A Nanostructured Degradable Hydrogel Composite for Osseous Regeneration

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Statement of Purpose: Bacterial cellulose (BC) is a hydrogel consisting of pure cellulose fibrils that are 3-4 nm thick, 70-80 nm wide, and 1-9 µm long; comparable to collagen nanofibrils[1]. BC is currently used in an FDA approved wound dressing (Xylos Corp, Langhorne, PA, USA) and has had promising results substituting a variety of tissues *in-vivo* and *in-vitro*[2]. In previous work, calcium-deficient hydroxyapatite (CdHAP) was biomimetically deposited in BC for potential use as an orthopedic biomaterial[3]. CdHAP is the main mineral component of bone and has been shown to stimulate bone growth when implanted in osseous defects[4]. A limitation to using BC for tissue substitution is its inability to degrade in mammalian systems. In this study, BC was oxidized to produce a degradable hydrogel that could precipitate CdHAP to produce a potential bone graft.

Methods: BC was produced from the bacterial strain *Gluconacetobacter hansenii* (ATCC 10821) by the method of Schramm and Hestrin[5]. BC was then chemically modified via oxidation (Ox BC). CdHAP was precipitated in native and oxidized BC as described previously[3]. The in-vitro degradation of these materials was tested by incubating BC, BC-CdHAP, Ox BC, and Ox BC-CdHAP in phosphate buffered saline (PBS) (pH 7.4) for 15d under static and dynamic conditions (Burrell Wrist Action Shaker: Pittsburgh, PA, USA). The samples were then rinsed in several changes of distilled H_2O, dried, and weighed. Oxidation was verified with FTIR (Biorad FTS6000: Randolph, MA, USA) while loss of crystallinity and formation of CdHAP was confirmed with XRD (Philips X'Pert, PANalytical: Almelo, Netherlands). SEM images of the samples were also obtained (LEO 1515, Zeiss: Oberkochen, Germany). Statistically significant mass differences after degradation was verified by performing a Tukey-Kramer Test at α=0.05 using JMP software (SAS: Cary, NC, USA).

Results/Discussion: FTIR confirmed oxidation of BC by the presence of carbonyl bands from aldehyde groups at 1652 and 1740cm^{-1}, as well a carboxylic acid band at 1543cm^{-1}. XRD verified that cellulose lost crystallinity after oxidation, rendering it more degradable. Despite chemical modification, oxidized cellulose retained its structure and the ability to biomimetically produce CdHAP. Figure 1 shows SEMs of BC, BC-CdHAP, Ox BC, and Ox BC-CdHAP. CdHAP develops into uniform 1µm clusters comprised of 10-50nm crystallites that are elongated in the c-axis like natural bone apatite. After in-vitro degradation, the Ox BC, Ox BC-CdHAP, and BC-CdHAP samples lost mass (Figure 2). The Ox BC-CdHAP mass was significantly decreased after being shaken in PBS for 15d. However, XRD analysis on the samples post-degradation detected NaCl salt in the oxidized samples despite several washings in distilled H_2O. This indicated that the oxidized samples had greater mass loss which was masked by NaCl deposition.

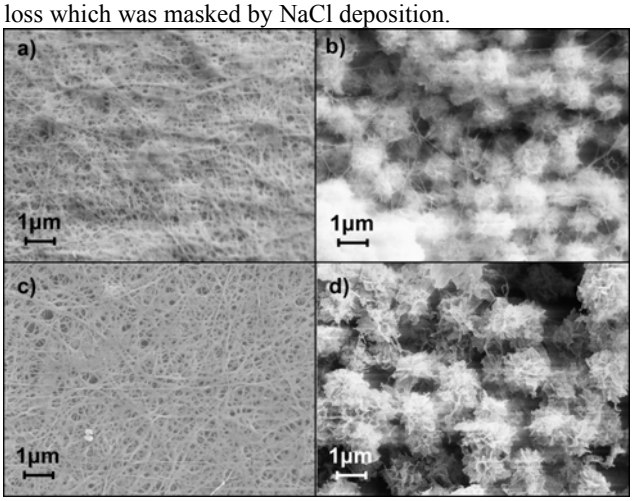

Figure 1: SEM images of a) native BC, b) native BC-CdHAP c) oxidized BC, d) oxidized BC-CdHAP

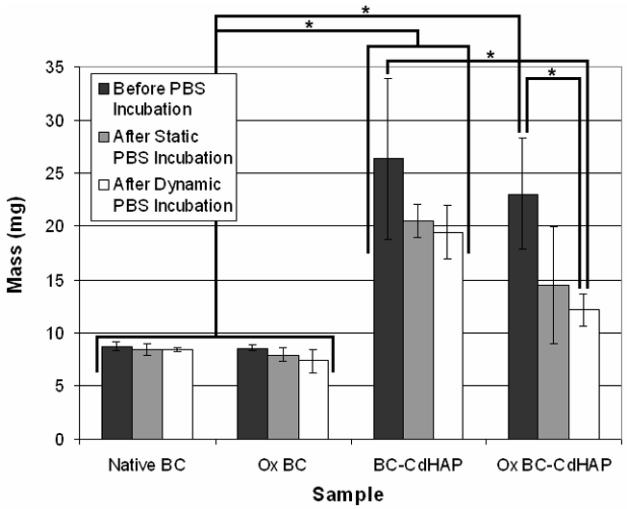

Figure 2: Weight loss of cellulose samples. Statistically significant mass differences at α=0.05 are indicated by *

Conclusions: Oxidized bacterial cellulose is a novel degradable hydrogel comprised of nanofibrils much like natural collagen. Oxidizing bacterial cellulose and biomimetically depositing CdHAP produces a new orthopedic biomaterial which has the potential to regenerate bone and degrade with new bone ingrowth.

References: (1) Bielecki et al. in VanDamme et al. (eds.) *Biopolymers*, Vol. 5. Weinham: Wiley, 2001;37-90. (2) Helenius et al. *J Biomed Mater Res* 2006;76A:431-438. (3) Hutchens et al. *Biomaterials* 2006;27:4661-4670. (4) Bourgeois et al. *J Biomed Mater Res* 2003;64A:402-408. (5) Schramm and Hestrin. *J Gen Microbiol* 1954;11:123-129.