



Thermoplastic starch/ethylene vinyl alcohol/forsterite nanocomposite as a candidate material for bone tissue engineering

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ABSTRACT

Recently, biodegradable polymers such as starch based blends have been well renowned in the biomedical field. Studies have considered them suitable for bone scaffolds, bone cements, tissue engineering scaffolds, drug delivery systems and hydrogels. The aim of this study was to synthesize nanocomposite biomaterial consisting a blend of thermoplastic starch and ethylene vinyl alcohol as the polymer matrix, and nano-structured forsterite as the ceramic reinforcing phase for bone tissue engineering applications. Furthermore, vitamin E was applied as a thermal stabilizer during melt compounding. Extrusion and injection molding were incorporated for melt blending and shaping of samples, respectively. With blending thermoplastic starch and ethylene vinyl alcohol, some properties of thermoplastic starch such as degradation rate and water absorption were modified. In addition, using nanoforsterite as the ceramic reinforcing phase resulted in the improvement of mechanical and biological traits. The addition of nanoforsterite decreased the weight loss of the thermoplastic starch and ethylene vinyl alcohol blend in simulated body fluid. Moreover, this addition modified the pH in the MTT (methyl thiazolyl tetrazolium) assay and stimulated the cell proliferation. Cell adhesion assays indicated a favorable interaction between cells and the biomaterial. The proposed nanocomposite has appropriate biocompatibility, as well as mechanical properties in order to be used in bone tissue engineering.

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1. Introduction

Orthopedic problems are growing and becoming more devastating and costly with increasingly active lifestyles, accidents and the ageing population. Synthesis of bone scaffolds is one approach to repair or replace damaged bone tissue that allows for overcoming the lack of donor tissue. Scaffolds are used as intermediate phase implants that stimulate bone growth and attract newly formed bone tissues before being remodeled. Therefore, material composition and characterization are important to the success of a scaffold. In this regard, different materials have been investigated, of which polymer-ceramic composites have drawn the most attention [1]. However, the ideal biomaterials are yet to be found.

Natural polymers such as starch are biodegradable, biocompatible and show good osteoconductive behavior [2]. Starch is a polysaccharide which is highly compatible with peptide and protein, promising for tissue engineering. It already has been reported that the starch-based materials are proper candidates for bone tissue engineering scaffolds [3–5].

However, processing and using starch as a biomaterial is challenging due to its low mechanical and thermal stability. Hence, starch based materials are commonly blended with more stable synthetic polymers, like ethylene vinyl alcohol, to make them less brittle and more easily processed [3]. Ethylene vinyl alcohol is a hydrophilic, nontoxic and biocompatible polymer with high mechanical strength. Blends of starch and ethylene vinyl alcohol have been proposed as a potential alternative biodegradable material for the wide range of biomedical applications, including bone cements, hydrogels for controlled drug delivery, and bone substitutes, showing promising mechanical properties, degradation behavior and biocompatibility [6–8]. However, thermal sensitivity of these polymeric blends during high temperature melt-based processes can intensify the degradation rates of scaffolds in simulated physiological solution due to production of low molecular weight components that leach to the solution [9]. This implies the need to use some type of antioxidant, such as vitamin E, to prevent thermomechanical degradation of the polymer blend during the manufacturing process.

Remarkably, polymer-ceramic composite scaffolds benefit from both biodegradable polymers and bioactive ceramics. Since mechanical properties of these artificial bones are affected by both ceramics and

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polymers, the properties of polymer–ceramic composite scaffolds resemble those of natural bone in terms of stiffness, strength and ductility simultaneously. Additionally, ceramic particles in these composites provide a pH buffering effect on the polymer's surface, improve cell seeding and modify polymer matrix degradation [10]. Different kinds of micro and nano-sized ceramic particles have been incorporated in synthesizing bone scaffolds [11–13].

The aim of the current study is to fabricate nanocomposite biomaterial for bone scaffold and orthopedic applications from a blend of thermoplastic starch (TPS) and ethylene vinyl alcohol (EVOH) as the polymer matrix and nanoforsterite (Mg_2SiO_4) as the ceramic reinforcing phase. Nanoforsterite is a promising new bioceramic with superior mechanical properties to calcium phosphate ceramics, glass and glass ceramics. Forsterite is biocompatible and has been claimed to be bioactive in nano-sized structure [14,15]. In order to suppress oxidation of polymer matrix during melt compounding, vitamin E (α -tocopherol) has been employed as the antioxidant. This is a natural chain-breaking antioxidant that has been shown to be an extremely effective melt stabilizing antioxidant for the polymers such as low density poly ethylene (LDPE), high density poly ethylene (HDPE) and poly propylene (PP) [16]. Moreover, ultra-high molecular weight polyethylene (UHMWPE) implants have been cross-linked and stabilized with vitamin E in order to improve the fatigue, wear and oxidative degradation resistance [17]. Extrusion and injection molding were incorporated for melt blending and shaping of samples, respectively. Structural and mechanical properties, as well as bioactivity, weight loss, cell viability and cell adhesion of samples were assessed. Furthermore, the effects of using vitamin E on the melt rheology and thermal stability were investigated.

2. Materials and methods

2.1. Materials

The starting materials used for synthesizing nanoforsterite in this research are nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$, Merck, 99.99% purity), colloidal silica (SiO_2 , 34 wt% solid fraction, Sigma), polyvinyl alcohol (Merck, molecular weight = 72,000), sucrose (Merck, 99.9% purity) and nitric acid (Merck, 65%). Moreover, unmodified food grade corn starch (Glucosan Company, Iran) was used as the base material for making thermoplastic starch. Glycerol (Mojallal Company, Iran) and water were chosen as plasticizers. Ethylene vinyl alcohol (Soarnol DC3205HB) with 32 mol% ethylene content was obtained from Nippon Gohsei Co., Japan, for preparing the polymer blend. Additionally, vitamin E (α -tocopherol, Hoffmann-La Roche Co., Switzerland) in powder form was used as the antioxidant.

2.2. Preparation of nanoforsterite

To prepare the nanoforsterite with particle size in the range of 25–45 nm, sol-gel method proposed by Kharziha and Fathi [14] was chosen due to the high purity of products, better composition homogeneity and simplicity. Aqueous precursor solution of the magnesium salts and colloidal silica with specified stoichiometric ratio was prepared. Then aqueous solution of sucrose was added gradually to the precursor solution and stirred for 2 h. Subsequently, polyvinyl alcohol aqueous solution was added to the final solution and the pH value was adjusted to 1 using nitric acid and stirred at 80 °C for 2 h. The prepared gel was aged for another 24 h and then heated at 100 °C for complete dehydration. Finally, the dried gel was calcined in a furnace at 800 °C for 2 h.

2.3. Blends preparation

To prepare the mixture of starch, glycerol and water (55, 30, 15 wt%), first glycerol and water were dissolved at 40 °C. Next, starch, which was previously dried for 3 h at 100 °C, was gradually added and thoroughly mixed for 2 h at 800 rpm with an overhead stirrer. Then

the starch mixture was sealed in a plastic bag for 48 h. Table 1 shows the compositions of the different samples. Vitamin E, EVOH and forsterite in specified weight ratios were added to the aged starch mixture and were manually mixed. Afterward, they were extruded by a co-rotating twin-screw extruder (Nanjing Giant machinery Co., China; length 1.2 m, diameter 53 mm). Screw speed was adjusted to 150 rpm, and the temperature range from feeder to nozzle was set at 120–140 °C for P-T and 175–195 °C for the other samples. Obtained strands were air cooled and pelletized, then molded in impact bars and dumbbell shaped tensile specimens using a Weissenburg/Bayern injection molding machine type mono80 with the same as extrusion temperature.

2.4. Torque rheometry

To find the optimum amount of required antioxidant, a laboratory internal roller mixer attached to Brabender Plasticorder (PLV-151, Brabender OHG, Germany) was employed. When the mixing chamber (capacity 60 ml) reached the desired temperature, 50 g of starch mixture (55 wt% starch, 30 wt% glycerol and 15 wt% water) with 0, 0.2 and 2 wt% of vitamin E were charged into the chamber and were mixed for 20 min. Rotor speed and temperature were set close to processing condition at 120 rpm and 170 °C, respectively. Torque versus time data was recorded as the indicator of polymer degradation. It is worth mentioning that the thermomechanical degradation process causes molecular breakage and consequently lowers the torque to maintain the screw speed.

2.5. Fourier transform infrared (FTIR) spectroscopy

In order to evaluate the effect of vitamin E in prohibiting the oxidation during the processing, the injected samples were analyzed by FTIR spectroscopy. The FTIR spectra of the injected samples were obtained by a Bruker-Equinox 55 FTIR spectrophotometer at 4 cm^{-1} resolution.

2.6. Thermal analysis

Thermal degradation of TPS without vitamin E and in presence of vitamin E was investigated using Thermogravimetry analysis (TGA) and Differential scanning calorimetry (DSC) measurements. Thermal analysis carried out by a Thermogravimeter/Differential Scanning Calorimeter (Mettler - Switzerland), coupled TA processor was used. Extruded granules (5 mg) of TPS without vitamin E and TPS containing 2 wt% vitamin E were heated from 20 to 850 °C at a heating rate of 10 °C/min under the air atmosphere.

2.7. Mechanical properties evaluation

Tensile properties of the samples were measured using a universal frame (H10KS, Hounsfield Co., England) equipped with an incremental extensometer. Tensile tests were carried out at a cross-head speed of 5 mm/min on dumbbell-shaped specimens at room temperature according to ASTM-D638. The notched impact strength was determined using a Santam impact tester according to ASTM-D256-8. At least five tests were carried out for each data point.

2.8. Scanning electron microscopy (SEM) observations

To study the morphology of specimens made, fractured surfaces of the impact samples were analyzed by scanning electron microscopy (SEM, Tescan Mira II) (Figs. 5-7 and 9-11) in the secondary electron mode, after being coated with a thin conductive layer of gold.

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