Combinatorial Design of Hydrolytically Degradable, Bone–like Biocomposites Based on PHEMA and Hydroxyapatite

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ABSTRACT

With advantages such as design flexibility in modifying degradation, surface chemistry, and topography, synthetic bone–graft substitutes are increasingly demanded in orthopedic tissue engineering to meet various requirements in the growing numbers of cases of skeletal impairment worldwide. Using a combinatorial approach, we developed a series of biocompatible, hydrolytically degradable, elastomeric, bone–like biocomposites, comprising 60 wt% poly(2–hydroxyethyl methacrylate–*co*–methacrylic acid), poly(HEMA–*co*–MA), and 40 wt% bioceramic hydroxyapatite (HA). Hydrolytic degradation of the biocomposites is rendered by a degradable macromer/crosslinker, dimethacrylated poly(lactide–*b*–ethylene glycol–*b*–lactide), which first degrades to break up 3–D hydrogel networks, followed by dissolution of linear pHEMA macromolecules and bioceramic particles. Swelling and degradation were examined at Hank's balanced salt solution at 37 $^{\circ}$C in a 12–week period of time. The degradation is strongly modulated by altering the concentration of the co–monomer of methacrylic acid and of the macromer, and chain length/molecular weight of the macromer. 95% weight loss in mass is achieved after degradation for 12 weeks in a composition consisting of HEMA/MA/Macromer = 0/60/40, while 90% weight loss is seen after degradation only for 4 weeks in a composition composed of HEMA/MA/Macromer = 27/13/60 using a longer chain macromer. For compositions without a co–monomer, only about 14% is achieved in weight loss after 12–week degradation. These novel biomaterials offer numerous possibilities as drug delivery carriers and bone grafts particularly for low and medium load–bearing applications.

1. Introduction

Polymers and bioceramics such as hydroxyapatite (HA), tricalcium phosphate (β–TCP), and bioglass may form biodegradable, biocompatible organic/inorganic composites that can be used as synthetic bone grafts. They are important to bone repair and regeneration, with structural and chemical properties that provide a biochemical environment to promote cellular responses and tissue integration [1, 2]. To meet the requirements of these functions, the most desirable synthetic organic/inorganic hybrid materials should be biocompatible, porous (with diameters of at least 100 μm), and hydrolytically or enzymatically biodegradable within a specific time frame (e.g., 4–6 months) [3-7]. They should also be both osteoconductive and osteoinductive, so that the cells can bind to the hybrid biomaterials, proliferate, migrate, and differentiate. Further, stability of mechanical properties in the materials is essential for cell survival and ingrowth of new bone tissue. Otherwise, stress shielding, inflammation, and other discomforts may result from a mismatch between mechanical properties of hybrid materials and those of surrounding tissue.

There is a significant body of literature that describes methods for fabrication of porous synthetic organic/inorganic biocomposites for bone repair and regeneration [3, 4, 6-13]. However, development of porous, biomimetic hybrid materials that simultaneously meet all requirements of bone repair and replacement still remains a challenge [14-16]. Furthermore, porous hybrid materials generally have low mechanical properties restricting their load–bearing applications [17]. In some applications, the hybrid biomaterials can be dense or non–porous. An example is the fixation of a bone fracture, where a rod of the synthetic polymer/bioceramic is inserted and fixed between the fractured bone pieces, or the rod is inserted within the broken bone [18, 19]. In this case, dense material that is both biocompatible and biodegradable is required to bear loads. Demand is increasing for both porous and dense organic/inorganic biomaterials, as evidenced by the growing number of cases of skeletal impairment [20, 21].

Recent reports have described the fabrication of non–degradable, dense, poly(2–hydroxyethyl methacrylate)/hydroxyapatite biocomposites (also known as FlexBone) based on in situ polymerization of 2–hydroxyethyl methacrylate in the presence of dispersed hydroxyapatite [22-24]. These newly formed biocomposites show elastomeric behavior. Because of the elastomeric nature of these biocomposites, they can be cut, bent, and machined into various shapes suitable for low and medium load bearing applications. However, these biomaterials are not degradable, limiting their usefulness in bone repair and replacement [6, 9, 13, 16, 25]. In certain applications, such as delivery of bone–morphogenetic proteins and antibiotics for bone repair, biomaterials are typically required to be biodegradable. To achieve biodegradability and

biocompatibility of hydrogel/HA biocomposites, we recently focused our efforts on development of pHEMA–type hydrogel/HA systems [26]. PHEMA hydrogel is widely known to be biocompatible, but not biodegradable [27, 28]. Our design of degradable, elastomeric pHEMA/HA biocomposites was inspired by the observation that a degradable crosslinker (also termed a macromer here) disintegrates by hydrolytic degradation the three–dimensional hydrogel network. Without the use of enzymes, the network decomposes into small structural molecules and generates water–soluble linear pHEMA macromolecular chains. Generally, this will occur if the molecular weight of linear pHEMA is below 8–10k Da [29]. HA is a bioactive ceramic and also resembles the inorganic phase of human bone, and thus may be gradually resorbed by the human body. Mindful of those traits, we purposely synthesized macromers/crosslinkers of dimethacrylated triblock copolymers, poly(lactide–*b*–ethylene glycol–*b*–lactide)(PLA–*b*–PEG–*b*–PLA), since these materials are biocompatible and hydrolytically degradable, and their degradation has been well investigated [30-37]. Interestingly, these dimethacrylated triblock macromers were not used as the crosslinkers in the synthesis of degradable pHEMA hydrogels [38-43]; nor were they used in the fabrication of degradable biocomposites comprising pHEMA and hydroxyapatite or other bioactive ceramics. Only few biopolymer/bioceramics (or bioglass) systems were reported in literature showing significant degradation in 4–6 months for bone tissue engineering [4-7, 44-46]. Our broad objective was to develop a series of degradable, bone–like pHEMA/HA biomaterials (not degradable pHEMA hydrogel because the pure hydrogel is useless without bioceramics for bone regeneration) as bone grafts/implants with controlled degradation especially in 4–6 months, which is highly recommended by surgeons in clinical trials [47]. Since there are various parameters in combination to determine degradation of the hybrid biomaterials such as crosslink density and wettability, we used a combinatorial approach that tuned the molecular structures of hydrogels for low to medium load–bearing applications in bone repair and regeneration [48-52]. Here, we report in detail how crosslink density, chain length (molecular weight), hydrophilicity of the macromer/crosslinker, and a polar co–monomer influence swelling and hydrolytic degradation of biomaterials.

2. Experimental section

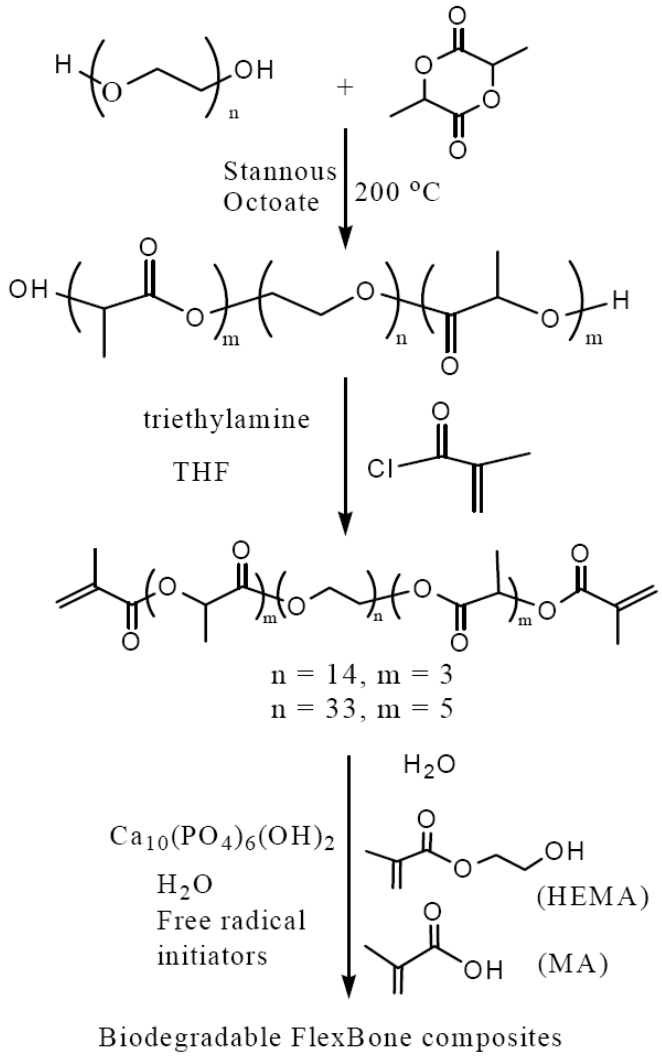
2.1 Materials

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without purification unless otherwise specified.

2.2 Synthesis of the macromer/crosslinker and fabrication of the poly(HEMA–co–MA)/HA biocomposites

Synthesis of the macromer/crosslinkers has been previously described [30, 33, 36, 37], but here we used a revised process. Prior to synthesis of the macromer/crosslinker, a lactide–co–polyethylene glycol (PEG)–co–lactide triblock copolymer was first synthesized, as shown in Scheme 1.

Scheme 1 Synthesis of degradable macromers and poly(HEMA-co-MA)/HA biocomposites



PEG600 or PEG1500, lactide, and stannous octoate were added sequentially according to a molar ratio of 93:279.5:1 (in the case of PEG600) or 93:465:1 (in the case of PEG1500) into a reaction flask at 23 ^{o}C. This mixture was purged with dry nitrogen for 30 min, and then stirred at 110 °C under vacuum for another 30 min. Next, dry nitrogen was charged to replace the vacuum and the reaction continued at 180 °C under nitrogen protection for 6 h. After cooling to 23 ^{o}C, the resultant triblock copolymer was dissolved in dichloromethane and further precipitated in anhydrous diethyl ether. The triblock copolymer

precipitate was separated and dried in a vacuum for subsequent synthesis of the macromer/crosslinker. During the synthesis of the macromer/crosslinker, the obtained lactide–co–PEG–co–lactide triblock copolymer and triethylamine (with a mole ratio of 1:6.94) were first dissolved in 230 mL dichloromethane in a reaction flask and then cooled in an ice bath to 0 °C. Subsequently, the mixture was purged with dry nitrogen for 30 min. The methacryloyl chloride, with the same molar ratio of triethylamine, was slowly added (dropwise, with stirring) into the flask and reacted for 9 h at 0 °C. The entire mixture was cooled to 23 ^{o}C and the reaction was maintained for an additional 14 h. The reaction mixture was filtered to remove triethylamine hydrochloride, and subsequently hexane was added for precipitation. The obtained crystalline solid (i.e., macromer) was further purified twice by dissolution and re–precipitation using dichloromethane and hexane. The macromer was dried at 50 °C for 5 h under vacuum.

Fabrication of the non–biodegradable pHEMA/HA composites was previously reported [23]; similar procedures were employed here to fabricate degradable pHEMA hydrogel/HA biomaterials (Scheme 1). The monomers (main monomer, co–monomer, and macromer/crosslinker with desired ratios, e.g., 20:20:60), ethanol, de–ionized water, ammonium persulfate (480 mg/mL water), sodium metabisulfite (180 mg/mL water) were mixed thoroughly at a ratio of 20:10:10:1:1 by mass. 40 wt.% HA (Trans–Tech, size 1–3 μm) relative to the total weight of all the components (i.e., 57.1 wt% relative to the total weight of monomers, macromere, and HA) was uniformly dispersed into the mixture and stirred at 5,000 rpm for 2 min using a homogenizer (Polytron PT 10–35). The resultant mixture was pumped into a syringe (20 mL, diameter of 18.32 mm) and capped. It was left overnight to gel at room temperature. As shown in Table 1, a series of hydrogel/HA materials with various formulations was fabricated using the process described above. These hydrogel/HA biomaterials were specifically labeled such as S–MA80–H0–Mac20 and L–MA0–H23–Mac77, where S represents the short chain of lactide/PEG600= 3, L the long chain of lactide/PEG1500 = 6, MA methacrylic acid, H HEMA, and Mac the macromer. The number right after MA, H or Mac represents the weight percentage of the associated component relative to the total weight (MA + H + Mac). For example, L–MA0–H23–Mac77 represents the biocomposite composed of 40 wt% HA and 60 wt% hydrogel which was synthesized using 0 wt% methacrylic acid, 23 wt% HEMA, and 77 wt% macromer based on the long chain of lactide/PEG1500 = 6.

Table 1 Formulations of degradable 60% poly(HEMA-co-MA)/40% HA biomaterials