mounting the samples on carbon tape and sputtering them with gold on a Spi Module Sputter Coater (Spi Supplies: Westchester, PA) at 20mA for 10s.

Results / Discussion: XRD verified the formation of crystalline calcium-deficient hydroxyapatite (CdHAP) within the bacterial cellulose. CdHAP is the natural mineral component of bone. It is an ideal material for bone grafts because it promotes bone colonization when implanted in osseous defects and degrades over time to be replaced by new bone. XRD confirmed that the CdHAP consisted of anisotropic 10-50 nm crystallites which are elongated in the c-axis mimicking the geometry of bone apatite crystals.

FTIR and peak deconvolution showed that the cellulose hydroxyl bands were shifted to lower wavenumbers after deposition of CdHAP. This suggests that the hydroxyapatite is chemically bonded to the cellulose. The presence of the phosphate ν_4 doublet in the BC-HAP composite confirms that the precursor phase to the hydroxyapatite is octacalcium phosphate (OCP) similar to physiological apatite. This implies that the calcium cations first complex with the cellulose (specifically the hydroxyl functional groups) before forming the apatite. The OCP precursor phase ensures a more crystalline and ordered hydroxyapatite structure.

Scanning electron micrographs showed that 1μm CdHAP spherical clusters had formed in the cellulose network (Fig 1a). Closer analysis revealed that the clusters were composed of nanosized crystallites with a needle and lamellar morphology (Fig 1b-d).

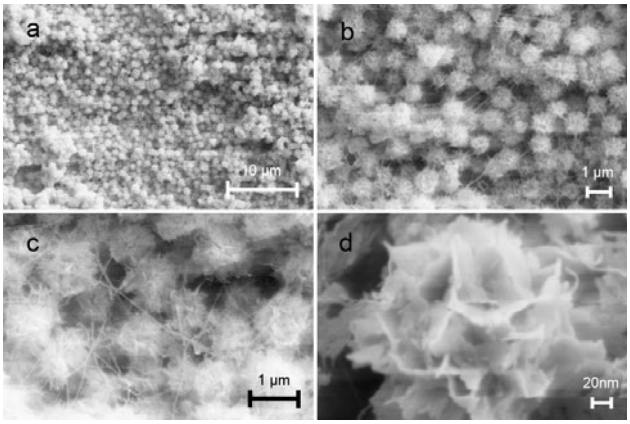

Figure 1: SEM images of BC-HAP at (a) 1kx (b) 5kx (c) 10kx (d) 50kx.

Conclusions: This method of hydroxyapatite formation mimics the biomineralization of bone. In physiological systems, calcium ions first complex with an intermediary matrix: either collagen fibrils or a matrix vesicle. These cations form regular nucleation sites which then associate with phosphate ions to produce an OCP precursor phase that eventually hydrolyzes to hydroxyapatite. The BC hydrogel provides a template for the biomimetic synthesis of ordered CdHAP crystallites. The formation of this material is similar to the physiological biomineralization of bone producing apatite crystals of comparable shape and size. The bioactivity of the CdHAP and the biocompatibility of the BC hydrogel substantiate this composite as a potential orthopedic biomaterial.

References: (1) Schramm M, Hestrin S: *J. Gen. Microbiol.* 11 (1954) 123-129 (2) Hutchens et al.: U.S. Patent Application No. 10/295,461 (2002)