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Effect of mixing ceramics with a thermosensitive biodegradable hydrogel as composite graft

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ABSTRACT

The composite of methoxy polyethylene glycol (mPEG) and poly(lactic-co-glycolic acid) (PLGA) thermosensitive hydrogel mixed with various portions of hydroxyapatite (HAP) or β-tricalcium phosphate (β-TCP) were used as bone graft substitutes. The physical properties of a series of composite gels, including the critical micelle concentration (CMC), particle sizes, zeta potential, rheological behavior, morphology of composite gels, and sol–gel transition, were characterized *in vitro*. These composite gels could form a gel at body temperature and could be controlled easily at room temperature, but showed only a small decline in pH, to between 6.33 and 6.66, whereas mPEG–PLGA gel without ceramic exhibited a more significant decrease in pH over a period of 5 days. The dissolution of ceramics results in an increase in the concentration of calcium and phosphate, which can buffer the degradation of mPEG–PLGA. Higher cell viability was observed in the composite gels with more bioceramics, as shown in the MTT assay and the live and dead stain. Mixing mPEG–PLGA with HAP or β-TCP may hold greater promise than mPEG–PLGA alone for repairing bone defects.

1. Introduction

Current methods for repairing bone defects include autograft, allograft, xenograft, and bone substitute using materials such as bioceramics and synthetic polymers. Autografts are the standard treatment but have limited applicability because of donor site morbidity. Allografts are associated with potential complications such as transmission of infectious diseases and induction of immune responses [1]. To solve these issues, recent research has focused on the use of synthetic bone grafts. These materials could degrade at a controlled rate to provide space for the formation of new bone [2], and the byproducts are not toxic to the implanted environment.

Aqueous solutions of some polymers undergo sol–gel transition in response to temperature changes [3]. The minimally invasive, in situ gel injection system is an advantageous alternative to surgical procedures. Polyethylene glycol (PEG) is highly hydrophilic and is often used to build a hydrophilic block of micelle-forming copolymers [4,5]. A series of thermosensitive biodegradable hydrogels were synthesized by ring-opening polymerization of methoxy polyethylene glycol (mPEG) with different kinds of ester monomers, including β-propiolactone, δ-valerolactone, ε-caprolactone [1], and poly(lactic-co-glycolic acid) (PLGA). The most researched biodegradable thermosensitive hydrogel is mPEG–PLGA because mPEG and PLGA have been approved by the Food and Drug Administration (FDA) for human use [6], they have excellent biocompatibility and biodegradability, and have been widely used as sutures, vascular grafts, drug carriers [7], and scaffolds for tissue engineering [8]. Because of all these advantages, our research team chose mPEG–PLGA as the polymeric material. In general, polylactic acid (PLA), polyglycolic acid (PGA), or PLGA degradation is accompanied by bond cleavage of the ester bond linkages in the polymer backbone because of hydrolytic attack of water molecules [9,10]. However, some problems need to be addressed for materials containing PLA, PGA or PLGA include low cell adhesion and local acidity [11]. pH value may lower to pH 3 after 35-day incubation [12]. Acidic environment around the hydrogel is known to be deleterious to bioactive proteins or cells cause a non-bacterial inflammation *in vivo* [13,14].

Calcium phosphate ceramics such as hydroxyapatite [15] and β-tricalcium phosphate (β-TCP) have been used in dental and

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orthopedic surgery and have been reported to result in excellent cell adhesion. These materials are highly biocompatible and osteoconductive and can stimulate bone regeneration [16]. A possible explanation for this increase in osteogenesis could be that the surface of calcium phosphate ceramics meets the electrical and spatial requirements for bone binding. However, their clinical application is limited because of brittleness, difficulty in molding, and the lack of drug delivery capability [17]. An important parameter of theses ceramic materials is its dissolution rate, which is affected by the Ca/P ratio. β-TCP (Ca/P < 1.67) dissolves 12.3 times faster than HAP (Ca/P = 1.7) in an acidic medium. The dissolution process results in an increase in the concentration of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) [18]. Yamada et al. investigated the osteoclastic resorption of calcium phosphate ceramic with different HAP/β-TCP rations [19]. β-TCP dissolves rapidly resulting in a high (Ca^{2+}) and therefore an ineffective resorption by the osteoclasts was seen. In contrast, β-TCP in combination with HAP dissolves more slowly. The release of anion contributes local basic environment.

Composite grafts with contents of HAP or β-TCP, and PLA have been reported to be effective at improving cell adhesion [18,20]. Polymer-bioceramic composites enhance structural integrity, fracture strength, and toughness of scaffolds [21]. However, biocompatibility as well as the chemical and physical properties of this kind of composite graft is unclear. The purpose of this study was to evaluate the performance of polymer-ceramic composites as bone substitutes in terms of sol–gel–sol transition, pH change and material toxicity.

2. Materials and methods

2.1. Materials

DL-lactide (LA) and glycolide (GA) were purchased from Purac. Methoxy polyethylene glycol (mPEG) (Mn = 550 g/mol) was obtained from Aldrich Chem, Co. Inc. β-TCP and HAP (<200 nm) were from Sigma; 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Fluka; and stannous 2-ethylhexanoate (stannous octoate), a catalyst, was purchased from Aldrich Chem.

2.2. Synthesis of mPEG–PLGA diblock polymer

A series of the methoxy polyethylene glycol-co-poly(lactic-co-glycolic acid) (mPEG–PLGA) diblock copolymers were synthesized by ring-opening polymerization of monomers and mPEG in the presence of stannous 2-ethylhexanoate [22]. A typical synthetic procedure is shown in Fig. 1. According to the previous studies in our laboratory [23], mPEG–PLGA (550–1405) copolymer has the most suitable thermosensitive and the most stable drug release profile. To prepare the diblock copolymer, mPEG (8.01 g) was mixed with lactide (16.67 g) and glycolide (3.80 g) in a dry three-neck reactor equipped with a mechanical stirrer, in a dry nitrogen atmosphere. An electric heater controlled the reactor temperature with the feedback sensor set to 160 °C. Stannous 2-ethylhexanoate (9 μL) was added to the reactor to catalyze the polymerization process that was performed at 160 °C for 8 h. The resulting copolymer was dissolved in 30 mL dimethyl sulfoxide (DMSO). The solution was then purified by dialysis (MWCO = 1000) for 3 days at 4 °C and lyophilized at −20 °C for 3 days.

2.3. Characterization of mPEG–PLGA copolymer

To study the molecular structure of the mPEG–PLGA copolymer, ^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed on a 500-MHz NMR spectrometer (Varian Unityinova 500 NMR) at room temperature using $CDCl_3$ as the solvent. Molecular weight and molecular weight distributions were determined by gel permeation chromatography (GPC; RI-2031, PU-2080, JASCO) and ^{1}H NMR, which used a ratio of δ = 3.6 ppm, δ = 4.8 ppm, and δ = 5.2 ppm for the calculation of molecular weight. Tetrahydrofuran (THF) was used as a solvent with a flow rate of 1 mL/min. Fourier transfer infrared spectra (FTIR) measurements were obtained using Perkin–Elmer system 2000 with KBr pellets.

2.4. Preparation of composite gel

The mPEG–PLGA copolymer-ceramic composite gels were formed by adding 20 wt.% mPEG–PLGA hydrogel with different weight ratios of HAP or β-TCP. The hydrogel (20 wt.%) was obtained by mixing 200 mg of mPEG–PLGA hydrogel with 0.8 mL deionized H_2O at 4 °C for 12 h. mPEG–PLGA hydrogel (20 wt.%) without ceramic was defined as G1C0. Different types and amounts of ceramics were subsequently added to the hydrogel, HAP 66.67 mg (defined as G3H1; polymer:HAP = 3:1), HAP 28.57 mg (defined as G7H1; polymer:HAP = 7:1), β-TCP 66.67 mg (defined as G3T1; polymer: β-TCP = 3:1), and β-TCP 28.57 mg (defined as G7T1; polymer: β-TCP = 7:1) at 4 °C and stirred for 4 h.

2.5. Dynamic laser scattering (DLS) and zeta potential

Particle sizes of polymeric micelles were measured using DLS. Measurements were carried out using a spectrophotometer (Nano Series Zeta Sizer; Malvern) equipped with a He–Ne laser at 633 nm, 25 °C, and a fixed scattering angle of 90°. The nano-micelle solution at a concentration of 0.1 wt.% was filtered through a 0.45-μm filter membrane before measurement.

2.6. Critical micellization concentration (CMC) determination

1 wt.% composite gel passed through a 0.45-μm filter and then serially diluted by a factor of 2 into 16 vials (1 wt.%, 0.5 wt.%, 0.025 wt.%,…, 3.05×10^{-5} wt.%). The fluorescent dye DPH was used at a concentration of 0.4 mM. 2 μL DPH solution was mixed with 100-μL copolymer solution at 4 °C overnight. The reaction was proceeded in the dark. Using an enzyme-linked immunosorbent assay (ELISA) reader set at an excitation wavelength of 360 nm and emission wavelength of 480 nm, absorption of fluorescence intensity was measured. The ratio of fluorescence intensity was plotted against the logarithm of copolymer concentrations to determine the CMC.

2.7. Determination of sol–gel–sol phase transition

Different concentrations of mPEG–PLGA solution (i.e., 10%, 15%, 20%, 25%, 30%, and 35%) were prepared at 4 °C, and then, different weight ratios of HAP or β-TCP were sequentially added, followed by mixing at 4 °C for 12 h. Samples were prepared in 1-mL Eppendorf tubes and incubated at 4 °C for 5 min until the temperature achieved equilibrium. The temperature was raised with an interval of 2 °C and maintained for 5 min before each sampling. The Eppendorf tubes were flipped upside down for 30 s to observe for any movement so to determine the sol–gel status. The temperatures of sol-to-gel and then gel-to-sol transformation were recorded for sol–gel–sol phase diagram using Gaussian regression.

2.8. Rheological measurement

The viscosity of diblock copolymer aqueous solutions with various concentrations of HAP/β-TCP were measured using a