Synthesis and Characterization of Cholic Acid-Containing Biodegradable Hydrogels by Photoinduced Copolymerization

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ABSTRACT: A cholic acid (CA)-containing biodegradable hydrogel (PLA-PEG-PLA-*co*-MACAH) was synthesized from the photoinduced copolymerization of a CA-modified methacrylate monomer (MACAH), bearing a spacer of hexane-1,6-diol spacer between the methacryloyl and the cholanoate moieties, and a macromonomer (PLA-PEG-PLA-DA), bearing two acryloyl end groups derived from a poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) triblock copolymer. The structure of MACAH was confirmed by FTIR, ^{1}H-NMR, and MS. The hydrogel PLA-PEG-PLA-*co*-MACAH was characterized by scanning electron microscopy and X-ray diffraction. The experiment results showed that the swelling ratios of the hydrogels decreased with the increase of the CA fraction. The investigation on the *in vitro* degradation of the hydrogel showed that the CA-containing hydrogels degraded much slower than the hydrogels without CA component. The bioactivity of the synthesized hydrogels was assessed by the simulated body fluid method. The observed formation of hydroxyapatite on the scaffold of the hydrogels indicated that the hydrogels possess good bioactivity. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2976–2980, 2009

Key words: hydrogels; cholic acid; PLA-*co*-PEG-*co*-PLA; photopolymerization; biodegradable

INTRODUCTION

The hydrogels based on block copolymers of poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) have been widely investigated as matrices for resorbable drug delivery carriers and tissue engineering scaffolds.[1,2] The widely used PLA-PEG-PLA triblock copolymer hydrogels reported to date have shown good biodegradability and hydrophilicity.[3–6] According to the degradation models previously reported, there are two main parameters that affect the degradation of chemically crosslinked PLA-*b*-PEG-*b*-PLA macromers.[7–9] One is the hydrolysis kinetics of the ester bonds of PLA within the hydrogels, and the other is the physical structure of the hydrogels. These two interdependent parameters make the degradation process of the gel very complicated. It was reported that increasing the molecular weight of the PEG segment decreases the crosslinking density (structural effect), which in turn increases the water content of PLA-*b*-PEG-*b*-PLA gels. At the same time, it also increases the hydrolysis rate of the ester bonds (kinetic effect) as well as the overall degradation rate.[10]

Cholic acid (CA) is a biogenic organic compound synthesized from the cholesterol in the liver of mammals. It consists of an amphiphilic steroid nucleus with a hydrophobic β-side and a hydrophilic α-side.[11] It is a useful natural modifier for the synthesis of liver-specific drugs.[12] The introduction of CA into polymers may improve the polymer's properties such as thermal properties,[13,14] biodegradation property,[15,16] biocompatibility,[17–19] and so forth. The investigation about the effect of introducing CA to the PLA-*b*-PEG-*b*-PLA gels over the gels' degradation rate and hydrophilicity is of academic information.

Here, we report our recent work on the synthesis, characterization of a CA-containing hydrogel, and the investigation of the formation of hydroxyapatite (HA) on the hydrogel.

EXPERIMENTAL

Materials

L-LA (97%, Aldrich, Shanghai, China), PEG (M_n 6000, Aldrich), CA (98%, Aldrich), hexane-1,6-diol (Acros, Organic, Belgium), and 2,2-dimethoxy-2-phenylacetophenone (DMPA, Acros) were used as

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available. Methacryloyl chloride (Aldrich) was freshly distilled before use. The triblock copolymers of PLA-PEG-PLA and PLA-PEG-PLA-DA were synthesized as described elsewhere.[10] Other reagents used were of analytical reagent grade. Oxygen and water-free reagents and solvent were used throughout the experiments using standard Schlenk technique.

Measurements

The FTIR spectra were recorded on a FTIR FTS-135 (Bio-Rad) with samples prepared from ground polymer powders mixed with KBr powder. The NMR spectra were recorded on an AV-300 spectrometer operating at 300 MHz using $CDCl_3$ for ^{1}H-NMR with TMS as an internal standard. Mass spectrometry (MS) was performed on a MS ZAB-HS apparatus. Scanning electron microscopy (SEM; JSM5610LV) on gold-coated specimens was used to examine the morphological and textural features of the hydrogels, using an accelerating voltage of 15 kV. X-ray diffraction (XRD) analysis was performed on a Rigaku D/Max-2500 X-ray apparatus under Cu 40 kV 100 mA (JPG).

Synthesis of the CA-containing monomer

The synthesis of the monomer, methacryloyl hexane-1,6-diol cholanoate (MACAH), involves first the synthesis of hexane-1,6-diol cholanoate (CAHE) and subsequently the methacryloylation of CAHE (Fig. 1).

CA (98 mmol, 40 g), hexane-1,6-diol (HE, 508 mmol, 60 g), and 2 mL HCl were added into a 250-mL flask. The mixture was allowed to react at 120°C for 24 h, and then excess HE was removed under reduced pressure. The products were poured into a 1000-mL beaker containing 800 mL distilled water and stirred for 24 h. The oil phase was separated, dried overnight in vacuum, and purified by column chromatography with silical gel ($CHCl_3$: CH_3OH = 10 : 1, v/v, R_f = 0.33). Pure CAHE was obtained in 65% yield.

A solution of CAHE (4.2 mmol, 2.14 g) and triethylamine (4.6 mmol, 0.66 mL) in THF (20 mL) was first cooled to 0°C. A solution of methacryloyl chloride (4.6 mmol, 0.46 mL) in THF (25 mL) was added dropwise over a period of 2 h while maintaining continuous stirring. The mixture was then gradually warmed to room temperature and stirred for 12 h. The hydrochloride salt of Et_3N was filtered off, and the product was concentrated and then purified over a chromatographic column of silica gel (100 mesh) using ethyl acetate as the eluent (R_f = 0.34). The monomer MACAH was obtained in 70% yield.

Synthesis of the copolymer hydrogels (PLA-PEG-PLA-*co*-MACAH)

The structure of the copolymer hydrogel is shown in Figure 2. On average, about 10 repeating units of oligo-

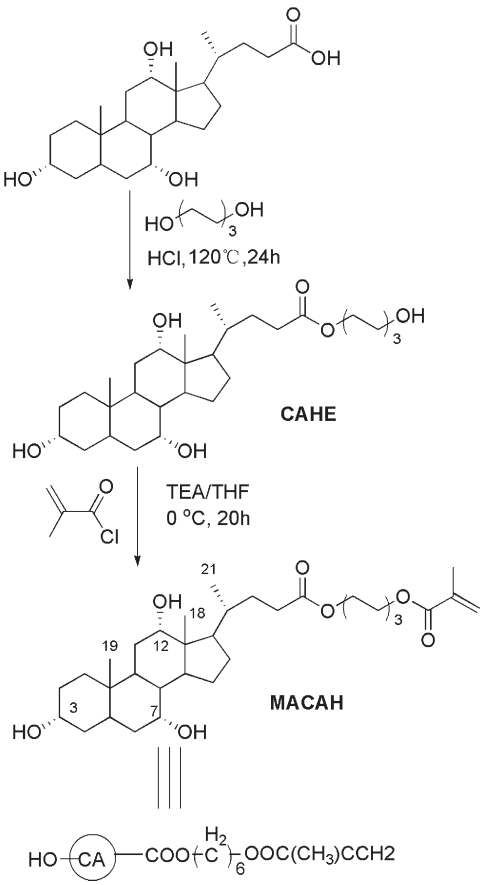

Figure 1 Synthetic route of the CA-containing monomer.

LA were attached to each side of the PEG (6000) core segment, as determined by ^{1}H-NMR spectroscopy. The macromer (PLA-PEG-PLA-DA) was dissolved in distilled water (conc. 35%, w/v). The UV photoinitiator (DMPA) was dissolved in ethanol to give a 25% (w/w) solution, and then the MACAH (weight ratios: 1 : 99 and 5 : 95 with MACAH over PLA-PEG-PLA-DA) was added to the ethanol solution. In this study, the macromer (PLA-PEG-PLA-DA) solution was added to the initiator solution, and the ratio of initiator/macromer was 5% (w/w). The solution was irradiated over 15 cm with LWUV (Model: EA-180/F) light at an intensity of 1700 $\mu w/cm^2$ for 30 min.

Swelling degree

To measure the swelling ratio (SR), preweighed dry samples were immersed in distilled water at room temperature for various duration times. After removal of excessive surface water with filter paper, the swollen samples were weighted at various time intervals. This procedure was repeated (up to five times) until no further weight increase was observed. The freshly prepared hydrogels were washed with distilled water, ethanol successively, and dried in vacuum (1 mmHg) at ambient temperature for 3 days, and then it was ready for the weight

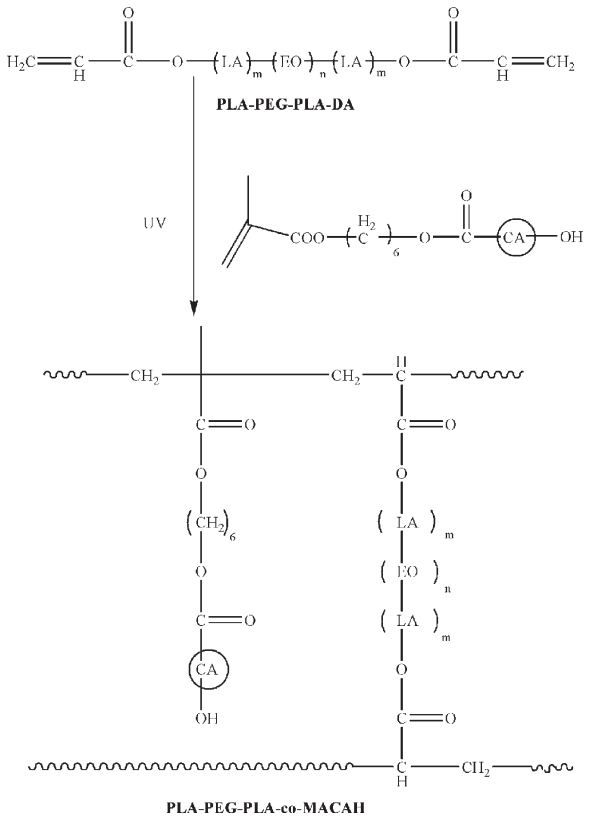

Figure 2 Synthesis of a biodegradable and resorbable CA-containing hydrogen.

determination. The SR was calculated by the following equation:

$$SR = (M_s - M_d)/M_d$$

where M_s and M_d denote the weights of swollen and dry samples, respectively.

In vitro bioactivity assessment

The *in vitro* bioactivity assessment was carried out by immersing 0.05 g of each hydrogels in 20 mL of simulated body fluid (SBF; pH 7.2) in polyethylene vials. The vials were placed in an orbital shaker rotating at 175 rpm at 37°C for 24, 36, and 48 h of time intervals. After various reaction times, the samples were removed and freeze-dried. The surfaces of the samples were characterized by SEM and XRD.

RESULTS AND DISCUSSION

Synthesis

The synthetic route of MACAH is shown in Figure 1. To increase the polymerization reactivity of the monomer, HE was selected as spacer between the methacryloyl group and CA.

The structures of CAHE and MACAH were confirmed with FTIR and ^{1}H-NMR spectroscopy. The IR spectrum of CAHE showed characteristic absorptions of the C=O carbonyl groups at 1727 cm^{-1}. The IR spectrum of MACAH also showed the absorption peaks of the C=O carbonyl group and the CH_2=CH— olefininc group at 1720 and 1668 cm^{-1}, respectively. For CAHE, ^{1}H-NMR ($CDCl_3$, δ, ppm, 300 MHz): δ = 0.68 (s; H-18), 0.89 (s; H-19), 0.98–1.00 (d; H-21), J = 5.1 Hz, 3.46 (m; H-3), 3.59–3.70 (t; CH_2CH_2OH), 3.85 (s; H-7), 3.97 (s; H-12), 3.95–4.10 (t; $COOCH_2$). MS: m/z = 509.8 ($M + H^+$) by FAB-MS. For MACAH, ^{1}H-NMR ($CDCl_3$, δ, ppm, 300 MHz): δ = 0.66 (s; H-18), 0.86 (s; H-19), 0.98 (d; H-21); J = 6.1 Hz, 1.98 (s; CH-3), 3.26 (m, H-3), 3.73 (s; H-7), 3.90 (s; H-12), 4.12–4.29 (m; $COOCH_2CH_2CH_2CH_2CH_2$-CH_2OOC) 5.56, 6.06 (d; ME-CH_2). MS data: m/z = 577.1 ($M + H^+$) by FAB-MS.

The IR spectra of the copolymerized hydrogel showed the disappearance of the double bonds (1613.14 cm^{-1}), retaining the other characteristic absorption signals of the monomer (data not shown).

Properties of the copolymerized hydrogel (PLA-PEG-PLA-*co*-MACAH)

Water-swelling ability

The water-swelling abilities of the hydrogels were measured as a function of time at room temperature. All the samples swelled rapidly and almost reached the steady equilibrium in about 120 min, showing a similar kinetic profile up to 400 min. The reason is possibly due to that with the increasing entering of the water into the network, the hydrogen bonds were becoming more weak and the water swelling capacities of the hydrogels were getting gradually close to each other. Thus after 120 min, the Samples B and C showed similar swelling rate. However, the SR of hydrogels is in the range of 1300–1570% and changes with the content of the CA in the network. The highest water absorption was observed for hydrogel A (1570% for A, 1300% for B and C, Fig. 3).

It was observed that an increase of CA fraction in the hydrogels led to a slight decrease in the equilibrium of SR. This was possibly attributed to the hydroxyl groups of CA that tend to form the physical crosslinking via hydrogen bonding. The water-swelling ability of these hydrogels was possibly not only governed by the chemical crosslinking degree in the network but also the amount of CA, which has more water-binding sites. For instance, with the increase in the ratio of MACAH, the apparent crosslinking density increased [as seen in Fig. 4(A,C)] and the hydrophilic functional group (—OH) also increased.

Bioactivity

Bioactivity is a critical factor for materials used in tissue engineering. The bioactivity of the hydrogels was investigated using SEM and XRD. Figure 4

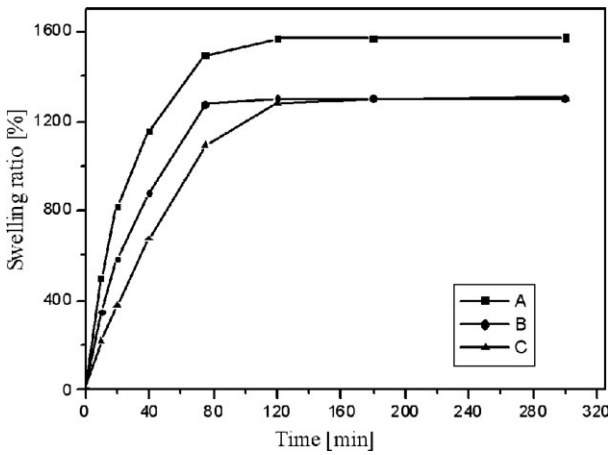

Figure 3 Swelling kinetics of the CA-containing hydrogels (CA-containing monomer weight ratio: A, 0%; B, 1%; and C, 5%).

shows the SEM images of the synthesized PLA-PEG-PLA-*co*-MACAH before and after soaking in SBF. A–C are the images of freeze-dried hydrogels' surface. Figure 4(A) clearly shows that the gel that does not contain the third monomer MACAH possesses the loose structure with some irregular holes. By increasing the amount of MACAH, the hydrogels get more and more compact bearing some honeycomb-like pores. Figure 4(A′–C′) are the images of freeze-dried surfaces of the hydrogels that were soaked in SBF; some deposits adhering to the degraded gel skeleton can be seen. In the experiment of *in vitro* bioactivity assessment, the following phenomenon was observed: The Sample A′ (having no CA component) was almost completely degraded and adhered to the bottom of the beaker. The Sample B′ became a seajelly-like gel, whereas Sample C′ (containing 5% MACAH) retained the shape well. It is deduced, from these observations, that introducing the third monomer MACAH into the skeleton of the block hydrogels has a positive effect over the degradation rate of the hydrogels.

The XRD spectra recorded for the hydrogel (CA-containing monomer, 1 wt %) in SBF with different immersing times are shown in Figure 5. The peaks at $2\theta = 30, 36$ (Fig. 5, curves b and c) was assigned for the crystalline of HA. However, after the

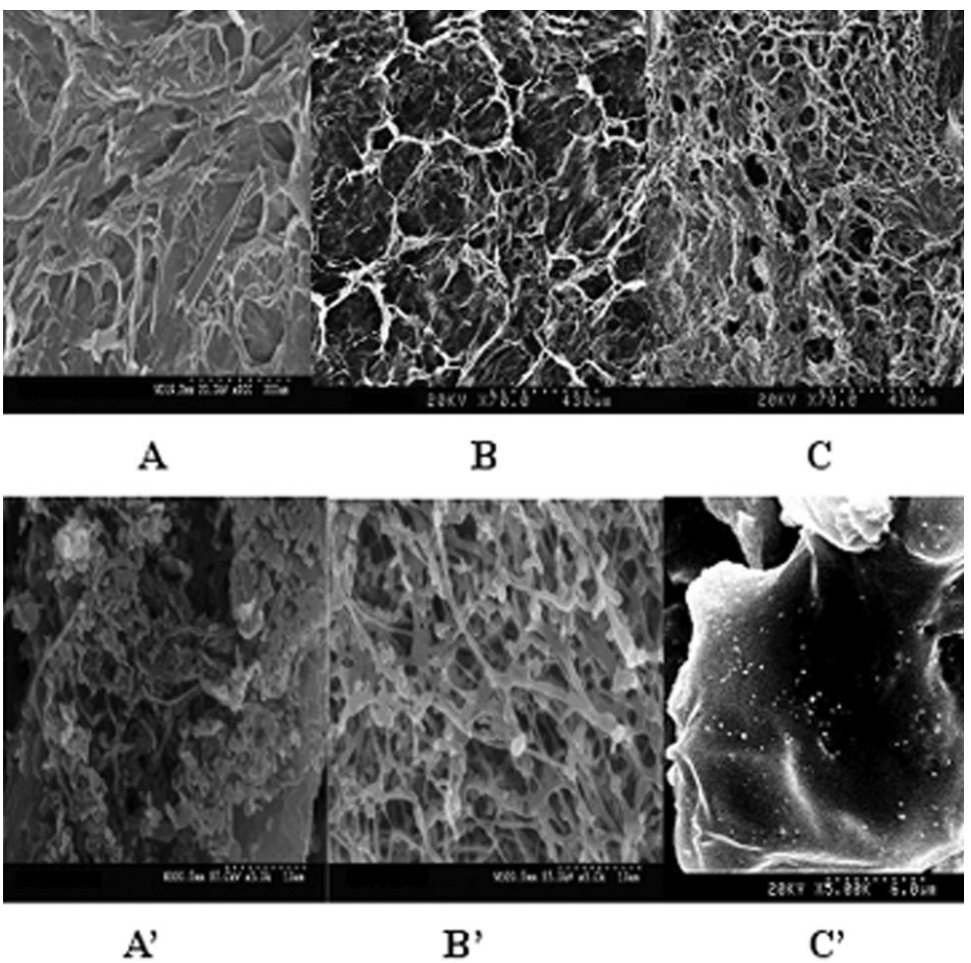

Figure 4 The scanning electron micrographs (SEM) of hydrogels (CA-containing monomer weight ratio: A, 0%; B, 1%; and C, 5%) compared with A′, B′, and C′ samples having been immersed in SBF for 48 h.

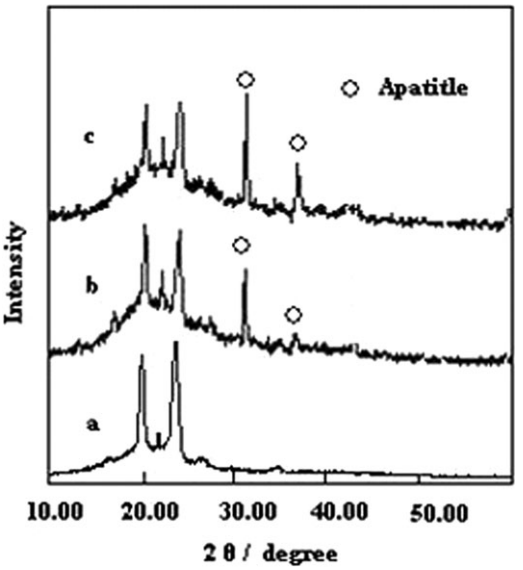

Figure 5 X-ray diffraction patterns of the CA-containing hydrogels (1%) after having been soaked in SBF for (a) 0 h, (b) 36 h, and (c) 48 h.

hydrogel was immersed for 36 h, the peaks could be observed in the XRD patterns, but were still not strong. It implied there was some defective structure in the HA-like layer. With the increase of the immersion time (48 h; Fig. 5, curve c), these peaks became stronger, which indicated that the HA crystal was getting more intact.

Based on the observations on the deposit formation from the SEM micrographs (Fig. 4) and the crystalline peaks of HA from XRD analysis, it could be concluded that the growth of the crystalline grains of HA was induced by the CA-containing component of the PLA-PEG-PLA-*co*-MACAH.

CONCLUSIONS

The CA-containing biodegradable hydrogels were synthesized by the photoinduced copolymerization of a CA modified methacrylate (MACAH) and a macromer bearing two vinyl end groups (PLA-PEG-PLA-DA). The experimental investigation indicated that the introduction of CA into the PLA-PEG-PLA hydrogels led to a slight decrease in the equilibrium of SR. SEM and XRD analysis of the hydrogels revealed that the growth of the crystalline grains of HA was induced by the CA-containing component of the PLA-PEG-PLA-*co*-MACAH.

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