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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 $CaCO_3$ microparticle hybrid hydrogels are facilely fabricated by in-situ release of Ca^{2+} from $CaCO_3$ microparticles induced by hydrolysis of D-glucono-δ-lactone to reduce pH. The mechanical strength of hybrid hydrogels is easily tunable by the alginate/$CaCO_3$ weight ratio. IBU was adsorbed into the porous $CaCO_3$ microparticles. The embedded porous $CaCO_3$ 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.

Keywords: Hybrid hydrogel; Alginate; In-situ gelation; Drug loading; Tunable mechanical strength

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 & Löbmann, 2004; Kuang, Wang, Gao, Hartmann, & Möhwald, 2005; Ogomi, Serizawa, & Akashi, 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 $CaCO_3$ and hydroxyapatite (HAp), are emerging as a new category of carrier for sustained release due to their large

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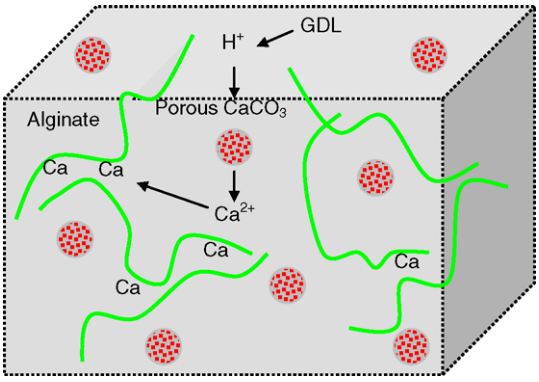
specific surface area, inclusion capability to various drugs, cell compatibility, and protein-adhesive property (Davis, 2002; Li, Wen, Shao, & Chen, 2004; Tosheva & Valtchev, 2005). Therefore, the conjugated composite of porous inorganic microparticles and hydrogels is attractive for preparing novel biomedical materials.

In this communication, we report a simple method as illustrated in Scheme 1 to fabricate alginate–$CaCO_3$ 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 $CaCO_3$ microparticles induced by hydrolysis of D-glucono-δ-lactone (GDL) to reduce pH. Embedding porous $CaCO_3$ 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. D-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.



Scheme 1. Schematic representation of alginate–$CaCO_3$ porous microparticle hybrid hydrogel fabricated by in-situ release of Ca^{2+} cations.

2.2. Preparation of porous $CaCO_3$ microparticles and drug loading

Uniform spherical $CaCO_3$ microparticles with an average diameter of 4–6 μm were prepared by rapid mixing of equal volume of $CaCl_2$ and Na_2CO_3 aqueous solutions. Typically, 0.2 M $CaCl_2$ solution was rapidly poured into an equal volume of 0.2 M Na_2CO_3 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 $CaCO_3$ 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 $CaCO_3$ 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 $CaCO_3$ microparticles were collected via centrifugation and dried. The IBU loading amount G was expressed as mg IBU/g $CaCO_3$ and determined by ultraviolet (UV) absorbance at 221 nm with a Hitachi U-3010 UV–Vis spectrometer.

2.3. Fabrication of alginate–$CaCO_3$ porous microparticle hybrid hydrogels

Two milliliters of 2 wt.% alginate solution and different weights of the $CaCO_3$ 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 Ca^{2+} 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 Ca^{2+} 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 $CaCO_3$ microparticles with narrowly distributed size from 4 to 6 μm have been prepared by colloidal crystallization from supersaturated (relative to $CaCO_3$) solution (Volodkin, Petrov, Prevot, & Sukhorukov, 2004; Wang et al., 2006). The $CaCO_3$ microparticle (Fig. 1a) appears with a rough and porous surface. The specific surface area, pore volume, average pore diameter of the $CaCO_3$ microparticle are 40 m^2/g, 0.23 cm^3/g and 22.3 nm, respectively, as determined by the Brunauer–Emmett–Teller (BET) method of nitrogen adsorption/desorption. Usually, the spherical $CaCO_3$ 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 $CaCO_3$ microparticles in water for more than 6 months. High stability of porous $CaCO_3$ microparticles in aqueous phase is important for their being embedded in the hydrogel and acting as a delivery source.

Typically, 50 mg of porous $CaCO_3$ 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 $CaCO_3$ is dissociated to release Ca^{2+}. Consequently the alginate chains are cross-linked to form a hybrid hydrogel containing the $CaCO_3$ 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 $CaCO_3$ microparticles. The blank alginate hydrogel without $CaCO_3$ microparticles, which is formed by in-situ release of Ca^{2+} 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 $CaCO_3$ microparticles becomes semitransparent or opaque (Fig. 1c) depending on the amount of $CaCO_3$. The $CaCO_3$ 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 $CaCO_3$ microparticles due to their porous nature, play important roles to prevent sedimentation of the $CaCO_3$ microparticles during gelation. Compared with the reported $CaCO_3$ 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 $CaCO_3$ microparticles.

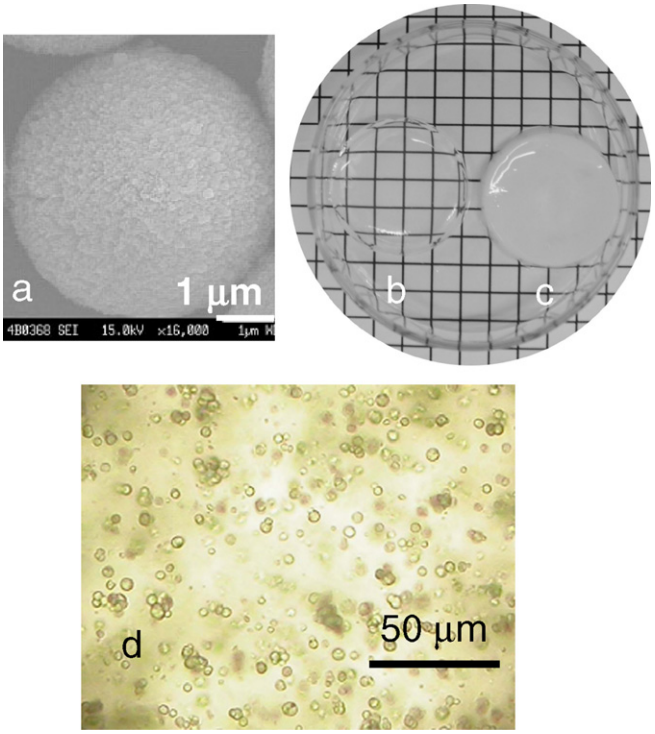


Fig. 1. (a) SEM image of porous $CaCO_3$ microparticle. (b) Photograph of the blank alginate hydrogel without $CaCO_3$ microparticles (transparent). (c) Photograph of alginate $CaCO_3$ microparticle hybrid hydrogel with $CaCO_3$ to alginate weight ratio of 1.25 (opaque). (d) Transmission optical microscope image of slice cross-sections of this hybrid hydrogel. The grid in the background of (b) and (c) is 0.5 cm × 0.5 cm.

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 Ca^{2+}. The blank alginate hydrogel is very soft with modulus lower than 10^3 Pa. Conjugation with $CaCO_3$ 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 $CaCO_3$ microparticles are about one order of magnitude higher than that of the blank alginate hydrogel. The addition of $CaCO_3$ microparticles strengthens the alginate hydrogels because the $CaCO_3$ microparticles in the gel act as cross-linking domains due to the enrichment of Ca^{2+} dissociated from the $CaCO_3$ 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 $CaCO_3$ microparticle hybrid hydrogel. Firstly, the $CaCO_3$ microparticles are allowed to adsorb IBU up to 40 mg IBU/g $CaCO_3$ determined by UV absorbance, then the IBU-loaded $CaCO_3$ microparticles are dispersed in alginate solution, finally the GDL solution is added to induce the gelation. IBU released from the alginate $CaCO_3$ 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

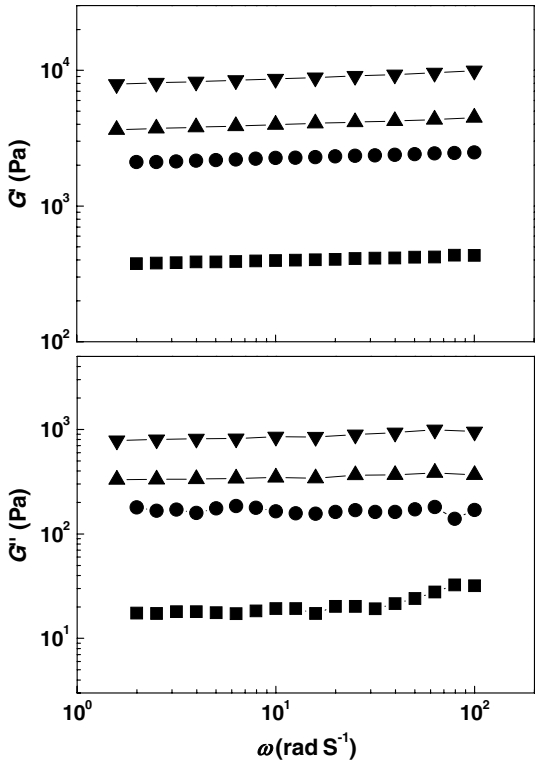


Fig. 2. Angular frequency ω dependence of storage modulus G' and loss modulus G'' at 25 °C for the hybrid hydrogel with $CaCO_3$/alginate weight ratio of 0 (square), 1.25 (circle), 2.5 (up triangle), and 12.5 (down triangle).

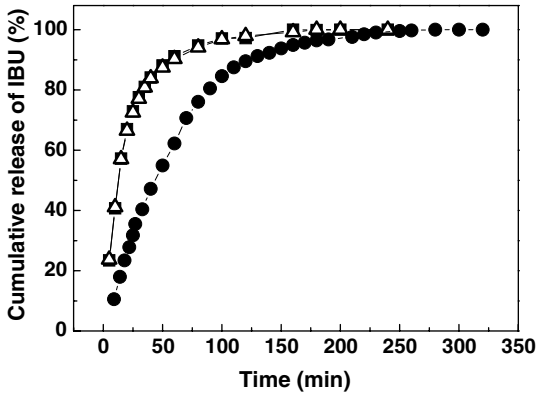


Fig. 3. Release profiles of pure IBU (close square), IBU–alginate hydrogel (open up triangle) and IBU–$CaCO_3$–alginate hybrid hydrogel with $CaCO_3$/alginate weight ratio of 1.25 (close circle) at pH 7.4 and 37 °C.

Fig. 3. 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.

Acknowledgements

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