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Effect of silane coupling agent treated bovine bone based carbonated hydroxyapatite on *in vitro* degradation behavior and bioactivity of PLA composites

Suriyan Rakmae, Yupaporn Ruksakulpiwat, Wimonlak Sutapun, Nitinat Suppakarn *

School of Polymer Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand
Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Bangkok, 10330, Thailand

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ABSTRACT

In this study, effect of treating bovine bone based carbonated hydroxyapatite (CHA) with silane coupling agent on *in vitro* degradation and bioactivity of PLA composites were investigated. PLA composite specimens containing CHA and silane-treated CHA were immersed in phosphate-buffered solution at 37 °C for the periods of time up to 8 weeks. The changes in specimen weights and morphologies, pH of PBS solution and PLA molecular weight were examined. The results showed that the strong interfacial bonding between silane-treated CHA and PLA matrix significantly delayed *in vitro* degradation of the PLA composites. However, the bioactivity of the PLA/silane treated CHA composites, determined by the formation of poorly crystalline calcium phosphate compounds on the specimen surface after immersion in simulated body fluid (SBF), seemed to be lower than that of the PLA/CHA composite.

1. Introduction

In the last decades, poly (lactic acid) (PLA)-based materials have been studied and used for temporary implant applications [1,2]. This polymer has many advantages such as being biodegradable and yielding nontoxic byproducts after degradation. The degradation of PLA in aqueous media both *in vitro* and *in vivo* is random hydrolysis of ester bonds by autocatalytic action of carboxylic acid end groups [3]. The mechanism depends on numerous factors, *e.g.* its initial molecular weight, mixing temperature, purity, shape of specimen and crystalline phase fraction [4–6]. This phenomenon produces intermediate acidic products, such as lactic acid and oligomers. Subsequently, these products are eliminated from human body through normal metabolic pathways such as tricarboxylic acid cycle [7,8]. Although several biodegradable polymers have been investigated for tissue engineering, no single polymer meets all the requirements for implant applications.

Since natural human bone consists of a complex organic/inorganic composite, much attention has been paid to the use of biodegradable polymer composites containing bioactive ceramics as an implant to promote bioactivity and adhesion between living tissue and the materials. Several works reported that physical properties as well as *in vitro* and *in vivo* degradation behaviors of the composites could be tailored by varying types of polymers and/or fillers used in the systems [9–11]. There are various bioactive ceramics that have been used as inorganic fillers for fabricating polymer composites *e.g.* bioactive glass, hydroxyapatite. Among those bioactive fillers, carbonated hydroxyapatite (CHA) is a novel biomaterial since CHA is very similar to the natural human hard tissue. As compared with HA, CHA has better biocompatibility, osteoconductivity, bone-bonding ability, cell attachment and proliferation [12,13]. CHA can be obtained from either chemical synthesis or natural sources, *e.g.* collophanite, bovine bone [14–18]. The PLA/CHA composite materials would guide osteoblast cell attachment, cell proliferation and bone tissue regeneration when they are implanted in human body. Subsequently, the implanted materials would gradually degrade at optimal rate and yield nontoxic byproducts after degradation.

However, the concerns in PLA/CHA composite processing are the dispersion of CHA and interfacial adhesion between CHA-PLA since these influence the composite strengths in physiological environment. Our previous works also showed that mechanical properties of PLA/CHA composites were affected by numerous factors, *i.e.* mixing technique, filler content and filler surface modification [17]. To lessen the problems, there are attempts to modify HA filler surface with silane coupling agents before fabricating polymer composites. Santos et al. and Deb et al. found that the water uptake of their dental matrix and bone cement decreased in the presence of silane treated HA [19,20]. Furthermore, various research groups suggested that the incorporation of silane-treated HA effectively improved mechanical properties and fracture behavior of their polymer composites, enhanced the resistance to dissolution in acid environments, exhibited the ability of forming bone-like apatite on their surface like other bioactive materials and had no toxicity to human

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* Corresponding author at: School of Polymer Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand. Tel.: +66 44 224439; fax: +66 44 224605.
E-mail address: nitinat@sut.ac.th (N. Suppakarn).

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osteoblast cells [18,21–25]. Some opinions about the occurring reactions between silane molecules and HA surfaces were also reported. Nishizawa et al. stated in their system that the silane coupling agent was covalently bonded to HA surface through the reaction with surface hydroxyl groups of the HA [26]. Yu-song suggested that the coupling mechanisms of γ-aminopropyltriethoxy silane (APES) with nano-HA were mainly predominated by three mechanisms, *i.e.* chemical bond, hydrogen bond and double electronic shell [27]. Labella et al. also believed that silane could be coupled to the HA surface through the reaction with the surface hydroxyl groups of HA [28]. However, they had no direct evidence that the surface hydroxyl groups were involved in these reactions.

PLA/CHA composite is often proposed as an implant material, so, long term *in vitro* study is still necessary for understanding degradation behavior of the composites and for searching the PLA/CHA composites with suitable degradation properties. Therefore, the present study used bovine bone based CHA powder as filler for producing PLA composites and focused on exploring influence of silane modified CHA on *in vitro* degradation of PLA composites. By *in vitro* study, it would be possible to predict the degradation behavior and the bioactivity of the PLA composites during the period of implanted time.

2. Materials and methods

2.1. Materials

PLA (4042D) was purchased from NatureWorks LLC Co. Ltd. Bovine bones were supplied by Limeiseng Co., Nakhon Ratchasima, Thailand. 3-Aminopropyltriethoxysilane (APES) and 3-methacryloxypropyltrimethoxysilane (MPTS) were purchased from Optimal Tech Co., Ltd. and Aldrich, respectively. Dibasic sodium phosphate ($NaHPO_4 \cdot 2H_2O$), monobasic sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$), potassium chloride (KCl) and dibasic potassium phosphate ($KHPO_4 \cdot 2H_2O$) were purchased from Carlo Erba Reagent Spa. Sodium chloride (NaCl) was purchased from VWR International buba/sprl. Sodium hydrogen carbonated ($NaHCO_3$), sodium sulfate (Na_2SO_4) and tris(hydroxymethyl) methylamine ($NH_2C(CH_2OH)_3$) were purchased from Fisher Scientific UK, Ltd. Magnesium dichloride ($MgCl_2 \cdot 6H_2O$) was purchased from Ajex chemicals. Calcium chloride ($CaCl_2$) was purchased from APS Ajex finechem.

2.2. Preparation and characterization of CHA and silane treated CHA powders

Bovine bones were burned in open air and were ground into powder using a ball milling machine. Then, the powder was heat treated at 1100 °C for 3 h. The obtained powder was characterized by an x-ray diffraction spectrometer (XRD) (Bruker axs/D5005), a scanning electron microscope (SEM) (Jeol/JSM-6400) which is equipped with an energy dispersive x-ray spectrometer (EDX) (Oxford instrument/Link ISIS6209) and a Fourier transform infrared spectrometer (FTIR) (diffuse reflectance technique (DRIFTS), BIO-RAD/FTS175C). According to our previous works, the results confirmed that the highly agglomerated and irregular shaped powder composed of crystalline carbonated hydroxyapatite (CHA) [18].

The CHA powder was modified by either APES or MPTS in acidic solution at pH of 3.5 for 3 h. Subsequently, each solution was suddenly neutralized with 0.1 N NaOH aqueous solution. The content of silane based on weight of CHA powder was 2.0 wt.%. After that, the silane-treated CHA powders were washed and dried overnight in an oven at 80 °C. APES treated CHA and MPTS treated CHA were called aCHA and mCHA, respectively. The presence of APES and MPTS on the CHA surface was confirmed by a FTIR (DRIFTS technique) and a thermogravimetric analyzer (TGA) (TA Instrument/ SDT2960). For TGA test, the sample was heated from room temperature to 1000 °C under a nitrogen atmosphere at a heating rate of 10 °C/min.

2.3. Preparation and characterization of PLA composites

PLA composites at various contents of filler, *i.e.* 0%, 20% and 40% by weight, were prepared by using an internal mixer (Haake/Rheomix) at 170 °C with a rotor speed of 70 rpm for 10 min. Each PLA composite was left at room temperature for 24 h and cut into small pieces. Various molding dies according to ASTM D256 standards were used to prepare composite specimens. PLA composites were heated in the dies from room temperature to 180 °C and maintained at that temperature for 15 min. Subsequently, it was hot-pressed by a compression molding machine (Gotech/GT-7014-A30) for 10 min under a pressure of 2000 psi and cooled to room temperature.

Fracture surfaces of the PLA composites were visualized using a SEM operating at 15 kV. All samples were coated with a thin layer of gold before examining.

Water contact angle on surface of the PLA composites was determined by sessile drop measurement using a telescopic goniometer (Ramé Hart/100-00). A distilled water droplet (5 µl) was placed on the sample surfaces and its contact angles were measured. Each reported angle was the averaged value. Five samples of each composite were used and three measurements were carried out for each sample.

2.4. Determination of in vitro degradation of PLA composites

A phosphated-buffered solution (PBS) at a concentration of 0.1 M and pH 7.4 was in-house prepared by mixing 81 ml of 0.2 M $Na_2HPO_4 \cdot 2H_2O$ solution together with 19 ml of 0.2 M $NaHPO_4 \cdot 2H_2O$ solution and adding distilled water to obtain total volume of 200 ml. *In vitro* hydrolytic degradation of PLA composites were determined by soaking PLA and PLA composites in a PBS. Three specimens ($4 \times 10 \times 60$ mm^3) from each sample were vertically placed in a 100 ml test tube filled with 35 ml PBS solution. The immersed specimens were incubated at 37 °C for 0, 1, 2, 3, 4, 6 and 8 weeks. The buffer solution in all test tubes was weekly replaced by fresh PBS. This was done in order to maintain a constant volume of PBS solution and to imitate, to some extent, *in vivo* flow model of continuously refreshing extracellular fluids.

At the end of each period, pH of PBS solution in each test tube was measured by pH meter (Jenway/3020). The specimens were removed from PBS and wiped with a filter paper to remove surface water. The wet weight (W_w) was measured. Then, these specimens were rinsed by distilled water for 3 times and vacuum dried at a temperature of 65 °C to a constant weight (W_d). Water absorption of the PLA composites from PBS solution was determined. The percentage of weight increase and percentage of weight loss of the specimen during immersion in PBS solution was calculated by the following equations:

$$\text{Weight increase}, \% = \left[\frac{(W_w - W_o)}{W_o}\right] \times 100$$

$$\text{Weight loss}, \% = \left[\frac{(W_o - W_d)}{W_o}\right] \times 100$$

Where W_o is an initial weight of the specimen; W_w is the wet weight of the specimen after removing from PBS; W_d is the weight of the specimen after removing from PBS and drying at 65 °C.

Additionally, the changes in PLA molecular weights and morphologies of the PLA composites after PBS immersion were investigated. Molecular weight of PLA in PLA composite was evaluated by a gel permeable chromatograph (GPC) using chloroform as an eluent. The chromatograph consisted of a styrene-divinylbenzene copolymer column (PLgel Mixed-C, 300 × 7.5 mm, 5 µm) and a differential refractometer detector