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Evaluation of alkaline pre-treatment of PLLA fibers for biomimetic hydroxyapatite coating

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ABSTRACT

A poly(L-lactic acid) (PLLA) and hydroxyapatite (HA) composite was formed via a biomimetic coating process. Before treatment in a modified-simulated body fluid (m-SBF), the surfaces of the PLLA fibers were etched with alkaline reagents to make the polymer surface easier for apatite to nucleate and grow. Several elements of this etching procedure (including the etching reagent, concentration, and length of pre-treatment) were varied to determine the optimal combination for producing a predictable HA coating with the desired properties of consistent coating distribution and thickness. The alkaline etching reagents used in this study were sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), calcium hydroxide ($Ca(OH)_2$), and calcium hypochlorite ($Ca(OCl)_2$). The weight gained through m-SBF coating, changes in the mechanical properties of the PLLA, and HA coating morphology were observed to determine the effects of different pre-treatment strategies on the biomimetic coating process. It was confirmed that the most effective etching reagents are $Ca(OH)_2$ and $Ca(OCl)_2$. It was also found that decreased etching reagent concentration leads to a decrease in HA coating and increased etching time increases HA deposition.

1. Introduction

The skeletal system, which includes bones, cartilages, ligaments, and other connective tissues, has several important functions in the human body, including structural support, storage of minerals and lipids, and production of red blood cells. Bone specifically serves to protect soft tissues and organs and to function as levers controlling the motion of the body [1]. The structure of bone, a material composed primarily of collagen, carbonated hydroxyapatite, and water, can be organized into seven levels. The second of these is the mineralized collagen fibril, the basic building block of bone, which consists of fine fibers of collagen protein-coated in several layers of plate-like hydroxyapatite (HA) crystals [2]. Collagen exhibits a triple helical structure, which provides it with high tensile strength as well as flexibility; the crystal coating supplies additional compressive strength and reinforcement to the collagen framework [3].

Bone fracture is a common and growing health problem in the United States. The prevalence of osteoporosis related fractures alone are expected to grow from about 2 million fractures in 2005 to greater than 3 million fractures in 2025 [4]. Autologous bone grafting remains the “gold standard” for bone repair and regeneration, but due to problems such as donor site morbidity and limited availability, there is a need for substitute materials to be developed [5]. As one of the primary components of bone, HA is an obvious candidate for use in substitute materials. The mineral is highly biocompatible and osteoconductive [5,6]. However, HA alone has poor tensile strength and fractures easily, and therefore, cannot provide the support required in load bearing situations [2,3,6].

The synthetic polymer poly(L-lactic acid) (PLLA), is biodegradable and does not release any toxic or harmful by-products as it disintegrates within the body. This important characteristic has already allowed it to garner FDA approval for use in humans [6]. Additionally, PLLA has versatile processing capabilities and may be suitable for a variety of applications [7]. Unfortunately, the surface properties of PLLA are not compatible with biological applications; the polymer is highly hydrophobic and lacks nucleation sites recognizable by biological molecules [6,8,9]. The solution to this problem is to coat the surface of the PLLA with HA and make use of the advantages of each material to generate promising bone repair materials.

Coating PLLA with HA is achieved through a biomimetic process: the polymer fibers are submerged in a modified simulated body fluid (m-SBF) solution, which contains inorganic ions in composition and concentrations near those found in human blood plasma. In this model body environment, the HA nucleates on the surface of the substrate, eventually forming an uninterrupted coating of apatite crystals [6,8–10]. Unfortunately, the hydrophobicity of PLLA makes this difficult and its smooth surface lacks nucleation sites for crystal development.

To achieve continuous bonding between the PLLA surface and the HA coating, the number of nucleation sites and the hydrophilicity of the PLLA must be improved. Various surface treatments have been

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applied to substrates in order to introduce functional groups to aid in biomimetic coating by acting as nucleation sites for HA [6,8,9,11–18].

One method through which this can be accomplished is the treatment of the PLLA fibers with basic solutions, which introduces carboxyl groups (—COOH) to the fiber surface. These groups are highly polar and negatively charged, so they improve both the wettability and act as HA nucleation sites, attracting positive ions, allowing for better HA coating [6,8]. In previous work done by this group, NaOH and NaOCl were used to pre-treat PLLA under mild conditions. It was found that more HA coating resulted with increased treatment time and etchant concentrations [6]. The objective of this study was to identify a reliable, consistent method for etching PLLA for improved biomimetic coating, and to evaluate whether the use of alkaline calcium compounds as etchants is more effective than sodium compounds by providing calcium ions on the surface of the PLLA to serve as apatite nucleation sites. In order to identify such a method, this study compared several etching reagents based on their effects on the surface of PLLA yarn and the HA coating, as well as the effect of etching time and etchant concentration.

2. Materials and methods

2.1. Preparation of PLLA samples

For etching and later SBF treatment, PLLA yarns obtained from Teleflex Medical were wrapped around a previously weighed Teflon frame, as shown in Fig. 1. The combined weight of the frame and PLLA fibers were then recorded to determine the total mass of fibers. PLLA films were cast for contact angle analysis.

2.2. Etching of PLLA fibers and films

Three separate etching experiments were performed in order to understand the relative advantages of different etching reagents, concentrations, and pre-treatment times. In the first etching experiment (later referred to as Experiment 1), four alkaline reagents were used to etch groups of PLLA fibers and films: 0.5 M NaOH (Fisher, 98 + %), 0.5 M NaOCl (Fisher, 4–6% w/v, purified grade), a saturated (0.01 89 M) solution of $Ca(OH)_2$ (Acros, 98 + %), and 0.25 M $Ca(OCl)_2$ (Fisher, 99 + %). The PLLA fibers and frame were stirred in 200 mL of one of the etching solutions at room temperature. The frame was removed after 1 h, rinsed with deionized water, and allowed to dry for 24 h. The frame and fibers were then weighed to detect the effects of etching on fiber weight.

In Experiment 2, the concentrations of the $Ca(OH)_2$ and $Ca(OCl)_2$ reagents were varied in pre-treatment to assess the optimal etchant concentrations. The previous etching solutions were diluted twice for each reagent to 0.0094 M and 0.0047 M for $Ca(OH)_2$ and to 0.125 M and 0.0625 M for $Ca(OCl)_2$. The fibers were then treated in the same manner and for the same length of time as above.

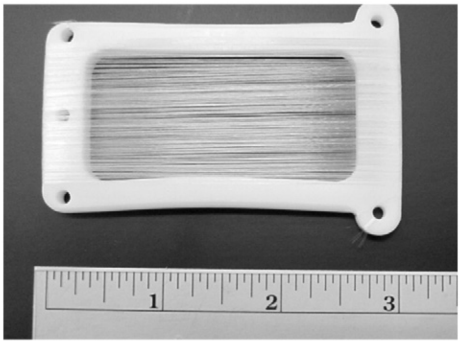

Fig. 1. PLLA fibers loaded onto a Teflon frame. The units of measure displayed on the ruler are cm.

To determine the optimum length of time for pre-treatment, the concentrations of $Ca(OH)_2$ and $Ca(OCl)_2$ from Experiment 1 were used. The PLLA fibers on frames were then stirred in 200 mL of the etching solution at room temperature as above, but half of the frames were removed after only 0.5 h, and the other frames remained in the etching solution for 2 h (Experiment 3). Films were etched under the same conditions as the fibers in Experiment 1. Fibers and films rinsed in deionized water for an equivalent amount of time as the samples treated in etchant solutions were used as control samples for all experiments.

2.3. Biomimetic coating of PLLA fibers

A biomimetic method was used to cover the PLLA fibers with HA. The m-SBF was prepared based on a protocol established in our previous studies [6,19]. The ion concentrations of these ions in the solution are listed in Table 1. The pH of the solution was adjusted to between 6.4 and 6.6. Then, each etched frame was immersed in 120 mL of static m-SBF at 40 °C. After 24 h, the frames were removed, rinsed with deionized water, and allowed to dry for 48 h. All frames from all etching experiments were coated with HA in this manner.

2.4. Sample characterization

2.4.1. Weight measurement

The weight of each frame was recorded after m-SBF treatment, drying, and removal of HA from the exposed sections of frame. This value was then used to determine the mass of HA coating gained and the percent weight gain for each fiber sample.

2.4.2. Static contact angle measurements

The static contact angles of the PLLA films were determined with a sessile drop test at room temperature. Three 2-μL drops of water were placed on each PLLA film, and the contact angle on each side of the drop recorded. This test was performed to compare the wettability of the etched and untreated PLLA films and confirm that etching had successfully increased the hydrophilicity of the polymer surface.

2.4.3. XPS analysis

X-ray photoelectron spectroscopy (XPS) was performed on fibers etched by each reagent in order to compare the surface properties of the PLLA treated with the various etchants to each other as well as untreated PLLA. A PHI Multiprobe with an X-ray photoelectron spectrometer was used to measure the binding energies. The hydrocarbon peak in the C1s spectra was set to 284.6 eV as a reference for the binding energy scale for the samples.

2.4.4. FESEM analysis

The appearance of etched PLLA fibers as compared to un-etched control fibers were observed with a field emission scanning electron microscope (FESEM) and images were recorded. A JEOL JSM 6335 F FESEM was used at an accelerating voltage of 7 kV. The morphology and thickness of the HA coating was also assessed via FESEM. Coated fibers from each etching condition from Experiment 1 (0.5 M NaOH, 0.5 M NaOCl, saturated $Ca(OH)_2$, and 0.25 M $Ca(OCl)_2$) were imaged at an accelerating

Table 1
Ion concentrations of the 3× m-SBF fluid used in this study.

Ion	Concentration in 3× m-SBF (mM)
Na^+	109.9
K^+	6.26
Mg^{2+}	2.75
Ca^{2+}	7.92
Cl^-	113.78
HCO_3^-	17.50
HPO_4^{2-}	3.13