Multifunctional Drug Delivery System Using Starch-Alginate Beads for Controlled Release

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Utilizing starch-containing alginate beads, a novel drug delivery system (DDS) was developed. With the starch inside, the composite bead could be dried in its original bead shape and handled in the dried state. By employing alginate multi-coating strategy on the starch-alginate beads, detained or controlled release was efficiently achieved and successfully demonstrated for a model peptide drug, L-phenylalanine. The initial latent time and release rate of the drug inside the beads were able to be controlled simply by varying the number of multi-coatings. While the latent time for the initial release was negligible for non-coated starch-alginate beads, the latent times of beads coated one, two, and four times increased to 15, 30, and 70 min, respectively. Furthermore, the alginate component of the composite beads could adsorb and remove heavy metals such as lead from the body. These multifunctional beads combined with the novel coating process will greatly benefit alginate gel-based DDS.

Key words alginate bead; drug delivery system; controlled release; coating; starch

Alginate is one of the polysaccharides abundantly found in the surface of seaweeds such as lavar (*Porphyra tenera*), green lavar (*Enteromorpha linza*), agaragar (*Gelidium amansii*), marine brown alga (*Undaria pinnatifida*), and tangleweed (*Kjellmaniella crassifolia*). Alginate becomes water-insoluble in the presence of polyvalent cations such as Ca^{2+}, Hg^{2+}, Be^{2+}, Cu^{2+}, Co^{2+}, Al^{3+}, and Fe^{3+}, resulting in an alginate gel. Bead-type of alginate gel can be easily prepared in one step without any harsh conditions for living cells inside the beads, and therefore, they have been extensively used for the immobilization of various biomolecules: immobilization of living cells in a fermentor; immobilization of hybridoma cells to produce monoclonal antibodies; and entrapment of mammalian cells for the implantation of artificial organs.[1—7)]

Alginate also has some useful characteristics that can be exploited for pharmaceutical purposes. In particular, the formation of a net-like lattice between the cations and the alginate within the gel is responsible for the slow release of the embedded drugs, which can be used for the development of alginate-based drug delivery systems (DDS).[8—10)] Due to its unique features, alginate gel has been extensively studied for the controlled release of drugs by many researchers.[11,12)] Especially, the Kawashima[13—17)] and Kanaya groups[18,19)] have published numerous reports on the key physical and forming properties of various alginate gels, which could be useful in the development of alginate gel-based DDS.

In DDS, slow release is an important factor to prolong the contact time between a drug and absorption surfaces in the body.[13,14,20)] The longer contact time is usually desirable to achieve stable physiological conditions by maintaining a constant drug concentration for an extended time. Currently, the slow-release property of drugs is mainly achieved either by the compressed extrusion of dried powder or by the addition of hydrophilic viscous additives to the drug tablets. Drug release from alginate beads is known to be blocked or sustained at low pH, such as in the stomach, by forming a surface gel cover, while drug delivery is accelerated at neutral pH, such as in the small intestine, by the swelling of alginate.[21)] Utilizing this manageable drug-release property, alginate has been applied to a variety of pharmaceutical DDS.[15—19)]

In this study, we developed a completely new strategy to achieve controlled or sustained release of drugs within alginate-based beads. First, we prepared starch-containing alginate beads in the dried state by dropping a solution containing sodium alginate and starch into a $CaCl_2$ solution, followed by drying. With the dried starch-alginate (SA) beads, an alginate-coating strategy was developed to control the initial latent time and the release rate of the embedded drug within the composite beads. Removal of heavy metal ion such as Pb^{2+} was also demonstrated for the alginate-coated SA beads.

MATERIALS AND METHODS

Materials Sodium alginate was obtained from Junsei Chemical Co., Japan. $CaCl_2$, $Pb(NO_3)_2$, phosphate-buffered saline (PBS) solution, and L-phenylalanine were purchased from Sigma (St. Louis, MO, U.S.A.). Wheat starch powder was purchased from a commercial vendor. Distilled water was generated using Waters Milli-Q (Biocel) (MA, U.S.A.). All other chemicals or reagents used in this study were of analytical grade.

Preparation of Alginate and SA Beads Four different mixture solutions were used to prepare alginate or SA beads: 1) sodium alginate 1.5% (w/w) alone; 2) starch powder 20% (w/w) and sodium alginate 1.5% (w/w); 3) sodium alginate 1.5% (w/w) and L-phenylalanine (3 mg/ml); and 4) starch powder 20% (w/w), sodium alginate 1.5% (w/w) and L-phenylalanine (3 mg/ml). First, the solution was treated in a sonicator for 10 min to remove air bubbles. Beads were prepared by dropping each solution through a 1000-μl

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Table 1. Changes in Physical Properties of Alginate Beads and SA Beads upon Drying and Swelling

	Alginate beads [diameter (mm)/mass (mg/each)]	SA beads [diameter (mm)/mass (mg/each)]
Before drying	5.0/60.0	6.5/190.0
After drying	1.9/4.3	3.6/26.5
After soaking in water	1.9/5.3	5.1/103.0

pipette tip into a $CaCl_2$ 1.0 M solution. Beads were formed immediately, left to harden in the $CaCl_2$ solution for 5—30 min, and washed with distilled water. The beads were dried overnight in an oven at 60 °C.

Measurements of Physical Properties of Alginate Beads and SA Beads The mean diameters of alginate beads and SA beads were measured with a conventional micrometer and their masses were measured using a conventional balance.

Alginate Gel Coating on SA Beads For the alginate gel coating, the dried SA beads were soaked in a 1.0% (w/w) sodium alginate solution and then put into $CaCl_2$ 1.0 M solution to form an alginate gel coating on the surface of the beads. The beads were left to harden in the $CaCl_2$ solution for 5—30 min, washed with distilled water, and dried overnight in an oven at 60 °C. The same procedure was repeated to produce up to four coats on the SA beads.

L-Phenylalanine Release Experiment Approximately 400 mg (based on dried mass) of alginate beads, non-coated SA beads, and alginate-coated SA beads containing L-phenylalanine were immersed in 40 ml of PBS (0.01 M, pH 7.4) solution contained in a 125-ml Erlenmeyer flask and the solution was stirred in a shaking incubator at 100 rpm and 25 °C. The dried masses of the beads were measured up to 0.1 mg accuracy to calculate the absorption rate later. At 5, 10, 15, 20, 30, 40, 50, 70, 100, and 130 min, 100 μl of the solution was sampled with a conventional 200-μl micropipette, diluted 10 times with PBS solution, and analyzed with UV/Vis Spectrophotometer (Jasco, V-530 (Japan)). The concentrations of L-phenylalanine released from the beads were determined by measuring the absorbance at 257 nm.

Lead Removal Analysis To evaluate the lead sorption capacity, approximately 400 mg of alginate beads, alginate-coated SA beads, and non-coated SA beads without L-phenylalanine were added to 100 ml of a 200 ppm Pb^{2+} solution and stirred at 25 °C. At 5, 10, 15, 20, 30, 40, 50, 70, 100, and 130 min, 100 μl of the solution was sampled, diluted 200 times with distilled water, and analyzed using an atomic absorption spectrophotometer (Perkin-Elmer, Model 3100, CT, U.S.A.) to determine the concentration of residual Pb^{2+}.

RESULTS AND DISCUSSION

Changes in Physical Properties of Alginate Beads and SA Beads upon Drying and Swelling To evaluate the extent of shrinkage upon drying, the mean diameters of alginate beads and SA beads were measured before and after drying. The swelling properties of the dried beads were also analyzed by soaking the beads in water, followed by measuring their diameters. With the starch inside, the SA beads could be dried in the original bead shape and handled in the dried state. The dried SA beads also swelled to some extent (3 times larger than the size of the dried beads) in water, as shown by the data in Table 1. Preparation in a dry form is essential for application as a commercial DDS, allowing the drug to be easily packed, transported, and stored. Starch was chosen as the structural component because it is inert to all kinds of biologically active drugs and also commercially available in powder form. Without the starch, the alginate beads became much smaller (18.2 times smaller than the original wet volume) and formed irregular particles upon drying, which did not swell even when soaked in water overnight (Table 1). The swelling property of SA beads is essential for application in DDS, leading to the release of the embedded drug, as described in the next section. Furthermore, the swelling of the SA beads could cause patients to feel satiety, providing an extra diet function to the beads.

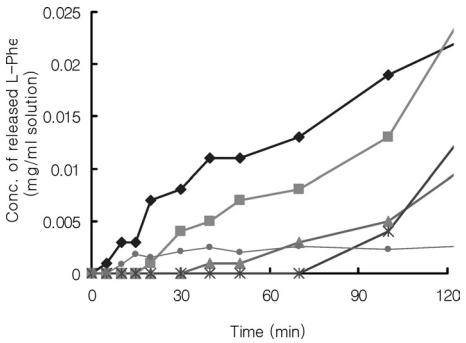

Fig. 1. Controlled Release of the Embedded L-Phenylalanine from Alginate Beads and Multi-coated SA Beads

—◆—, non-coated; —■—, coated once; —▲—, coated twice; —∗—, coated four times; —●—, alginate beads.

Controlled Release of L-Phenylalanine L-Phenylalanine was employed as a model peptide drug to evaluate the drug delivery characteristics of the alginate beads and multi-coated SA beads, *i.e.*, the initial latent time and release rate. With the dried alginate beads containing L-phenylalanine, the concentrations of the released L-phenylalanine were measured at predetermined time intervals. Within approximately 15 min, L-phenylalanine was released from the beads up to a concentration of 0.0025 mg/ml and remained almost the same until 120 min, indicating that no additional L-phenylalanine was released after the initial release (Fig. 1). This is due to the lack of swelling of the alginate beads, which prevents the release of the embedded drug.

L-Phenylalanine was released from the non-coated SA beads at a relatively rapid rate, in comparison with the release from alginate-coated SA beads, without any latent time for initial release (Fig. 1).

Upon alginate coating of the SA beads, however, latency of the initial release was observed. The difference between

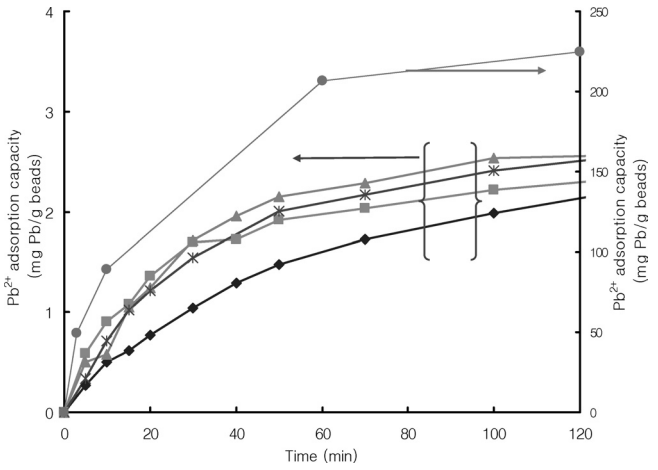

Fig. 2. Pb^{2+} Adsorption Capacities of Alginate Beads (Used Wet without Drying) and Multi-coated SA Beads

—◆—, non-coated; —■—, coated once; —▲—, coated twice; —∗—, coated four times; —●—, alginate beads.

the alginate beads and SA beads is ascribed to the presence of the starch component within the SA beads as explained above. Starch is believed to function as a structural component and prevent the SA beads from shrinking too much. The length of the initial latent time depended on the number of coatings, as expected. The latent times for SA beads coated one, two, and four times were 15, 30, and 70 min, respectively, indicating that the latent time can be controlled simply by the number of the alginate coatings. Using this strategy, it would be possible to develop an acid-labile drug to target a site in the gastrointestinal (GI) tract. The drug can be protected in the stomach preventing its release until it arrives at the desired site in the GI tract by embedding the drug in SA beads with an appropriate number of alginate coatings.

The initial release rates of L-phenylalanine were 9.1×10^{-5} M/h, 5.8×10^{-5} M/h, and 2.7×10^{-5} M/h for non-coated, once-coated, and twice-coated beads, respectively, indicating that a greater than 60% decrease in the rate was caused by each coating. A slow release rate is sometimes desirable for drugs sensitive to the target body. This is also important when the effect of a defined amount of drug must continue for an extended time. The release rate of drugs could be also controlled by changing the percentage of starch in the SA beads. A slower release rate can be achieved simply by increasing the starch percentage of the SA beads.

Lead Adsorption Capacity of SA Beads Alginate is widely known to be a potent metal adsorbent and is used to remove various heavy metal ions.[22—24] For example, marine brown alga contains the polysaccharide up to 40 wt%, leading to its sorption capacity of up to 350 mg of lead/g biomass.[22] Therefore, we envisioned that the alginate component of the SA beads could adsorb and remove contaminating heavy metals from the body. We performed an experiment to evaluate the adsorption capacity of the SA beads for lead ion. The lead adsorption capacity of the beads was determined to be about 2 mg Pb^{2+}/g of dried SA (Fig. 2.). The capacity is much lower than that of wet alginate beads (230 mg Pb^{2+}/g of dried alginate) due to the much lower content of alginate per unit mass of SA beads. However, the level of the contaminating heavy metal in human body is usually extremely low and the adsorption capacity of the SA beads is sufficient to remove such heavy metals. Furthermore, the adsorption capacity was increased by approximately 10% by alginate coating of the beads. Since there is no enzyme that digests alginate found in the human body, the alginate with absorbed heavy metals is believed to be discharged from the body through the excretory system. The results demonstrated that in addition to the DDS function, SA beads could function as an adsorbent to remove heavy metals from the body.

CONCLUSIONS

We developed a novel DDS using composite beads composed of starch and alginate. Employing the starch as a structural component, the resulting SA beads had some advantages, including greater degree of swelling in water, which would be useful for their application in DDS. To achieve a controlled or sustained DDS based on the SA beads, we developed an alginate-coating strategy. Using the strategy, efficient control of the initial latent time and the release rate was successfully demonstrated with the model peptide drug L-phenylalanine. Since the strategy developed in this study can be simply achieved without any cumbersome procedures requiring technical expertise, it has enormous potential applicability in alginate-based DDS.

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