Short communication

Alginate–calcium carbonate porous microparticle hybrid hydrogels with versatile drug loading capabilities and variable mechanical strengths

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Abstract

Novel alginate/porous CaCO₃ microparticle hybrid hydrogels are facilely fabricated by in-situ release of Ca²⁺ from CaCO₃ microparticles induced by hydrolysis of D-glucono-δ-lactone to reduce pH. The mechanical strength of hybrid hydrogels is easily tunable by the alginate/CaCO₃ weight ratio. IBU was adsorbed into the porous CaCO₃ microparticles. The embedded porous CaCO₃ microparticles endow alginate hydrogels versatile drug loading capability and sustained release, especially for less water-soluble substances. This methodology provides a novel way to create new hybrid hydrogels for biomedical applications.

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1. Introduction

Hydrogels with reversible volume response to external stimuli have been studied extensively as biomaterials for tissue engineering, cell encapsulation, and carrier of drugs, peptides or proteins due to their hydrophilic character and possible biocompatibility (Chen, Jo, & Park, 1995; Lee, Kung, & Lee, 2005; Peppas, 1987; Peppas & Langer, 1994). They have been designed to control the release of drugs responding to the environmental change in pH, temperature, and so on (Beebe et al., 2000; Chen & Hoffman, 1995; Osada, Okuzaki, & Hori, 1992; Singh, Chauhan, Kumar, & Chauhan, 2007; Tanaka, Nishio, Sun, & Ueno-Nishio, 1982). However, sustained release over a long period cannot be expected from hydrogels because the release from hydrogels is generally diffusion-controlled, which is fast for passing through hydrogels due to their loose network structure (Tabata & Ikada, 1998). Meanwhile, water-insoluble substances are difficult to be incorporated into hydrogels because of their incompatibility and phase separation. Thus, it is highly desired to modify hydrogels for these purposes.

Hydrogels designed for the tissue engineering scaffolds should contain lots of pores large enough to accommodate living cells and should be biodegradable, releasing growth factors and creating pores, into which living cells penetrate and proliferate (Hoffman, 2002; Lee & Mooney, 2001). However, such disadvantages of porous hydrogels as low cell affinity and low mechanical strength cause difficulties in handling and cell culture (Hutmacher, 2001).

Artificial conjugation of hydrogels and inorganic compounds is interesting for preparing novel functional materials (Grassmann & Lübmann, 2004; Kuang, Wang, Gao, Hartmann, & Möhwald, 2005; Ogomi, Serizawa, & Akaishi, 2005; Ribeiro, Barrias, & Barbosa, 2004; Schnepp, Gonzalez-McQuire, & Mann, 2006). Hybrid composite hydrogels possess both properties of the hydrogel and inorganic compound. Porous inorganic microparticles, such as CaCO₃ and hydroxyapatite (HAp), are emerging as a new category of carrier for sustained release due to their large...
specific surface area, inclusion capability to various drugs, cell compatibility, and protein-adhesive property (Davis, 2002; Li, Wen, Shao, & Chen, 2004; Tosheva & Valtchev, 2005). Therefore, the conjugated composite of porous inorganic microparticles and hydrogels is attractive for preparing novel biomedical materials.

In this communication, we report a simple method as illustrated in Scheme 1 to fabricate alginate–CaCO₃ porous microparticle hybrid hydrogels with versatile water-insoluble drug loading capability and favorable mechanical strength. The alginate chains were cross-linked to form an infinite network by in-situ release of calcium cations from CaCO₃ microparticles induced by hydrolysis of α-glucono-δ-lactone (GDL) to reduce pH. Embedding porous CaCO₃ microparticles endows the alginate hydrogel a new potential to load water-insoluble drugs, proteins, and cells with appropriate toughness for the delivery and tissue engineering purposes. To authors’ knowledge, this is the first to use the inorganic porous microparticles directly in hydrogel formation as a reservoir with two effects of cross-linkers and released substances. Our methodology provides a novel way to create new hybrid hydrogels for biomedical applications.

2. Materials and methods

2.1. Materials

Sodium alginate (Kimitsu Chemical Industries Co., Japan, Mw 120,000) was dialyzed and freeze-dried before use. α-Glucono-δ-lactone (GDL, Sigma), ibuprofen (IBU, Juhua Group Corporation Pharmaceutical Factory, China), and sodium poly(styrenesulfonate) (PSS, Aldrich, MW 70,000) were used without further purification. Highly purified water was obtained by deionization and filtration with a Millipore apparatus (resistivity higher than 18.2 MΩ cm). Other chemicals were all analytical reagents and used as received.

2.2. Preparation of porous CaCO₃ microparticles and drug loading

Uniform spherical CaCO₃ microparticles with an average diameter of 4–6 µm were prepared by rapid mixing of equal volume of CaCl₂ and Na₂CO₃ aqueous solutions. Typically, 0.2 M CaCl₂ solution was rapidly poured into an equal volume of 0.2 M Na₂CO₃ solution (containing 4 g/l of PSS) at room temperature. After vigorous agitation, the precipitate was filtered off, thoroughly washed with pure water, and dried. The porous structure was observed by scanning electron microscopy (SEM) with a Philips XL 30 at the acceleration voltage of 15 kV. The specific surface area, porous volume, and porous size distribution of CaCO₃ microparticles were determined following the Brunauer–Emmett–Teller (BET) method of nitrogen adsorption/desorption at −196 °C with an ASAP2010 surface area analyzer (Micromeritics Instrument, USA).

The acetone-immersed dry CaCO₃ microparticles (0.2 g) were added into 5 ml of an IBU ethanol solution of 50 mg/ml and the suspension was kept to adsorbing equilibrium under gentle stirring for 24 h. Subsequently, the IBU-loaded CaCO₃ microparticles were collected via centrifugation and dried. The IBU loading amount G was expressed as mg IBU/g CaCO₃ and determined by ultraviolet (UV) absorbance at 221 nm with a Hitachi U-3010 UV–Vis spectrometer.

2.3. Fabrication of alginate–CaCO₃ porous microparticle hybrid hydrogels

Two milliliters of 2 wt.% alginate solution and different weights of the CaCO₃ microparticles with or without IBU were mixed, 100 ml freshly prepared 0.2 g/ml GDL aqueous solution was added, and the alginate hydrogel was formed in a few minutes. For comparison, a blank alginate hydrogel was prepared by in-situ release of Ca²⁺ from Ca–EDTA chelate using 100 ml freshly prepared 0.2 g/ml GDL aqueous solution. IBU–alginate hydrogel was also prepared by in-situ release of Ca²⁺ from Ca–EDTA chelate when IBU was dispersed in the alginate solution. The dynamic modulus was measured with a RFS-II rheometer (Rheometrics Ltd.) at 25 ± 0.1 °C using a cone-plate fixture. The diameter and angle of the cone were 50 mm and 0.04 rad, respectively. Optical images of slice cross-sections of hybrid hydrogels were observed with an AxioLab Pol polarizing microscope (Carl Zeiss, German).

2.4. In vitro release

A certain weight of either the IBU-loaded bare CaCO₃ microparticles or the hybrid hydrogel containing IBU-loaded CaCO₃ microparticles was put in a dialysis bag (cut-off MW was 8000) and immersed in the release buffers (PBS, pH 7.4) with continuously stirring at 37 °C. The release solution of 3 ml was drawn from the release medium at each certain time intervals, the IBU concentration...
was determined with UV absorbance, and the solution was then returned.

3. Results and discussion

Uniform spherical porous CaCO₃ microparticles with narrowly distributed size from 4 to 6 μm have been prepared by colloidal crystallization from supersaturated (relative to CaCO₃) solution (Volodkin, Petrov, Prevot, & Sukhorukov, 2004; Wang et al., 2006). The CaCO₃ microparticle (Fig. 1a) appears with a rough and porous surface. The specific surface area, pore volume, average pore diameter of the CaCO₃ microparticle are 40 m²/g, 0.23 cm³/g and 22.3 nm, respectively, as determined by the Brunauer–Emmett–Teller (BET) method of nitrogen adsorption/desorption. Usually, the spherical CaCO₃ microparticle turns to rhombohedral calcite microcrystal after several week storage in water at room temperature because of recrystallization (Volodkin et al., 2004). By using poly(styrene sulfonate) (PSS) as the dispersant to prevent recrystallization, we are successful in keeping the spherical shape of CaCO₃ microparticles in aqueous phase is important for their being embedded in the hydrogel and acting as a delivery source.

Typically, 50 mg of porous CaCO₃ microparticles are dispersed in 2 ml of alginate solution with 2 wt%, 100 ml of freshly prepared 0.2 g/ml GDL aqueous solution is added to lower the solution pH value to ca. 2.5 due to its slow hydrolysis. A trace of CaCO₃ is dissociated to release Ca²⁺. Consequently the alginate chains are cross-linked to form a hybrid hydrogel containing the CaCO₃ microparticles. This gelation process lasts about several minutes, which can be tuned by changing the amount of added GDL. Without adding of GDL, no gel is formed by merely mixing alginate solution with CaCO₃ microparticles. The blank alginate hydrogel without CaCO₃ microparticles, which is formed by in-situ release of Ca²⁺ from Ca–EDTA (Kong, Lee, & Mooney, 2003; Lu, Liu, Dai, & Tong, 2005; Stokke et al., 2000), is transparent (Fig. 1b) and the alginate hybrid hydrogel containing CaCO₃ microparticles becomes semitransparent or opaque (Fig. 1c) depending on the amount of CaCO₃. The CaCO₃ microparticles are embedded in the alginate hydrogels, which is obviously shown in Fig. 1d. Some factors, such as fast gelation, high polymer (also acting as the dispersant) concentration, and low density of CaCO₃ microparticles due to their porous nature, play important roles to prevent sedimentation of the CaCO₃ microparticles during gelation. Compared with the reported CaCO₃ hybrid hydrogels from in-situ mineralization (Kuang et al., 2005; Ogomi et al., 2005), the present method has some advantages of rapid process within several minutes, easy adjustment to the gel strength, and versatile loading capability of CaCO₃ microparticles.

Fig. 2 shows the storage modulus G’ and loss modulus G’’ for the blank alginate hydrogel and hybrid alginate hydrogels. All hydrogels are prepared with the same amount of GDL in order that all hydrogel are cross-linked with the same amount of Ca²⁺. The blank alginate hydrogel is very soft with modulus lower than 10³ Pa. Conjugation with CaCO₃ microparticles, however, enhances the strength of the as-prepared alginate hydrogel. For both hydrogel samples, G’ is always higher than G’’ and almost independent of angular frequency ω, indicating the formation of infinite network. The plateau G’ values for the alginate hydrogels containing CaCO₃ microparticles are about one order of magnitude higher than that of the blank alginate hydrogel. The addition of CaCO₃ microparticles strengthens the alginate hydrogels because the CaCO₃ microparticles in the gel act as cross-linking domains due to the enrichment of Ca²⁺ dissociated from the CaCO₃ microparticles.

An anti-inflammatory drug of ibuprofen (IBU) is used as the model drug with very low water solubility to demonstrate the loading capacity and release property of the alginate CaCO₃ microparticle hybrid hydrogel. Firstly, the CaCO₃ microparticles are allowed to adsorb IBU up to 40 mg IBU/g CaCO₃ determined by UV absorbance, then the IBU-loaded CaCO₃ microparticles are dispersed in alginate solution, finally the GDL solution is added to induce the gelation. IBU released from the alginate CaCO₃ microparticle hybrid hydrogel is monitored by UV absorbance of the release medium. The release profiles in the simulated intestinal fluid (PBS, pH 7.4) at 37 °C are depicted in
The release of IBU–alginate hydrogel is similar to that of pure IBU powders. The half release time $t_{1/2}$ for both pure IBU powders and IBU–alginate hydrogel without CaCO$_3$ is 13 min. The total release time for both pure IBU powders and IBU–alginate hydrogel without CaCO$_3$ is 160 min. However, the release of IBU–CaCO$_3$–alginate hybrid hydrogel is slower compared with those of pure IBU powders and IBU–alginate hydrogel without CaCO$_3$. The half release time $t_{1/2}$ for IBU–CaCO$_3$–alginate hybrid hydrogel is 45 min and the total release time for IBU–CaCO$_3$–alginate hybrid hydrogel is 260 min. This fact indicates that the release of the encapsulated IBU is dominantly controlled by porous CaCO$_3$ and the coverage of alginate hydrogel has no detectable delay to the release rate. Consequently, the less water-soluble substances can be included in hydrogels with the help of porous inorganic microparticles as the reservoir for delivery.

4. Conclusions

The alginate hybrid hydrogel has been fabricated with CaCO$_3$ microparticles when the pH is lowered to dissociate Ca$^{2+}$ in-situ induced by the gradual hydrolysis of GDL. The porous CaCO$_3$ microparticles behaves as a reservoir for both purposes of calcium cations for cross-linking and adsorbed substances for delivery. Conjugation with CaCO$_3$ microparticles also enhances the mechanical strength of the alginate hydrogel, probably due to the formation of a percolation network cross-linked with the microparticles. This facile approach endows alginate hydrogels with an enhanced loading capability and sustainable release, especially for less water-soluble substances. The hydrogels and porous CaCO$_3$ microparticles can behave as a good carrier for cells and cell growth factors, respectively. Therefore, the CaCO$_3$ microparticle hybrid hydrogel will have some very spectacular applications in tissue engineering owing to the reservoir and affinity effects of the CaCO$_3$ microparticles.

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References


