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Iron Oxide Nanoparticle-incorporated Alginate Capsules as Magnetic Field-assisted Potential Delivery Platforms for Agriculture Pesticides and Biocontrol Agents

Dohyeon Lee[1], Kyoung Soon Choi[2], Daun Kim[1], Sunho Park[1], Woochan Kim[1], Kyoung-Je Jang[3], Ki-Taek Lim[4], Jong Hoon Chung[3], Hoon Seonwoo[5]*, Jangho Kim[1]*

[1]*Department of Rural and Biosystems Engineering, Chonnam National University, Gwangju 61186, Republic of Korea*
[2]*Advanced Nano-Surface Research Group, Korea Basic Science Institute (KBSI), Daejeon 34133, Republic of Korea*
[3]*Department of Biosystems & Biomaterials Science and Engineering, Seoul National University, Seoul 08826, Republic of Korea*
[4]*Department of Biosystems Engineering, Kangwon National University, Cuncheon 24341, Republic of Korea*
[5]*Department of Industrial Machinery Engineering, Sunchon National University, Suncheon 57922, Republic of Korea*

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Abstract

Purpose: Biocompatible capsules have recently been highlighted as a novel platform for delivering various components, such as drug, food, and agriculture pesticides, to overcome the current limitations of living systems, such as those in agriculture, biology, the environment, and foods. However, few active targeting systems using biocompatible capsules and physical forces simultaneously have been developed in the agricultural engineering field. **Methods:** Here, we developed an active targeting delivery platform that uses biocompatible alginate capsules and controls movements by magnetic forces for agricultural and biological engineering applications. We designed and fabricated large-scale biocompatible capsules, using custom-made nozzles ejecting alginate solutions for encapsulation. **Results:** To develop the active target delivery platforms, we incorporated iron oxide nanoparticles in the large-scale alginate capsules. The sizes of alginate capsules were controlled by regulating the working conditions, such as concentrations of alginate solutions and iron oxide nanoparticles. **Conclusions:** We confirmed that the iron oxide particle-incorporated large-scale alginate capsules moved actively in response to magnetic fields, which will be a good strategy for active targeted delivery platforms for agriculture and biological engineering applications, such as for the controlled delivery of agriculture pesticides and biocontrol agents.

Keywords: Alginate, Biocompatible capsule, Iron oxide, Magnetic field, Target delivery system

Introduction

There is always a demand to maximize agricultural crop yields of major food sources (Curtis and Halford, 2014). Crop yields have been improved using modified high-yield crops and synthetic fertilizers (Ashfaq et al., 2015). However, productivity remains limited owing to pests that cause significant and total crop losses; at least one-third of food resources are estimated to be lost because of the effects of pests before harvesting (Popp et al., 2012). Thus, pesticides play a key role in the agricultural industry because they help prevent crop losses from pests. The use of pesticides has had a major impact on the qualitative and quantitative improvement of food resources, facilitating growth of the world population. However, most insecticides have a fatal adverse effect on non-targeted creatures, as less than 0.1% of the applied insecticide reaches the target insects

*Corresponding author: Jangho Kim, Hoon Seonwoo
Tel: +82-62-530-5181, +82-61-750-3261;
Fax: +82-62-530-2159, +82-61-750-3260
E-mail: rain2000@jnu.ac.kr, uhun906@scnu.ac.kr

(Kumar et al., 2013). Furthermore, improperly used insecticides can contaminate the air, surface water, and groundwater. Therefore, safe and efficient pesticide application is essential for preventing side effects caused by pesticides (Oliveira et al., 2014).

To overcome current problems related to the use of pesticides and biocontrol agents, novel delivery systems for use with pesticides and biocontrol agents must be developed. Currently, various drug delivery systems (DDSs) have been widely investigated for various applications such as biological engineering, biomedical, environmental, and food engineering. DDSs typically use biomaterial-based carriers to encapsulate specific materials (e.g., cells, drugs, genes) for applications in living systems such as human, animals, and plants. Using the DDSs, specific materials can be delivered and released at specific targets. For example, when the carriers undergo degradation, the incorporated drugs can be diffused in a controlled manner. Various biocompatible materials have been used as drug carriers, such as poly (lactic-co-glycolic acid) (Kapoor et al., 2015), polycaprolactone, polyethylene glycol (Wang et al., 2014), and gelatin (Elzoghby, 2013). Alginate has a wide variety of biological applications and shows no toxicity, minimal immunogenicity, and tunable biodegradability (Paques et al., 2014). Additionally, the properties of alginate can be controlled according to the intended application. In addition, substances that react under specific conditions can be coated on the surface or guided to a desired location by being placed inside the DDS. Because of these advantages, DDSs are widely used in various fields such as medicines and the food industry (Farokhzad and Langer, 2009, Fathi et al., 2014).

Several studies have reported the use of DDS-based platforms (DDSP) in agriculture. Kim (2016) carried out a study to prevent fire blight, which is a serious disease in apples and pears, using capsules that released *Pantoea agglomerans* strain E325. Strains of *P. agglomerans* have attracted attention as potential antagonists for fire blight. Recently, studies to decrease the side effects of DDSP for agricultural use have been conducted. Ultraviolet light was used for irradiation during the stage of capsule formation for photodegradation of residual pesticides to reduce the toxicity of capsules, increasing the effectiveness of encapsulated insecticide application (Guan et al., 2008). However, the application of DDSs in agriculture remains limited because of the side effects caused by excess drugs after use. To use DDSP in agriculture, drug-loaded carriers must be actively controlled to induce drug release at a desired location. Active targeting using a magnetic field may be a good strategy for improving DDSP. Iron oxide nanoparticles (IONs) are widely used with a magnetic field (Demirer et al., 2015). However, few active DDSP have been applied in the agricultural engineering field.

In this study, we prepared large-scale ION-incorporated

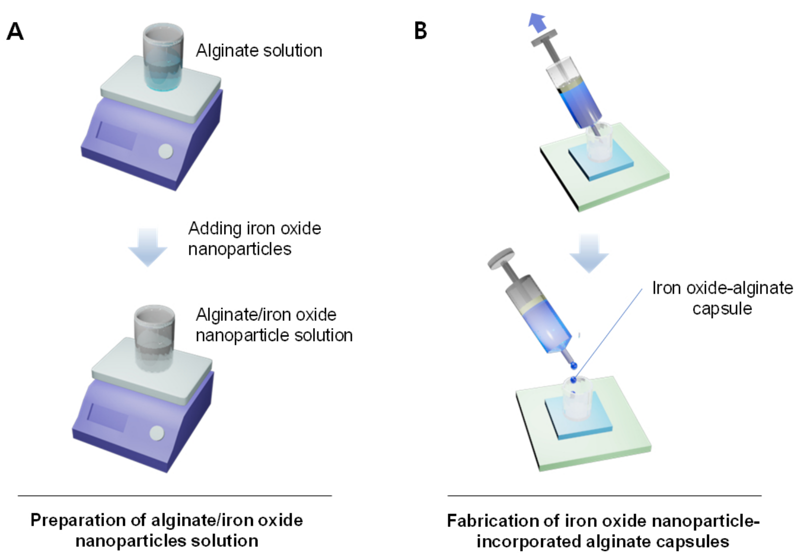

Fig. 1. (A) Schematic of preparation of alginate/iron oxide nanoparticles solution and (B) fabrication of of iron oxide nanoparticle-incorporated alginate capsules. First, 0.05–0.5 wt% iron oxide nanoparticles and 1–2 wt% sodium alginates were added to 50 mL deionized water. The solution was dissolved in a beaker equipped with a magnetic stirrer at 1500 rpm overnight. The iron oxide nanoparticles-alginate solutions were placed in a syringe. The iron oxide nanoparticle-alginate solutions were injected into gelling solution.

alginate capsules (ION-ACs) and investigated the reactivity of the capsules under magnetic fields (Fig. 1). The capsules were made by ionic cross-linking of alginate, and various concentrations of IONs were inserted into the capsules to optimize the ION concentration. Next, we analysed their morphological properties, such as average diameters. Finally, the reactivity of ION-ACs to magnetic fields was analysed to verify the spatial control of capsules.

Materials and Methods

Materials

Iron (II, III) oxide (97%, MKBZ5546V) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium alginate (9005-38-3), calcium chloride anhydrous (10043-52-4), and Tween® 20 (9305-64-5) were purchased from DAEJUNG (Busan, Korea).

Design and fabrication of ION-ACs

First, 0.05–0.5 wt% iron oxide and 1–2 wt% sodium alginates were added to 50 mL deionized water, and the solution was stirred at 1500 rpm overnight. After stirring, the solution was loaded into a 1-mL syringe fitted with a 21-gauge needle, and the syringe was installed on a syringe pump. In addition, 0.2 wt% calcium chloride and 0.05 wt% Tween® 20 were added to 50 mL DI water and the calcium chloride solution was stirred at 1500 rpm for 1 h. The iron oxide and alginate solution-loaded 1-mL syringe fitted with a 21-gauge needle was fixed 10 cm from the surface of the calcium chloride bath. An ION/alginate droplet was formed by ejecting the solutions pumped by the syringe pump, and ION-ACs were fabricated by deposition of the droplets into the calcium chloride solution bath while stirring at 800 rpm. Finally, the resulting mixture was sonicated for 10 min.

Capsule characterisation

The size and morphology of samples were observed using an optical image stabilisation camera (SM-N920K, Samsung, Seoul, Korea). The mean particle size was obtained from capsule images by counting more than 30 particles, using Image J software (1.50i) (NIH, Bethesda, MD, USA).

Magnetic performance

The fabricated ION-ACs were dispersed in deionized water, and magnetic fields generating rotating movement at 1500 rpm were applied to the solution, using a magnetic stirrer (CW.001.515, Cowie Technology, Middlesbrough, UK). The rotation of the ION-ACs by the magnetic fields was photographed using a video camera (SM-N920K, Samsung), and the number of revolutions of the capsule was measured from the movies. A video playback program was used to slow down the speed of play (x 0.2). Then, numbers of rotation of each capsules per minute were counted and used as rpm calculation.

Statistical analysis

Statistical analysis was carried out using the SAS program (SAS, Inc., Cary, NC, USA). Capsule diameter was measured and compared at different concentrations, and the reaction by the magnetic field was measured as the number of revolutions of the capsule. These results were analysed to determine whether the capsules showed statistically significant differences. Duncan's least significant difference (LSD) method was used to compare the means of the various properties of ION-ACs. The level of significance was set at $p < 0.05$. Shapiro-Wilk test was used to evaluate the normal distribution of ION-ACs ($p < 0.05$).

Results and Discussion

Effects of alginate and iron oxide concentrations on capsule sizes

The morphological properties of ION-ACs were characterised. Figure 2A shows the morphologies of the fabricated ION-ACs. All ION-ACs showed reactivity to the magnetic forces. Their reactivity increased as the concentration of IONs increased. The ION-ACs showed better responses to the magnetic bars with increasing ION concentrations. The size of ION-ACs was measured by image analyses of OIS images, using Image J software. The effects of alginate concentration and ION concentration on capsule diameter were also examined (Fig. 2B). As a result, the diameter of the capsule was adjusted according to the concentration. Capsule diameters were found to depend on the concentration of alginate and IONs. An increase in both alginate and ION concentrations gradually increased capsule diameter. Although not evaluated in this study, capsule diameters can be adjusted to wider ranges by adjusting the diameters of

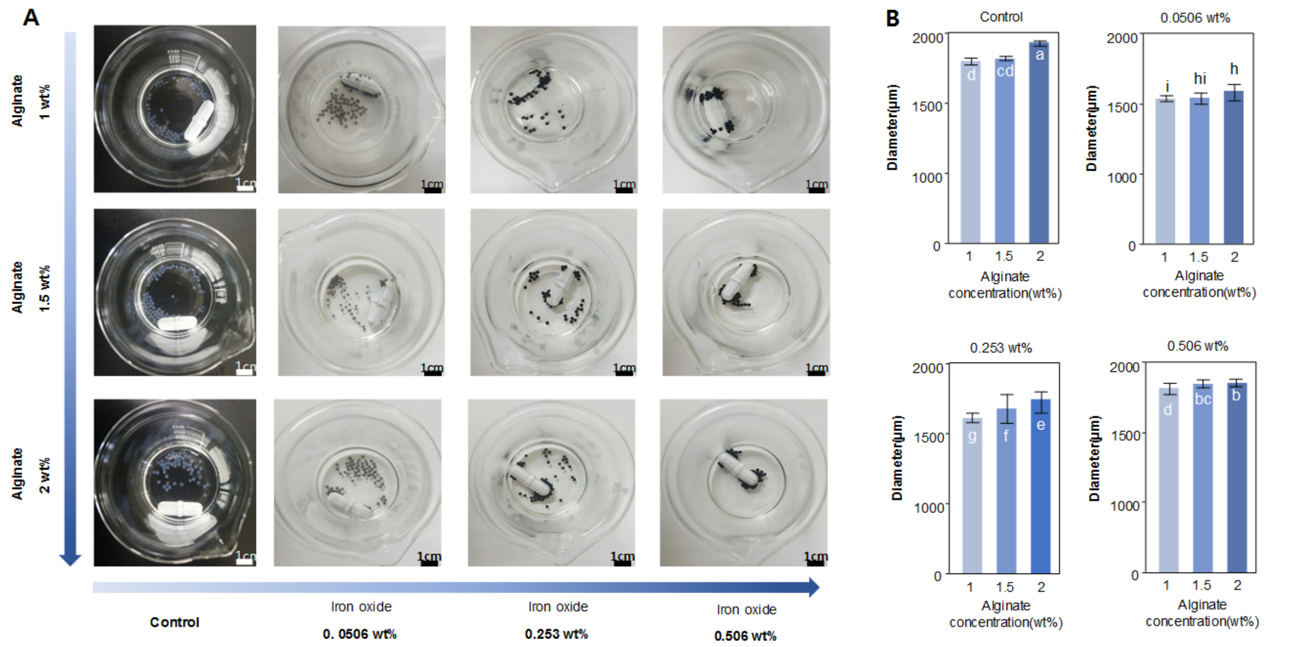

Fig. 2. (A) Sample size and morphology characterised using an OIS camera. As the concentration of iron oxide increased, aggregation occurred in response to the magnetic bar. (B) The effects of alginate and iron oxide concentration on the capsule were examined by graphing. The mean particle size was obtained from capsule images by counting more than 30 particles using Image J software. The alginate capsule diameters increased as iron oxide and alginate concentrations increased. Each of error bars mean standard deviation, and Duncan's LSD method was used for statistical analysis (n = 30, $p < 0.05$). Means with same letters are not significantly different.

the nozzles used and release rates.

The DDSPs have been used to various fields, such as biological and agricultural engineering as well as medicine. One of important factors for DDSPs are the size of capsules, because the size of capsules influences the release of agents such as drugs from them. Furthermore, the usage of capsules affects largely according to the size of system. For example, nano- or micro-scale capsules are appropriate to small-sized systems such as medicine or veterinary fields. In contrast, large-scale capsules are appropriate to big-sized systems such as environmental and agricultural fields. Our study is to reveal the potential of ION-ACs as a carrier for controlled delivery for pesticides or biocontrol agents. Thus large-scale capsules would be appropriate to the purpose of study. The current work is a basic study to confirm the reactivity of the ION-ACs to the magnetic fields. For fulfilling the purpose, large scale capsules as a model platform can achieve the characterization and reactivity to magnetic fields easily. Therefore, the large scale capsules were chosen as an experimental group in this study.

Effects of alginate and iron oxide concentrations on capsule uniformity

Statistical analysis was performed using SAS program. Capsule uniformity changed according to the concentration of iron oxide nanoparticle and alginate. However, at higher concentrations, the results were more unstable. The diameter distribution of capsule was plotted using the SAS program (Fig. 3). Shapiro-Wilk test was conducted to evaluate the normal distribution of experimental groups. The results were expressed in each of distribution charts. P-value represent the possibility of normal distribution, that is, lower p-value than 0.05 is far from normal distribution. Capsule uniformity was greatly affected by the concentrations of iron oxide nanoparticle and alginate. As a result, we evaluated that the ION-ACs which has 1.5 wt% alginate concentration generally showed normal distribution. Among them, 0.0506 and 0.253 wt% ION concentration showed very high normal distribution, that is, high uniformity. Generally, when fabricating capsules or spheres, it is known there is deep correlation between nozzle size and solution viscosity. Because solution viscosity is affected by solute concent-ration, it is able to say that nozzle size and solute concentration decide the capsule size and uniformity. In

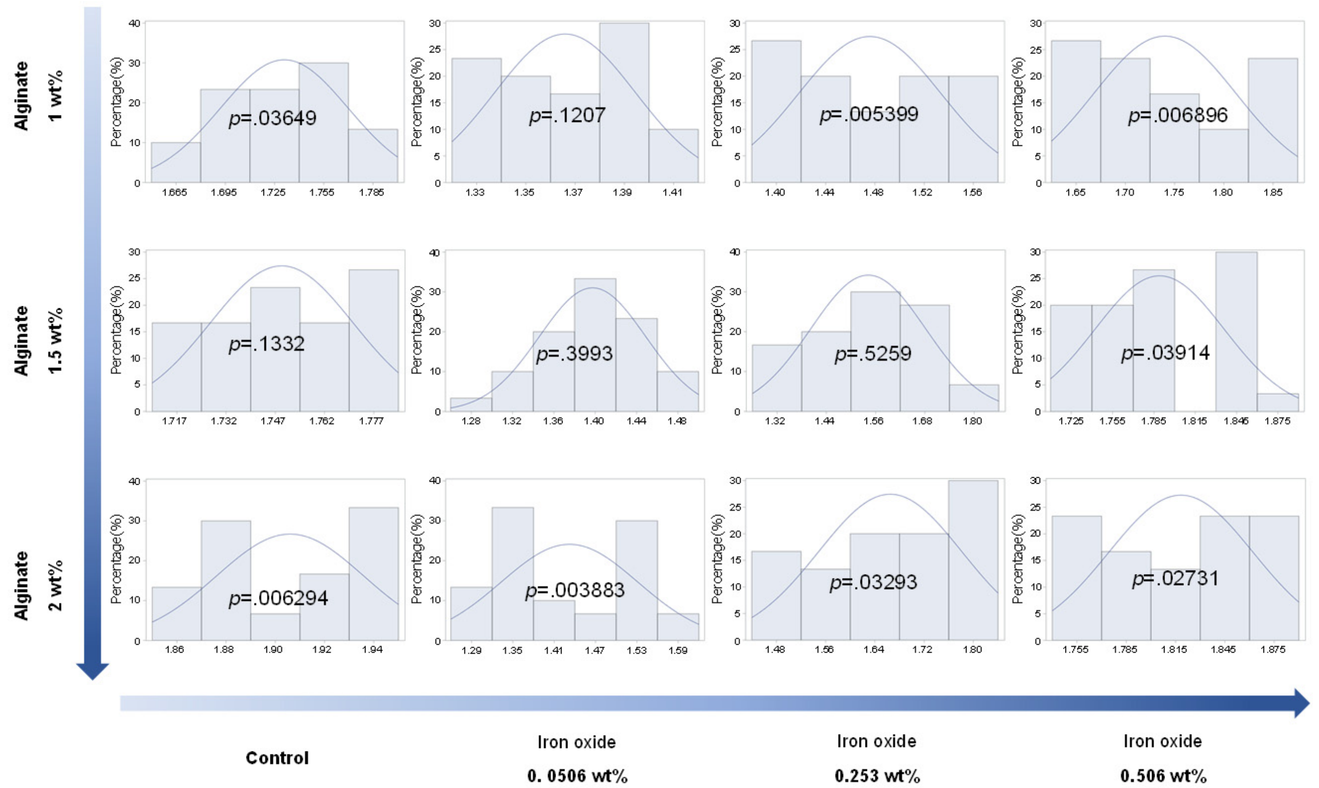

Fig. 3. Analysis of capsule diameter distributions using the SAS program. The results of Shapiro-Wilk test were expressed in each of distribution charts. P-value represent the possibility of normal distribution. In other words, the groups which has p-value lower than 0.05 is far from normal distribution. The analysis showed that the uniformity of the capsule was greatly affected by the concentrations of iron oxide nanoparticles and alginate. Thus, the concentrations of iron oxide nanoparticles and alginate are very important considerations for controlling the sizes of large-scale capsules.

this study, 22-gauge needle was used in capsule fabrication. In this setting, the appropriate concentrations of alginate and IONs seemed to be 1.5 and 0.0506-0.253 wt%, respectively. Thus, the concentrations of iron oxide nanoparticles and alginate are very important for controlling the sizes of large-scale capsules. However, many conditions should be tried to fabricate various sized ION-ACs. Additional studies therefore are still needed to develop a system for producing uniform capsules.

Effect of magnetic fields on movement of ION-ACs

To examine the reactivity of the prepared ION-ACs against magnetic forces, magnetic fields were formed using a magnetic stirrer. The magnetic forces were invoked by the rotation of a stirrer, and capsules were rotated in response to 1500-rpm stirring. The number of magnetic force-reactive capsules was measured and compared (Fig. 4). As the concentration of IONs increased, the number of reactive capsules increased. In contrast, as the concentration of alginate increased, the number of reactive capsules decreased. Therefore, the reactivity of the magnetic field can be controlled by adjusting the concentrations of IONs and alginate. The response to the magnetic field can be applied in a variety of agricultural fields. However, customized capsules should be manufactured by determining the sizes suitable for application in fields. A compromise may be required between capsule uniformity and magnetic reactivity.

These capsules will be useful in a variety of applications that require flexible, on-demand control over the timing and dose of deliveries. Current insect-proof is based on non-targeting method, that is, passive DDS. This method has a serious problem; the rest of unused pesticides can influence the surrounds, resulting in severe environmental pollution. Therefore, the insecticides should be used with appropriate dose and location. The size and concentration of alginate capsule may solve the problem related to dose. In addition, the use of active targeting method can solve the problems related to location. If magnetic fields properly guide the location of ION-ACs, the effects of

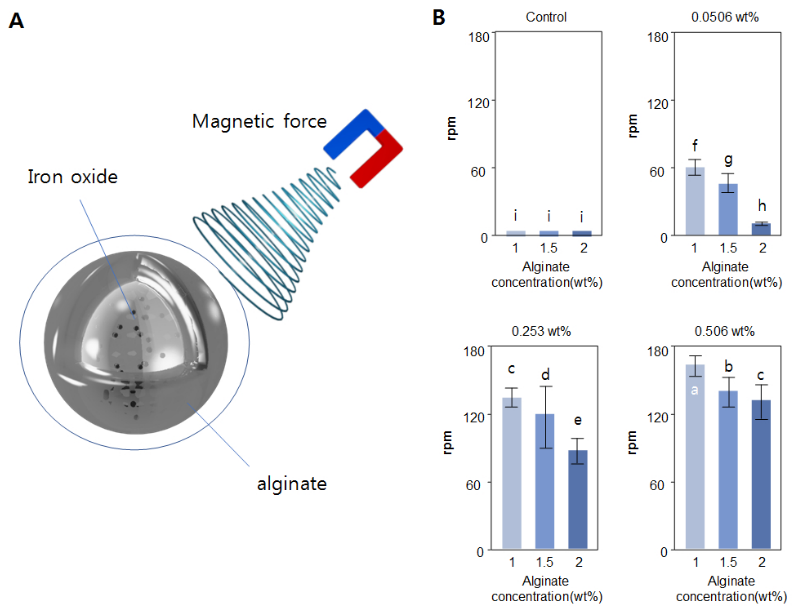

Fig. 4. (A) Schematic of effect of magnetic fields on movement of iron oxide-alginate capsules. Iron oxide in the alginate capsules moved actively with the magnetic field. (B) Controlled rotations of the capsules by the magnetic fields. Using video camera-based photograph images, the number of revolutions of the capsules was measured. The iron oxide-alginate capsules were subjected to magnetic fields at 1500 rpm. Iron oxide-alginate capsules in the magnetic field were rotated at different speeds depending on the iron oxide nanoparticle and alginate concentrations. Each of error bars mean standard deviation, and Duncan's LSD method was used for statistical analysis (n = 30, $p < 0.05$). Means with same letters are not significantly different.

pesticide can be limited in the region of interest. The capsules can overcome limitations related to pesticide use. Because of their high toxicity, existing pesticide treatments have adverse effects over wide areas. The active targeting method using ION-ACs can control the pesticide release into desired areas. Targeted delivery may also be useful as a delivery platform for biocontrol agents (e.g., bacterial antagonists) as an alternative to antibiotics in agriculture applications. The development of portable magnetic field inducer will be able to control selective and targeted pesticides. The researches related to active targeting delivery will be carried out as a new study. Furthermore, the effectiveness of alginate as a drug carrier material is well known. It was used as drug carriers for oral inhalation (Martin et al., 2015), as well as target delivery for cancer therapy (Boekhoven et al., 2015). Jain et al., (2014) well review the case in which alginate was used as drug carriers and the efficacy of alginate based DDS. Consequently, the ION-ACs will accomplish their role as biological or pesticide agents. In this work as a preliminary study, the alginate capsules were used as potential platfroms for the application of pesticides and biological agents. Thus we mainly focused on the magnetic sensitivity of ION-ACs. Although the release property of ION-ACs was not experimentally proven in this study, we have demonstrated that the ION-ACs could be a potential platform for pesticides or biological agents. We will carry out a new study to reveal the ION-ACs as a delivery platform for biological reagents or pesticides.

Conclusions

In this study, the large-scale ION-ACs were developed as a platform for a magnetic field-assisted active targeted delivery system. The capsule sizes were sensitively controlled by altering the concentrations of alginate and IONs. Furthermore, the large-scale capsules were actively moved by magnetic fields. Although further studies are still needed to enhance capsule precision and uniformity of ION-ACs, we have demonstrated that the ION-ACs could be a potential delivery platform for pesticides or biological agents for agricultural applications in various fields.

Conflict of Interest

The authors have no conflicting financial or other interests.

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