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Biomimetic synthesis of calcium-deficient hydroxyapatite in a natural hydrogel

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Abstract

A novel composite material consisting of calcium-deficient hydroxyapatite (CdHAP) biomimetically deposited in a bacterial cellulose hydrogel was synthesized and characterized. Cellulose produced by *Gluconacetobacter hansenii* was purified and sequentially incubated in solutions of calcium chloride followed by sodium phosphate dibasic. A substantial amount of apatite (50–90% of total dry weight) was homogeneously incorporated throughout the hydrogel after this treatment. X-ray diffractometry (XRD) showed that CdHAP crystallites had formed in the cellulose. XRD further demonstrated that the CdHAP was comprised of 10–50 nm anisotropic crystallites elongated in the *c*-axis, similar to natural bone apatite. Fourier transform infrared (FTIR) spectroscopy demonstrated that hydroxyl IR bands of the cellulose shifted to lower wave numbers indicating that a coordinate bond had possibly formed between the CdHAP and the cellulose hydroxyl groups. FTIR also suggested that the CdHAP had formed from an octacalcium phosphate precursor similar to physiological bone. Scanning electron microscopy (SEM) images confirmed that uniform ~1 μm spherical CdHAP particles comprised of nanosized crystallites with a lamellar morphology had formed in the cellulose. The synthesis of the composite mimics the natural biomineralization of bone indicating that bacterial cellulose can be used as a template for biomimetic apatite formation. This composite may have potential use as an orthopedic biomaterial.

Keywords: Bacterial cellulose; Hydrogel; Hydroxyapatite composite; Biomimetic material

1. Introduction

In nature, the nucleation and growth of mineralized materials are often controlled by organic macromolecules such as proteins and polysaccharides. Bone and teeth consist of a small amount of organic matrix which manipulates the formation of apatite into distinct microstructures suitable for the mechanical forces they encounter in vivo [1]. A new development in biomaterials is the biomimetic synthesis of calcium phosphate in polymer matrices to produce composites that can initiate osteogenesis when implanted in bony sites [2]. Polymers with distinct molecular organizations may be used as a template to control the geometry of the apatite to mimic that found in bone [1].

Hyaluronic acid and glycosaminoglycans are polysaccharides found in mammalian tissues [3]. Other polysaccharides such as chitosan, alginate and starch are currently being investigated to substitute skin, cartilage, nerves, blood vessels and bone [4]. These natural polymers are biocompatible, abundant in source, have low cost and can be modified to host a variety of chemical, physical and biological properties [5]. Polysaccharides are able to form cross-linked polymer network structures called hydrogels

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which are highly hydrated and porous like living tissue [4]. When implanted, these hydrophilic materials allow the permeation of water, metabolic products and chemical signals in the aqueous physiological environment [4].

Cellulose is a polysaccharide currently used in medicine to produce dialysis membranes, drug coatings and blood coagulants, among many other products [6]. As the primary component of plant cell walls, cellulose is the most abundant natural polymer in the world with an estimated 10^{10}–10^{11} tons produced each year [7]. Plant cellulose is generally associated with other biopolymers such as hemicellulose and lignin to form a laminate. Extracting these polymers from cellulose requires harsh chemical processes, such as treatment with sulfur dioxide, sodium hydroxide and bleaching agents. This often causes the cellulose to lose strength. Cellulose used in medicine is typically regenerated using the viscose process or modified into derivatives such as cellulose acetate or carboxymethyl cellulose. These celluloses have lost almost all of their crystallinity and native form [8].

Certain bacterial species possess the ability to secrete pure cellulose in the form of a hydrogel [9]. Bacterial cellulose (BC) is synthesized by nonpathogenic microbial strains such as *Gluconacetobacter*, which are commonly found on naturally grown fruits and fruit products [10]. *Gluconacetobacter* has been used in vinegar production and also in production of a Filipino food, nata-de-coco [10]. When grown under static cultures in liquid medium, the bacteria synthesize a layer of pure cellulose hydrogel called a pellicle at the air–liquid interface. The pellicle enables the aerobic bacteria to obtain oxygen at the surface and serves as protection against drying and ultraviolet radiation [10]. The bacteria extrude nanofibrils of pure cellulose which bond together to form ribbons that are typically 3–4 nm thick, 70–80 nm wide and 1–9 μm long. The ribbons twist and overlap to form parallel crystal lattice planes which stack on top of each other to form the thickness of the pellicle [9].

Though chemically identical to plant cellulose, BC is morphologically very different. Produced in a pure form, BC does not have to be isolated with extensive chemical treatment. The bacteria naturally synthesize the cellulose into a crystalline three-dimensional network. BC fibrils are up to 200 times finer than plant cellulose fibrils producing substantial contact area [11]. This permits a high density of inter- and intra-fibrillar hydrogen bonds which gives the cellulose its hydrogel structure. Hydrogen bonding enables the BC to hold water in its interstitial spaces allowing immense water retention [12]. Water makes up 99.8% of the total volume of the matrix with pure cellulose comprising the remaining 0.2% [13]. Hydrogen bonding also grants the cellulose high strength. Nishi et al. [12] claimed that dried BC has the highest Young's modulus (15–30 GPa) ever known in two-dimensional organic materials.

BC is a biocompatible material currently being investigated for a variety of medical applications. A skin substitute based on BC called XCell® has been approved by the Food and Drug Administration and is currently on the market (Xylos Corporation: Langhorne, PA, USA) [11]. XCell® is a high performance dressing which maintains the moisture balance in the wound to speed up healing and epithelialization [11]. Results from Svensson et al. [14] established BC as a prospective scaffold in tissue engineering, while Helenius et al. [15] confirmed its excellent in vivo biocompatibility. Specific in vivo studies have shown the potential of BC in the substitution of blood vessels [16], nerves [16], gingiva [17] and the dura mater [18]. Although cellulose degrades slowly in mammalian systems, it can be rendered more degradable by chemical modification [11,15].

In this study, a novel composite was characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The composite consists of calcium-deficient hydroxyapatite (CdHAP) biomimetically incorporated into a BC hydrogel [19]. The composite is being developed for potential use as an orthopedic biomaterial. 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 [20].

2. Sample preparation and experimental procedures

2.1. Synthesis of BC

For the present study, the bacterial strain *Gluconacetobacter hansenii* was obtained from the American Type Culture Collection (Manassas, VA, USA) (ATCC 10821). The cellulose was cultivated by the method of Schramm and Hestrin with a substitution of mannitol for glucose in the nutritional medium [21]. The cellulose was purified as reported by Evans et al. [22].

2.2. Deposition of CdHAP

CdHAP was formed in BC by performing alternating incubation cycles with calcium and phosphate solutions [19]. An incubation cycle is defined as suspending the cellulose pellicle in 100 mM $CaCl_2$ (pH 4.83) under agitation in an orbital shaker for 24 h (23 °C), rinsing the BC briefly in deionized water, then transferring the pellicle to 60 mM Na_2HPO_4 (pH 8.36) under agitation for another 24 h (23 °C). Six groups of four BC pellicles each were synthesized with zero, one, two, three, four and five incubation cycles. The group with no incubation cycles (native cellulose) was used as a control.

Four replicates of each sample were made so that they could later be dried and weighed to determine the amount of CdHAP in each composite. It was assumed that approximately the same amount of cellulose existed in all the composites since they were synthesized under identical conditions. The mass of CdHAP in the cellulose was