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Fabrication and evaluation of bioresorbable PLLA/magnesium and PLLA/magnesium fluoride hybrid composites for orthopedic implants

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ABSTRACT

Polymers, such as poly-L-lactide (PLLA) were the first materials to be used as commercial biodegradable and bioresorbable implant materials. However, the limitations were focused on low mechanical properties and acid degradation by-products which were concerned as the source of inflammation. In this work, hybrid composites incorporated PLLA with 3–7 wt% magnesium and magnesium fluoride particles were developed to overcome drawbacks mentioned above as novel bioresorbable orthopedic implants. The morphology, mechanical and thermal properties, *in vitro* degradation and cytotoxicity assessment were analyzed by SEM, DSC, UTM, pH value monitoring and MTT. It was found that the tensile strength was slightly decreased with addition of Mg particles. The tensile fracture morphologies indicated that the interface adhesion between Mg particles and PLLA matrix could be contributed to the influence on mechanism property. The addition of Mg and MgF_2 into PLLA was effective in neutralizing the acid environment caused by degradation by-products, which was reflected by a close pH value to body fluid. Moreover, cell viability showed better cytocompatibility of composites with the beneficial Mg ions release. Therefore, PLLA/magnesium and PLLA/magnesium fluoride hybrid composites had a promising potential for orthopedic implant application.

1. Introduction

Bioresorbable materials are widely used for bone fixation and repair applications overcoming the need of a second surgery for implant removal after the bone heals in comparison with metallic implants. Among the bioresorbable materials, the biodegradable polymers are widely studied and used as medical materials. They have been demonstrated to be biocompatible *in vivo* with non-toxic leaches and could degrade in a controllable rate with tissue recovery, which have been approved for clinical use as surgical suture and bone fixation device [1–4].

Poly-L-lactide (PLLA) is a biodegradable aliphatic polyester, which is derived from renewable resources and is considered to be one of the most promising polymers as fracture fixation materials due to its comparatively good strength and slow degradation rate [5,6]. However, PLLA has some shortcomings such as lower hydrophilicity, acidic degradation products, and the poor compressive strength. The adverse clinical effect is found that the *in vivo* degraded acidic monomers may cause inflammatory or allergic reactions [7]. To overcome those drawbacks mentioned above, the chemical modification of PLLA including copolymerization and blending modification are extensively studied [8–11]. Among these methods, synthesization of polymer based composites has gained more and more interests in recent years. He et al. [12] synthesized β-CD-PLA-mPEG copolymer, which could be used as a biocompatible drug delivery system. A biocomposite of carbon fiber reinforced hydroxyapatite/polylactide was prepared by hot pressing, and showed excellent mechanical properties [13]. Zhang et al. [14] developed a PLLA/multiwalled carbon nanotube composite and found that the young's modulus was increased whereas the growth of fibroblasts was inhibited. Addition of bioglass increased water absorption and weight loss in comparison to pure polymer. PLLA/bioglass composite retained its structural integrity for implantation of 16 weeks, indicating degradation was still in the early stages [15]. The porous PLLA/HA hybrid composite showed better osteoconductivity, bone regeneration, and mineralization and had moderately high strength [16].

With regards to bone repair and regeneration, magnesium is considered as an alternative approach to enhance the bioactivity and control the degradation of bioresorbable polymer. Magnesium and its alloys are expected to be a type of revolutionary degradable metallic materials. Interest in research and application for bone

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fixation and coronary stents has been growing in the last decade [17]. Mg is an essential trace element for human metabolism and its deficiency has been linked to various pathological conditions [18]. Prior studies showed the benefits of magnesium are attributed its ability to simulated bone growth and healing [19]. Thus, the incorporation of Mg particle into PLLA could be expected to provide beneficial biological effects on bone regeneration. The alkaline circumstance, which resulted from the degradation of magnesium, could neutralize the degraded acidic products of PLLA, which consequently relieved the inflammation. Kum et al. [20] utilized OLA-grafted magnesium hydroxide as a reinforcing material for a composite of PLLA. It was found that PLLA/Mg-OLA showed twofold higher tensile strength compared with pure PLLA, as well as the inflammatory response was reduced. Cifuentes et al. [21] developed a PLLA composite with 30 wt% Mg for orthopedic application and found that reinforcing the polymer matrix with Mg particle increased its yield strength from 58 to 101 MPa, approaching value to that of human cortical bone. However, there is a lack of relevant combined evaluation of mechanical, degradation properties and biocompatibility of the composite.

In this work, two types of composite were designed: poly-L-lactide (PLLA) matrix loaded with pure magnesium particles; PLLA loaded with magnesium fluoride particles, in which the degradation of magnesium was controlled by fluoride treatment [22]. The composites were prepared using a two-step process: master batch preparation using a solvent mixture followed by extrusion process and injection molding. The morphology, mechanical property and thermal stability of composites were investigated to find the influence of Mg incorporation. The degradation and cytotoxicy were evaluated for the potential application as orthopedic implants.

2. Materials and method

2.1. Sample preparation

A medical-grade poly-L-lactide (PLLA) (PL-20, SinoBiomaterials Co., Ltd., China) was dissolved in chloroform, and loaded with pure Mg (99%) or fluoride treated Mg particles (⩽60 μm) to prepare the master batch. Then the mixtures were kept under magnetic stirring to attain uniform dispersion and casted in Petri dishes. The solvents were allowed to evaporate at room temperature overnight followed by oven vacuum-drying at 55 °C for 8 h. The crushed master batch was dry-mixed with PLLA, diluting it to 3, 5 and 7 wt% magnesium contents. The composites were manufactured using a twin-screw extruder (SJSZ-07A, Wuhan Ruiming Polymer Machine Co., Ltd., China) with a screw speed of 20 rpm and the temperature was varied from 170 °C at the feeding zone to 185 °C at the die. The pelletizing step was followed after extrusion.

The extruded pellets were dried at 80 °C for 3 h prior to injection molding (FM 1202, Beijing Future Material Sci-tech Co., Ltd.) of the test samples according to ASTM D638-05, testing method. The barrel temperature of the injection molding machine was set at 180 °C. The injection pressure was kept constant at 40 MPa and the mold temperature was 70 °C. The injection-molded samples were used for evaluation of the mechanical and thermal properties. The samples used for immersion and cytotoxicity tests were cut to dimensions of 10 mm × 10 mm × 4 mm and ground with SiC abrasive paper up to 1200 grit, followed by ultrasonic cleaning in absolute ethanol for three times.

2.2. Microstructure characterization

Morphology of the composite surfaces and the distribution of magnesium/magnesium fluoride particles in the PLLA matrix were observed with an optical microscope (Axiovert 200 MAT, Zeiss, Germany) and a scanning electron microscope (SEM, HITACHI S-3400N) equipped with energy-dispersive spectrometry (EDS, Oxford INCA energy 300). The molecular weight of the hybrid composites was measured with size exclusion chromatography (SEC) analysis by gel permeation chromatography (GPC, Waters 1515, USA).

2.3. Mechanical properties

According to ASTM D638-03, the tensile and bending tests were carried out on an Instron 5582 testing machine at room temperature using a load cell of 50 kN and a strain rate of 2 mm/min until failure. After the tests, the fragments of samples were collected for fracture analysis.

2.4. Thermal properties

The thermal characteristics of PLLA, PLLA/Mg and PLLA/MgF_2 hybrid composites were measured by a differential scanning calorimetry (DSC, Q20 TA Instrument) under argon atmosphere. The experimental design was based on two temperature cycles: a first heating from 10 to 200 °C, allowing to see the effect of composite processing, was followed by a fast cooling to 10 °C intended to quench the polymer in its vitreous state; finally, a second heating from 10 to 200 °C allows to see the effect of composition on the crystallization and melting temperatures, all three of them at a rate of 20 °C/min.

2.5. In vitro degradation evaluation

Degradation evaluation of the composites was performed by immersion tests according to ASTM G31-72. All the samples were immersed in the simulated body fluid (SBF) (8.035 g NaCl, 0.225 g KCl, 0.072 g Na_2SO_4, 0.231 g $KH_2PO_4{\cdot}3H_2O$, 0.292 g $CaCl_2$, 0.311 g $MgCl_2{\cdot}6H_2O$, 0.355 g $NaHCO_3$, 39 mL 1 M HCl and 6.118 g Tris) [23] at 37.5 ± 0.5 °C for four weeks. The pH of the initial solution was 7.4. The solution was changed every 24 h to simulate the metabolic environment of the human body. The pH value of the solution was measured during the immersion test. Samples were removed after 30 days rinsed in distilled water and air dried overnight. The changes in surface morphology and composition of samples after degradation in the solution were analyzed by SEM and EDS. The release of Mg ions during immersion was measured by an atomic absorption spectrometer (Analyst AA800, PerkinElmer, USA).

2.6. In vitro cytotoxicity assessments

L929 fibroblast cells were cultured in a Dulbecco's Modified Eagle's Medium (DMEM, Hyclone, USA) which contained 10% fetal bovine serum (FBS, Hyclone, USA), 100 U/mL penicillin (sigma) and 100 μg/mL streptomycin (sigma) at 37 °C in a humidified tmosphere of 5% CO_2. When the cells reached 80% confluency, the cells were passaged at a 1:4 split ratio and those between the third and seventh passages were used for all the experiments. Prior to the cells experiment the samples were sterilized using ultraviolet radiation for at least 2 h.

The cytotoxicity test was carried out by the extract assay according to ISO10993-5: 2009. Extracts were prepared with a surface area to extraction medium ratio of 1.25 cm^2/mL in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h incubation. Cells were incubated in 96-well flat bottomed cell culture plates at 3×10^3 cells per 100 μL in each well and incubated for 24 h to allow attachment. The medium was then replaced with 100 μL of extract, 100 μl of negative control (medium alone) or 100 μl of