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Multifunctional hydrogels based on β-cyclodextrin with both biomineralization and anti-inflammatory properties

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ABSTRACT

In this study, a series of carboxylated hydrogels are synthesized using β-cyclodextrin, epichlorohydrin and succinic anhydride. It is found that the carboxyl groups on the β-cyclodextrin hydrogels could facilitate the biomineralization process in the simulated body fluid. The new generated minerals are apatite and similar to that of bones. Meanwhile, the carboxylated β-cyclodextrin hydrogels can also load and release anti-inflammatory drug (using indomethacin as a model) in a controlled manner during the biomineralization process for 28 days. The carboxylated β-cyclodextrin hydrogels also have low cytotoxicity and are promising for bone tissue engineering.

1. Introduction

The treatment of bone defect such as osteoporosis or fracture remains a critical challenge (Sarah et al., 2013; Ulrich et al., 2013). The application of biomaterials has improved healing and the treatment outcome for patients with osteoporotic and fractures (Goldhahn et al., 2008). Recently, quite a few researchers have turned their attention to the bioinspired mineralization process for bone repair or bone tissue engineering. The biomineralization process has been widely studied for the fabrication of organic/inorganic composite biomaterials, and polymeric materials are found to be effective candidates to act as scaffolds or organic matrix to obtain bone-like restorative materials (Li et al., 2013; Phadke, Zhang, Hwang, Vecchio, & Varghese, 2010; Song, Saiz, & Bertozzi, 2003; Wu et al., 2013). As inspired by the protein-mediated nucleation of apatite *in vivo*, e.g., the bone sialoprotein induced bone generation (Goldberg, Warner, Stillman, & Hunter, 1996; Hunter & Goldberg, 1994) and the amelogenin induced dental enamel mineralization (Aichmayer et al., 2010; Beniash, Simmer, & Margolis, 2005), a well-known strategy of inducing nucleation of mineral phases is to utilize polymers bearing electrolyte groups (polyelectrolytes) to initiate nucleation through binding of calcium or phosphate ions to their anionic or cationic functional groups, respectively, to control the crystal nucleation and growth of the inorganic component (Kawashita et al., 2003; Michel et al., 2006; Ngankam et al., 2000; Phadke et al., 2010; Shkilnyy et al., 2008; Suzuki, Whittaker, Grondahl, Monteiro, & Wentrup-Byrne, 2006).

Structurally, natural bone is a composite of collagen, a protein-based hydrogel template and inorganic crystals: the combination of the hard inorganic material and the elastic hydrogel network endows bone excellent mechanical properties such as low stiffness, resistance to tensile/compressive forces and high fracture toughness (Song et al., 2003; Weiner & Wagner, 1998). Thus, a lot of natural or synthetic hydrogels have been investigated for bone tissue engineering. Hydrogels can swell by large quantities of water without the dissolution of the three-dimensional networks due to their hydrophilic groups but crosslinked structure, which gives them physical characteristics (such as structural integrity and elasticity) similar to that of soft tissues (Li et al., 2006; Nguyen & West, 2002; Wu et al., 2011; Xin et al., 2010). In the research area of bone tissue engineering, hydrogels have been increasingly applied as scaffolds due to their good biocompatibility and mechanical properties (Lin, Cao, Chen, Wu, & Li, 2013). Meanwhile, after specific molecular design and modification, hydrogel can not only act as the scaffolds which resemble living tissues closely because of their relatively high water content, soft and rubbery consistency, but also play an important role in controlling of crystal nucleation and growth of the inorganic component in biomineralization (Phadke et al., 2010; Sargeant, Aparicio, Goldberger, Cui, & Stupp, 2012). Nevertheless, inflammatory responses to the heterogeneous

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implant materials often occur during surgical implantation (Kim, Mauck, & Burdick, 2011). Thus, it is necessary to obtain hydrogel systems that can simultaneously induce biomineralization and have anti-inflammatory property. Also, since drugs have been widely studied to inhibit the inflammation for bone diseases (Rodan & Martin, 2000), it is reasonable and valuable to design above multifunctional hydrogel systems with materials which can encapsulate and release anti-inflammatory drug in a controlled manner during biomineralization process.

The cyclodextrin (CD) based hydrogels could be ideal for above purpose due to its specific properties. Cyclodextrins are cyclic oligosaccharides consisting of six to eight glucose unites linked by α-1,4-glucosidic bonds, which are named as α-, β- and γ-CD, respectively. CDs have a specific shape of truncated cone with a hydrophobic inner cavity, by which CDs could facilitate the inclusion of a number of drugs/genes to improve their solubility, chemical stability and bioavailability, or gene transfection efficiency (Hedges, 1998; Huang, Xin, Guo, & Li, 2010; Li & Loh, 2008; Li, Xiao, Li, & Zhong, 2004; Szejtli, 1998; Xu & Yang, 2011; Zhang et al., 2010). CD based hydrogels could be obtained by crosslinking agents such as epichlorohydrin. The hydrogels not only retain the CD hydrophobic cavity but also have the three-dimensional hydrophilic network which can resemble the physical characteristics of soft tissues. Thus, CD based hydrogels have been widely used as drug/gene carriers and tissue engineering scaffolds in the biomaterials field (He et al., 2013; Kopecek, 2007; Li, Yin, Zhang, Liu, & Li, 2012; Machin, Lsasi, & Velaz, 2012; Xin et al., 2010; Wu et al., 2011). Meanwhile, since it has been reported that CD or CD based hydrogels have the capability to induce mineralization (Shchipunov, Karpenko, Krekoten, & Postnova, 2005; Xiao, Liu, Qiu, Zhu, & Liu, 2009), it is reasonable to design multifunctional hydrogels based on CD, which can simultaneously induce biomineralization and load/release anti-inflammatory drugs, for bone repair.

In this work, a series of hydrogels were synthesized using β-cyclodextrin (β-CD) and epichlorohydrin, and then modified with succinic anhydride (SA) to make them bearing different amounts of carboxyl groups, which could facilitate their capability to induce biomineralization. Then, a kind of nonsteroidal anti-inflammatory drug, i.e., indomethacin (IDM), was chosen as the model drug since it is widely used as anodyne in the treatment of rheumatoid arthritis and other degenerative joint diseases. As shown in Fig. 1, the drug (IDM) loaded within the β-CD based hydrogels could be gradually released into the simulated body fluid (SBF) during the biomineralization process. The hydrogel characterization, drug controlled release profile and the biomineralization process are described.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) and dimethylsulfoxide (DMSO) was purchased from Chengdu Kelong Chemical Reagent Plant, China. Succinic anhydride (SA) and epichlorohydrin (EP) were supplied by Tianjin Bodi Chemical Co., Ltd., China. Indomethacin (IDM) was provided by Wuhan Hezhong Bio-Chemical Co., Ltd., China. All other reagents and solvents were of AR grade and used as received without further purification. Distilled water was used throughout.

2.2. Synthesis of carboxylated β-CD hydrogels

β-CD hydrogels were synthesized in a strong alkali condition using epichlorohydrin as the crosslinking agent following the procedure reported in a previous work (Yang & Kim, 2010). In this work, at a high NaOH concentration (33% w/w), the epoxy group and chlorine atom of epichlorohydrin mainly react with the C_6—OH of β-CD on the primary surface, and rarely at hydroxyl groups of C_2—OH and C_3—OH (Renard, Deratani, Volet, & Sebille, 1997). The crosslinking density, i.e., the number of crosslinked points per volume could be controlled by the feeding ratio and reaction time. Usually, a higher EP/β-CD ratio will result in a higher cross-linking density. In this work, the EP/β-CD ratio was fixed thus we could investigate the effect of the carboxyl groups on the functions of the hydrogels. After that, the hydrogels were modified with succinic anhydride (SA) to obtain the carboxylated β-CD hydrogels. In this work, the feeding mass ratio of β-CD gel/SA was fixed at 1:0 (Gel-1/0), 1:4.6 (Gel-1/4.6) and 1:9.2 (Gel-1/9.2), respectively. A typical synthesis procedure of Gel-1/4.6 is described below: 5.748 g β-CD was dissolved in 10 mL NaOH solution (33%, w/w) and stirrer overnight at room temperature. Then, 5.166 mL EP were added dropwisely with vigorous stirring. After the adding of EP, the mixture was heated to 30 °C for polymerization for 4 h. The mixture was removed to a petri dish for curing at 30 °C. After that, the gel was washed by a large amount of distilled water to remove the impurities and then dried in vacuum oven at 60 °C for 12 h. Then, 0.5 g dry β-CD hydrogels was immersed in 10 mL DMSO in a 50 mL round-bottom flask. 10 mL of DMSO solution containing 2.3 g succinic anhydride was added to this mixed solution under vigorous stirring and reacted at 35 °C for 24 h. The DMSO solution was dialyzed against water to remove the excess succinic anhydride as well as the organic solvent. Finally, the product of Gel-1/4.6 was obtained by lyophilization.

2.3. Characterization of carboxylated β-CD hydrogels

The Fourier transform infrared spectroscopy (FTIR) of hydrogels were performed on a Nicolet Nexus 670 FTIR spectrometer (USA). Dried hydrogels were scanned from 500 to 4000 cm^{-1} at the reflex mode for their extremely thin and transparent characters. X-ray photoelectron spectroscopy (XPS) data were obtained from ESCALAB 220i (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The base pressure was 3×10^{-9} mbar. The binding energies were referenced to the C1s line at 284.8 eV from adventitious carbon. The preparation of samples involved removal of impurity by dialysis and the washed sample was collected and dried in the freeze dryer overnight before use. The swelling ratios of β-CD based hydrogels were investigated at pH7.4. The freeze-dried hydrogels were immersed in 50 mL of SBF (pH 7.4) at 37 °C for 24 h. The hydrogels were weighed after removing the excess surface water with filter paper. The swelling ratio is defined as follows:

$$\text{Swelling ratio} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \quad (1)$$

2.4. Adsorption test

In order to quantify the content of carboxyl groups in the hydrogel, toluidine blue O (TBO) adsorption was carried out. 0.01 g carboxylated β-CD hydrogels was immersed in a 20 mL TBO solutions (pH 10) with a given concentration under stirring at 25 °C for 12 h. The TBO solution was substituted by fresh ones every 3 h to keep the constant concentration. Then the hydrogels was taken out, washed by NaOH solution (pH 10) for 10 min to remove the free TBO, and then immersed into 50% HAc to dissolve the captured TBO to get a blue colored solution. The absorbance of the solution at 620 nm was measured and the amount of the TBO adsorbed by the hydrogels was calculated by a standard curve obtained using TBO solution of different concentrations.

2.5. Drug loading of hydrogels

2 g indomethacin (IDM) powder was dissolved in 10 mL DMSO. And then 0.3 g carboxylated β-CD hydrogels were added to the

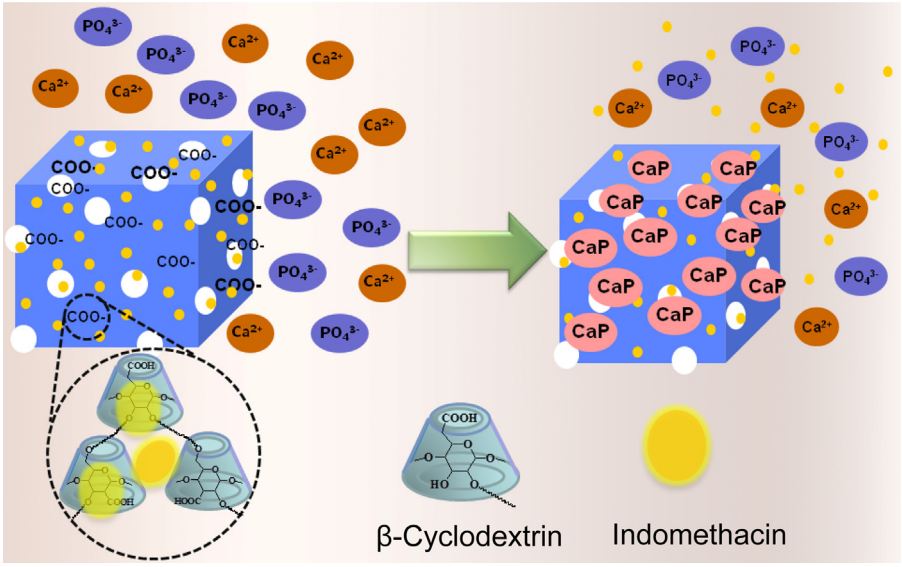

Fig. 1. Schematic illustration of the biomineralization and drug release process of the carboxylated β-CD hydrogels.

solution. After left to stand for 24 h, the IDM-loaded hydrogels were collected and washed by acetone to remove IDM on the surface. Then IDM-loaded hydrogels were dried in vacuum at 50 °C to remove DMSO.

2.6. Biomineralization and drug release studies in vitro

The carboxylated β-CD hydrogels were placed in a plastic beaker containing 100 mL 1.5 SBF and incubated in an oven at 37 ± 0.5 °C for four weeks. The SBF solution was refreshed every 24 h. The released IDM concentration of all collected solutions was recorded based on absorbance intensity at 320 nm by UV (MAPADA UV-1800PC Spectro Photometer). After four weeks, the carboxylated β-CD hydrogels were rinsed thoroughly with deionized water and dried by lyophilization. Before scanning electron microscopy (SEM) measurement, the surface of hydrogels was coated with a thin layer of gold. The SEM test was carried out at an accelerating voltage of 10 kV (Inspect F, FEI, USA). Element analysis was measured by an energy dispersive X-ray analyzer (EDS, INCA350, Oxford, UK) connected to SEM directly. X-ray diffraction analysis (XRD) analysis of hydrogels was performed on a D/max 2200 (Rigaku Corporation, Japan) X-ray diffractometer with a thinfilm analyzer. Cu Kα radiation was used for the diffraction with a voltage of 40 kV and a current of 30 mA. A step size of 0.02° (2θ) and a scan speed of 3° min^{-1} were used and the diffraction data were collected from 15° to 45° (2θ).

2.7. Cytotoxicity studies

The cytotoxicity of the carboxylated β-CD hydrogels was evaluated via MTT assay in the COS7 cell line. The cells were cultured in Dulbecco's modified eagle medium (DMEM) with high glucose, containing 100 U/mL penicillin, 100 mg/mL streptomycin and 10% fetal bovine serum (FBS), at 37 °C in 5%-CO_2 air atmosphere. The cells were seeded in a 96-well microtiter plate (Nunc Co., Wiesbaden, Germany) at a density of 1×10^4 cells/well and incubated in 100 μL of DMEM/well for 24 h to evaluate the cytotoxicity of the extract from hydrogels. The culture media were replaced with fresh culture media containing serial dilutions of extract from hydrogels and DMEM, and the cells were incubated for 24 h. After incubation for 4 h, 25 μL of MTT solution in PBS (5 mg/mL) was added to each well and the cells were incubated for another 4 h. Finally, the formazan crystals dissolved in DMSO were added at the quantity of 100 μL per well and followed by shaking for 15 min. The absorbance of each well was measured at 570 nm.

3. Results and discussion

As shown in Fig. 2, the carboxylated β-CD hydrogels were synthesized using epichlorohydrin as cross-linking agent and then modified by succinic anhydride. The EP is a cross-linking agent with bifunctional structure thus it can connect β-CD units to form hydrogels by polycondensation. According to the previous report, these nucleophilic substitution such as epichlorohydrin, ethylene glycol and bisepoxy reactions usually occur in alkaline conditions, which is required for the deprotonation of hydroxyl groups of β-CD (Xin et al., 2010). The SA was used to obtain carboxylated β-CD hydrogels with different number of carboxyl groups. In anhydrous DMSO, SA can easily react with —OH thus it can introduce carboxyl groups into the β-CD hydrogels (Shi, Thomas, Mye, Kotlyar, & Baker, 2007). The FTIR spectroscopy is used to confirm the structure of carboxylated β-CD hydrogels and also the drug-loaded hydrogels. As shown in Fig. 3(a), peaks in the range of 1028–1159 cm^{-1} is due to the C—O and C—O—C stretching, and the peak at 3340 cm^{-1} is ascribed to the stretching of the hydroxyl group of β-CD hydrogel. As for the Gel-1/4.6 (Fig. 3b), the strong characteristic absorption peak observed at 1736 cm^{-1} is attributed to the stretching vibration of C=O, indicating the successful introduction of carboxyl groups into the β-CD hydrogel. After the loading of IDM as the anti-inflammatory drug, there are two weak peaks at 1600 and 751 cm^{-1}, which are attributed to the stretching vibration of benzene and chlorine, respectively (Fig. 3c). These absorption bands were characteristic functional groups of IDM. Since the samples were thoroughly rinsed and lyophilized before FTIR measurement, this result indicates that the IDM has been incorporated into the carboxylated β-CD hydrogels.

Then, the samples of Gel-1/0, Gel-1/4.6 and Gel-1/9.2 were analyzed by X-ray photoelectron spectroscopy (XPS). XPS has been proved as a powerful technique for the chemical analysis and elemental composition study of a large variety of materials. In brief, the C1s XPS spectrum of hydrogels (Table S1) clearly indicated a considerable degree of modification with four components that correspond to carbon atoms in different functional groups: the nonoxygenated ring C (—C—C), the C atom in C—O bond (—C—O),

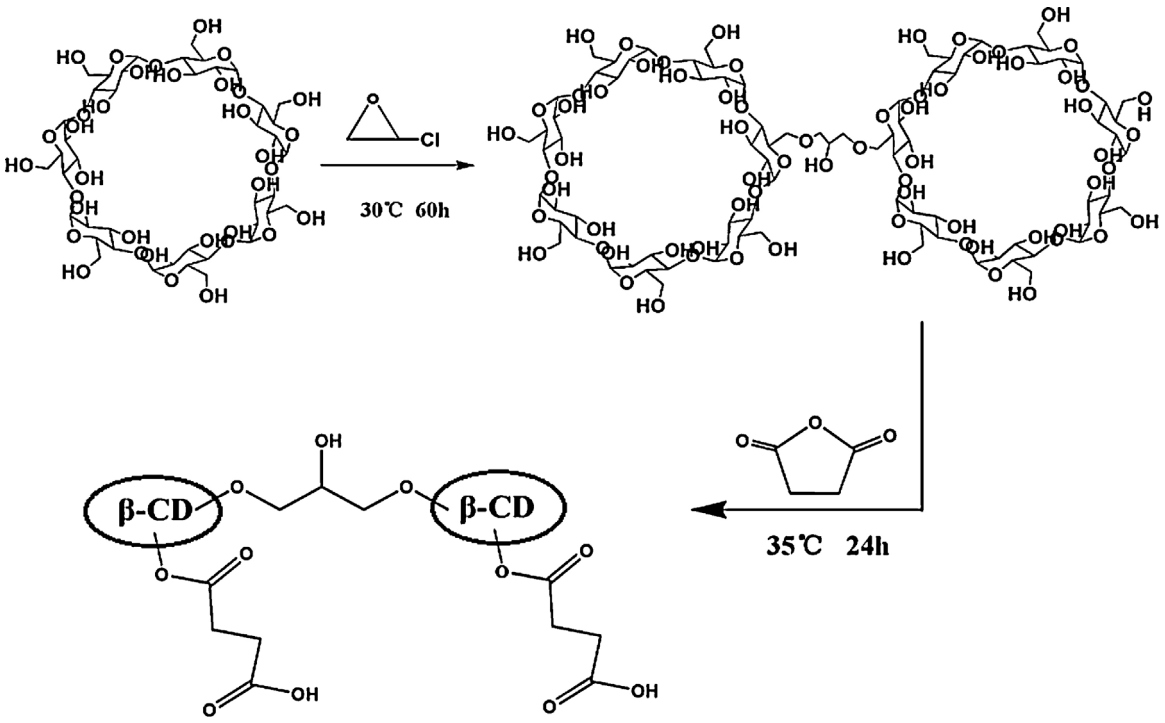

Fig. 2. The synthetic route of carboxylated β-CD hydrogels.

the C atom connected by ester bond (O—C—O), and the carboxylate carbon (—COOH). Their identities and atomic percentages are presented in Table S1. All C atomic percentages were obtained by calculating of the areas shown in Fig. 4. The C1s spectra of the Gel-1/4.6 and Gel-1/9.2 are compared in Table S1. As can be seen, the proportion of —COOR in Gel-1/9.2 is more than that in Gel-1/4.6. It suggests that more carboxyl groups have been introduced into the β-CD hydrogels with the increase of the feeding amount of SA.

TBO can form a stable complex with carboxyl groups in solutions through electrostatic interactions with the complex ratio of 1:1. Therefore, the binding of TBO by the carboxylated β-CD hydrogels was studied to quantify the content of carboxyl groups in hydrogels. The standard curve was drawn with TBO solutions

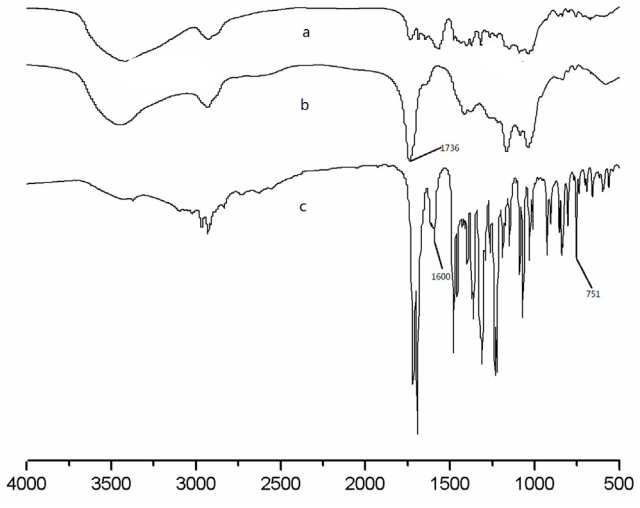

Fig. 3. The FTIR spectra of unmodified Gel-1/0 (a), Gel-1/4.6 (b) and IDM-loaded Gel-1/4.6 (c).

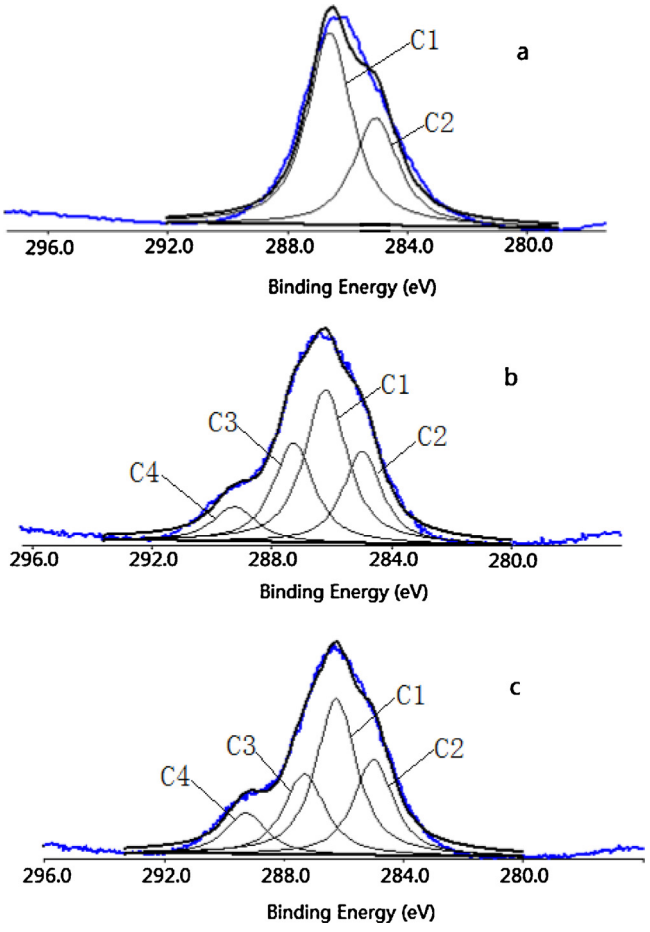

Fig. 4. The XPS spectra and scan of C 1s region of Gel-1/0 (a), Gel-1/4.6 (b) and Gel-1/9.2 (c).

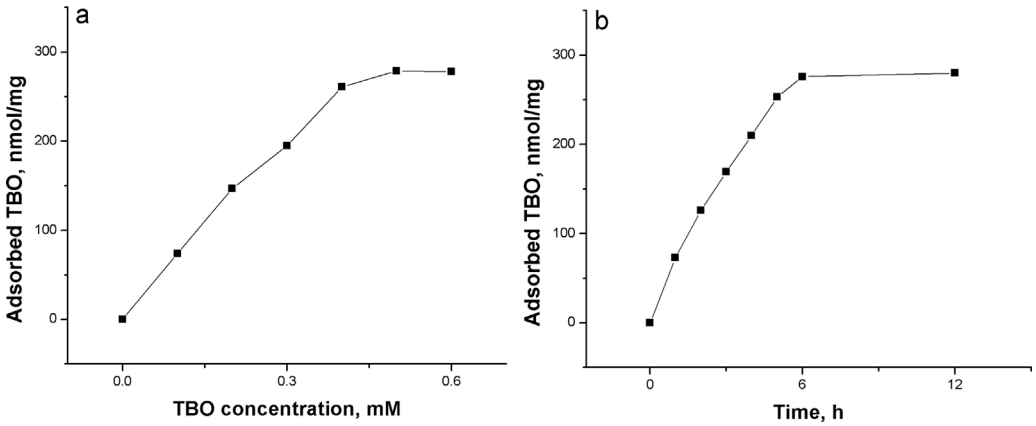

Fig. 5. (a) The effect of TBO concentration on the TBO adsorption capacity of Gel-1/9.2. (b) TBO adsorption rate on Gel-1/9.2 from TBO solution with a concentration of 0.5 mM.

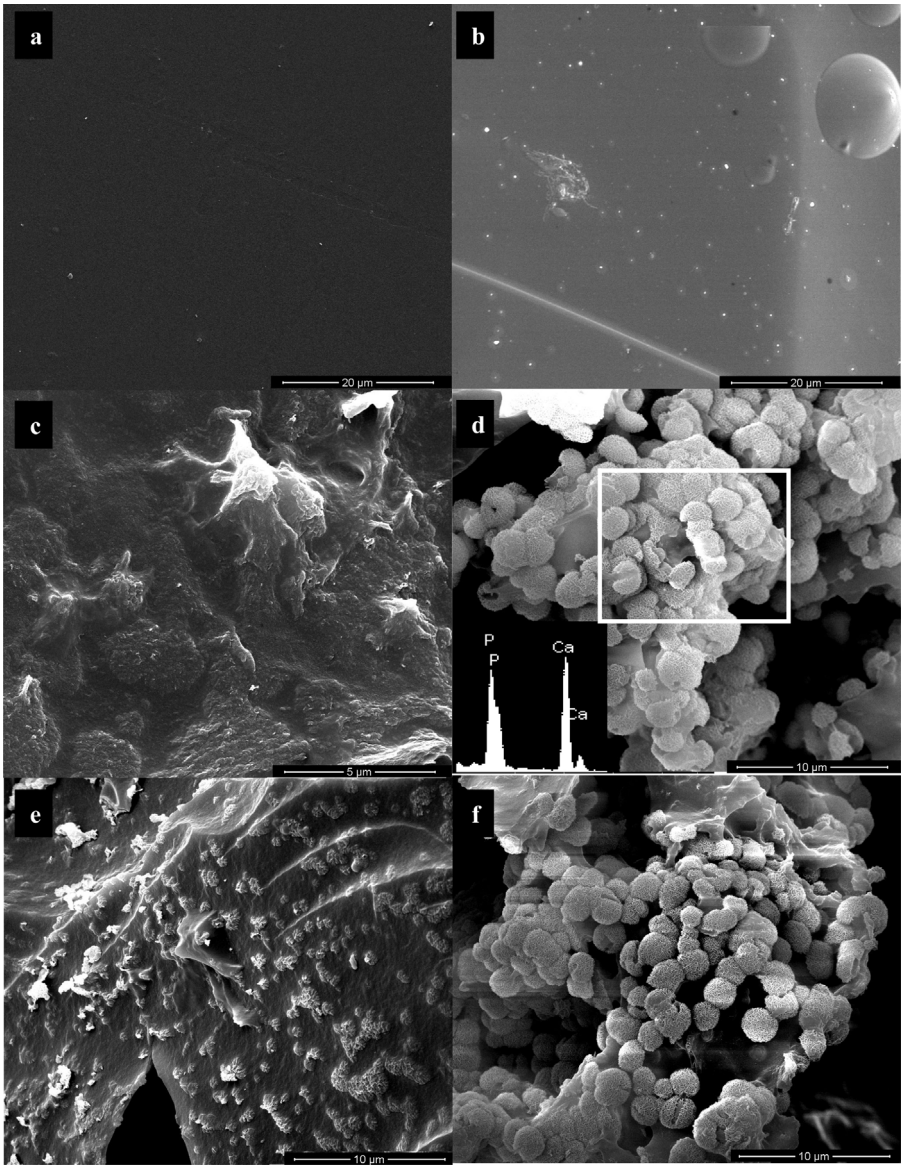

Fig. 6. SEM images of hydrogels after being soaked in SBF: (a) Gel-1/0, 0 day; (b) Gel-1/0, 28 days; (c) Gel-1/4.6, 14 days; (d) Gel-1/4.6, 28 days, insert is the energy disperse spectrum (EDS) analysis; (e) Gel-1/9.2, 14 days and (f) Gel-1/9.2, 28 days.

of different concentrations. Fig. 5a displays the equilibrium TBO adsorption capacity as a function of the equilibrium TBO concentration. The maximum adsorption capacity (279 nmol/mg) of Gel-1/9.2 is reached. Fig. 5b indicates that the adsorption process of Gel-1/9.2 is completed within 6 h. From the equilibrium adsorption capacity of the TBO, and the 1:1 (TBO:COO—) complex ratio, the carboxyl groups of Gel-1/4.6 and Gel-1/9.2 can be calculated as 211.5 nmol and 279 nmol per milligram of the carboxylated hydrogels, respectively.

The swelling capability of hydrogel can be described in terms of the maximal amount of absorbed water and the swelling ratio at balance, which depends on the network structure of polymers and pH of the solution. The swelling ratios of three hydrogels without or with different content of —COOH at different pH conditions are shown in Fig. S1. Each numerical value represents the average data of three measurements. As shown in Fig. S1a, the swelling ratio of uncarboxylated β-CD hydrogel (Gel-1/0) is almost stable at about 4.5 at different pH conditions. Gel-1/0 does not have carboxyl groups which are essential for the deprotonation process, thus its swelling ratio is independent of the pH value. On the other hand, the swelling ratio of Gel-1/4.6 and Gel-1/9.2 are 4.2 and 3.5 at pH 4.0, respectively. It looks that the swelling ratio of the hydrogels is decreased with the increase of carboxyl group contents at pH 4.0. It should because that —COOH exhibits electric neutrality under acidic conditions and it is easy to form hydrogen bonds by itself so that it enhances the cross-linking degree of the molecular networks within the hydrogels. These reasons lead to the increase of intermolecular interactions and rigidity, resulting in slow relaxing of network, which make Gel-1/9.2 to be the one with the lowest swelling ratio at pH 4.0. The swelling ratios of carboxylated β-CD hydrogels increase dramatically with the increase of —COOH contents at pH 7.4. At a higher pH condition, the deprotonation of —COOH results in more negatively charged —COO^-, thus the carboxylated β-CD hydrogels could be more stretched and expanded due to the electrostatic repulsion between —COO^- groups. It is also noted that the swelling ratios of both Gel-1/4.6 and Gel-1/9.2 do not show obvious changes while the pH increased from 7.4 to 11. This should because that most of the carboxyl groups of hydrogels have been deprotonated at pH 7.4, thus a further increase of pH could not obviously increase the electrostatic repulsion and subsequent swelling ratio.

As shown in Table S2, the loading efficiency (LE) of IDM in hydrogels decreases with the increase of carboxyl groups in the hydrogels. Gel-1/0, as the control sample without carboxyl groups, exhibits the highest LE of 27.36%. And Gel-1/9.2 shows the lowest LE of 15.38%. This result should due to two reasons. First, IDM is a negatively charged molecule, thus the electrostatic repulsion between IDM and carboxylated β-CD hydrogels could increase with the increase of —COOH content, which should decrease the final LE of IDM in hydrogels (Masson, Loftsson, Jonsdottir, Fridriksdottir, & Petersen, 1998; Xin et al., 2010). Meanwhile, since the hydrophobic IDM is mainly encapsulated in the hydrophobic cavities of CD, the increase of —COOH side groups might decrease the encapsulation of the drug molecules into CD's cavities, which is consistent with previous reports (Harada, Furue, & Nozakura, 1981).

The SEM morphologies of biomineralized hydrogels are shown in Fig. 6. There is no obvious mineralization on the unmodified β-CD hydrogel after being immersed in SBF solution for 28 days as compared to its original status (Fig. 6a and b). It indicates that β-CD hydrogels without carboxyl groups does not have the ability to induce the growth of minerals. On the other hand, new generated minerals were observed on Gel-1/4.6 and Gel-1/9.2 after being immersed in SBF solution for 14 days, while the latter one induced more new minerals (Fig. 6c and e). It looks like that the growth of new mineral crystals on Gel-1/9.2 l hydrogels are faster than that on Gel-1/4.6 within the scope of 14 days. This means that more

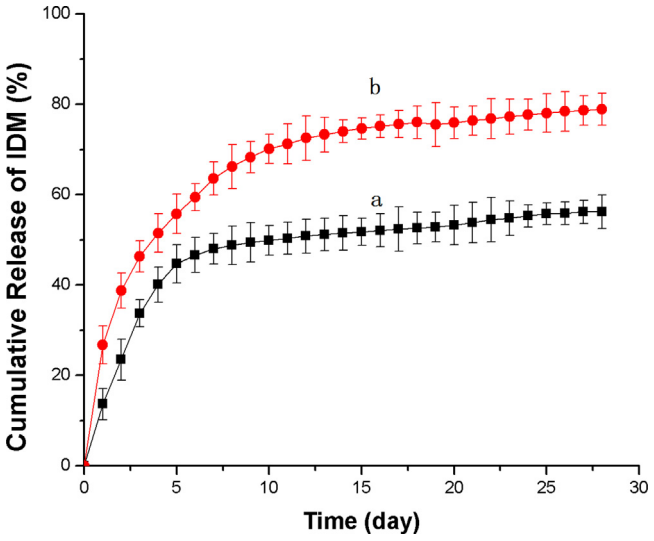

Fig. 7. Drug release behaviors of IDM from Gel-1/4.6 (a) and Gel-1/9.2 (b) at pH = 7.4.

carboxyl groups on the β-CD hydrogels could facilitate the biomineralization process in SBF solution. After a longer immersion time of 28 days in SBF solution, new generated crystals covered almost all the surface regions of the hydrogels (Fig. 6d and f). And the mineral crystals appeared to be a lot of tortoise-shell like particles consisting of many flake crystallites (Gu, Khor, & Cheang, 2003). The energy dispersive spectroscopy (EDS) analysis performed on the mineral surface of the composite revealed a Ca/P ratio (1.8) similar to that of HA (insert of Fig. 6d), indicating that the phase and composition are similar to the mineral phase of the bone (Wu, Su, Zhang, & Wang, 2008). XRD was also used to determine the type of mineral crystals formed on the hydrogels surface. It looks that there is obvious differences in mineralization depending on whether the β-CD hydrogel was modified by SA. As shown in Fig. S2, after being immersed in SBF solution for 28 days, Gel-1/0 does not show mineralization phenomenon (Fig. S2a). On the other hand, Gel-1/4.6 exhibits one peak between 25.5° and 26.5°, and another peak between 31.5° and 33°, which could prove the formation of apatite (Fig. S2b). The broad apatite peaks also indicates the low crystallinity of apatite formed *in vitro*. Above results suggest the carboxylated β-CD hydrogels might be applied for bone repair.

β-CD based hydrogels have been widely applied in drug controlled release systems due to the formation of drug/CD complexes (Salmaso et al., 2007). The *in vitro* release profiles of IDM from carboxylated β-CD hydrogels were performed in SBF solution as shown in Fig. 7. As can be seen, the IDM release from both Gel-1/4.6 and Gel-1/9.2 are rapid during the first phase (0–5 days). Meanwhile, we could find that the release rate of IDM from Gel-1/9.2 is higher than that from Gel-1/4.6, although Gel-1/9.2 exhibits more biomineralization that Gel-1/4.6 in the first 14 days (Fig. 7c and e). The drug release behavior from the hydrogels is usually modulated by the swelling/erosion process and electrostatic interaction between drug and hydrogels. Since the carboxylated β-CD hydrogels and IDM are both negatively charged, we speculate that a higher electrostatic repulsion between them could be the reason for the faster release rate of Gel-1/9.2 (with more carboxyl groups). The data obtained during the first phase (0–5 day) of both hydrogels is found to fit linear $(M_t/M_\infty)/t^n$ plots and n is between 0.5 and 1.0, indicating that it is an anomalous transport kinetics (non-Fickian diffusion) (Kevadiya, Joshi, & Bajaj, 2010). After 5 days, the IDM release from carboxylated β-CD hydrogels was slow down but it can still last for the whole biomineralization period (28 days). It is also found that the biomineralized hydrogels have some benefits to obtain a long-term release system since only part of the loaded

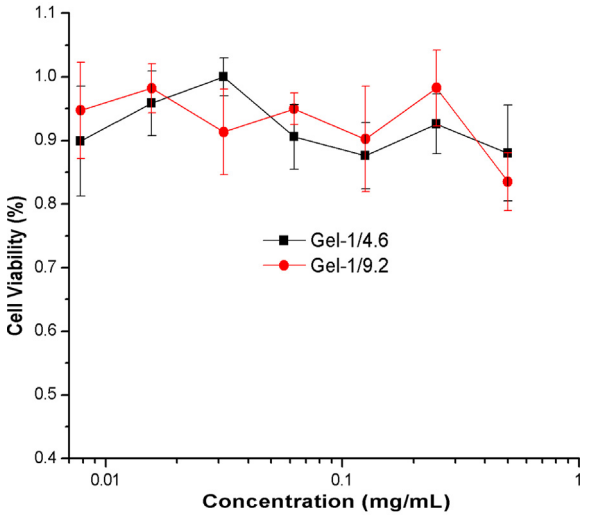

Fig. 8. Cytotoxicity test determined by MTT assay in COS7 cells. Cells were treated with the extract of Gel-1/4.6 and Gel-1/9.2 at different concentrations ($n = 6$).

drug are released within 28 days (56.2% for Gel-1/4.6 and 78.8% for Gel-1/9.2), which may due to the inhibition of drug release by new generated minerals. Base on above observations, carboxylated β-CD hydrogels are capable of releasing anti-inflammatory drugs during the biomineralization process of bone repair.

Further, although β-CD is well-known for its biocompatibility and has been approved as pharmaceutic adjuvant, it is still desirable to evaluate the cytotoxicity of the carboxylated β-CD hydrogels, which was tested by MTT assay in this work. The exact of hydrogels were prepared at different concentrations from 0.008 to 0.5 mg/mL. As shown in Fig. 8, both Gel-1/4.6 and Gel-1/9.2 exhibits low cytotoxicity on COS7 cells. Cell viability higher than 80% was obtained for both samples at all investigated concentrations. The low cytotoxicity indicates that the carboxylated β-CD hydrogels have good biocompatibility for biomedical applications.

4. Conclusion

In this study, carboxylated β-CD hydrogels are successfully synthesized using β-cyclodextrin and epichlorohydrin, and then modified with succinic anhydride. Gel-1/9.2 exhibits faster biomineralization rate in the SBF solution during the first 14 days than Gel-1/4.2, which is due to its higher content of carboxyl groups. The new generated minerals are apatite and similar to the mineral phase of the bone. Meanwhile, the carboxylated β-CD hydrogels can also load and release anti-inflammatory drug in a controlled manner during the biomineralization process. The release rate of IDM from Gel-1/9.2 is higher than that from Gel-1/4.6 due to the higher electrostatic repulsion between IDM and carboxyl groups. The hydrogels also exhibits low cytotoxicity in MTT assay. In summary, the multifunctional carboxylated β-CD hydrogels is promising for bone repair as it can induce biomineralization and release anti-inflammatory drug at the same time.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.10.076.

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