Colloids and Surfaces B: Biointerfaces 109 (2013) 301–306

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Facile fabrication of uniform size-controlled microparticles and potentiality for tandem drug delivery system of micro/nanoparticles

Shintaroh Iwanaga[a,b,*], Noriaki Saito[b], Hidetoshi Sanae[c], Makoto Nakamura[b,c,**]

[a] Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Tokyo 153-8904, Japan
[b] Graduate School of Science and Engineering for Research, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan
[c] Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan

ARTICLE INFO

Article history:
Received 12 January 2013
Received in revised form 15 March 2013
Accepted 15 April 2013
Available online 19 April 2013

Keywords:
Inkjet printing technology
Drying process
Micro/nano particle
Drug delivery system

ABSTRACT

This article describes a rapid and facile method for manufacturing various size-controlled gel particles with utilizing inkjet printing technology. Generally, the size of droplets could be controlled by changing nozzle heads of inkjet printer, from which ink solution is ejected. However, this method uses drying process before gelling microparticles, and with that, the size of microparticles was easily controlled by only altering the concentration of ejected solution. When sodium alginate solution with various concentrations was ejected from inkjet printer, we found that the concentration of alginate solution vs. the volume of dried alginate particle showed an almost linear relationship in the concentration range from 0.1 to 3.0%. After dried alginate particles were soaked into calcium chloride solution, the size of microgel beads were obtained almost without increasing their size. The microparticles including various sizes of nanoparticles were easily manufactured by ejecting nanoparticle-dispersed alginate solution. The release of 25-nm sized nanoparticles from alginate microgel beads was finished in a relatively-rapid manner, whereas 100-nm sized nanoparticles were partially released from those ones. Moreover, most of 250-nm sized nanoparticles were not released from alginate microgel beads even after 24-h soaking. This particle fabricating method would enable the tandem drug delivery system with a combination of the release from nano and microparticles, and be expected for the biological and tissue engineering application.

1. Introduction

Functional micro or nano particles are paid attention in the field of medical and biological engineering such as medicine or drug development, not applied only to industrial applications [1–4]. In medical field, some drugs are supported to these fine particles, and they will be used for releasing drugs in vitro or in vivo, which is called Drug Delivery System (DDS). To support the objective drugs on the fine particles, the interaction between them is one of quite important factors. In general, drugs will be supported on carriers with covalent bond [5–7], hydrogen bond, electrostatic interaction or hydrophobic interaction [8–16]. Controlling drug release from carriers will be directly dependent on force of interaction between drugs and carriers [5,15]. Therefore, controlling interactions is one of key factors for drug release.

On another front, drugs supported on carriers would be released from a surface of carriers by diffusion. Thus, speed of release will be dependent on size of particles to a great extent [12,17,18]. If a particle has a perfect spherical shape, as volume of particles is proportional to the cube of particle diameter, surface area is proportional to the square of particle diameter, and thus specific surface area is inversely proportional to particle diameter. Therefore, the smaller size of particles would become, the quicker speed of drug releasing from carriers would become [12,19]. Moreover, a supporting amount of drugs within carrier is one of important factors for time of drug release, and thus, porosity of particles, that is density, would have a big role for the time of drug releasing [20].

The large amounts of fabricating method of microparticles are reported. Roughly, grinding technique with a mill [21–23], spray drying method [24–26] and emulsification method [9,27,28] are quite famous for fabricating microparticles. In the case of grinding with a mill, fabricated microparticles have awkward shapes because of crashing by physical force, and they have different surface area even if size of them is similar. When surface area or porosity of microparticles is different, drug release rate would

* Corresponding author at: Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Tokyo 153-8904, Japan. Tel.: +81 3 5452 6616; fax: +81 3 5452 6617.
** Corresponding author at: Graduate School of Science and Engineering for Research, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan. Tel.: +81 76 445 6884; fax: +81 76 445 6884.
E-mail addresses: iwa@iis.u-tokyo.ac.jp (S. Iwanaga), maknaka@eng.u-toyama.ac.jp (M. Nakamura).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.04.007

dramatically change even though particle size is same [29,30]. This is one of causes for difficulty of controlling drug release. Although numerous microparticles enable to be prepared by spray drying method, it is difficult to fabricate uniform-sized microparticles because irregular droplets in size will be frequently ejected from spray nozzle [25,31]. With emulsification method, quite uniform microparticles could be fabricated by using microfluidics such as T-junction [32–35]. However, it gets difficult to manufacture microparticles with less than several micrometers because particle size was affected by channel size of microfluidics [36,37]. Moreover, it is hard to prepare the same-sized microparticles with general mixing method of emulsification [9,38,39].

In addition, although it is comparatively easy to fabricate microparticles with from tens of μm to hundreds of μm, or nanoparticles with less than hundreds of nm, it is extremely difficult to prepare monodispersed microparticles with several micrometers without any separation [40–42]. Most of particles with tens of μm or hundreds of nm would be applied for DDS in vivo [11,16,40,43]. However, there are some troubles for using microparticles with these sizes in vivo. Microparticles over tens of μm cannot pass through within capillary vessels, and these particles release drugs within large blood vessels because these microvessels have approximately 10 μm [44,45]. On the other hand, although nanoparticles less than hundreds of nm are able to release the drug effectively within capillary vessels, they would accumulate in liver or spleen in a relatively-rapid manner, and be metabolized soon [16].

We had previously reported a fabrication method of uniform-sized microgel particles utilizing with inkjet printing system [46–50]. Inkjet printer enables high resolution printing thanks to ejecting extremely uniform-sized droplets [51]. Thus, we focused on this property of inkjet printing system to be applied for fabricating monodisperse microparticles. In this article, we described a fabricating method for uniform microparticles with our inkjet printing system and drying process (Fig. 1), and a release property of nanoparticles from fabricated alginate microgel particles. We showed that different uniform-sized microparticles were easily fabricated with changing alginate concentration. And we also confirmed that fabricated alginate microgel particles had different release ratio with various size of nanoparticles. Drying process would be expected to enable microparticles to include involatile drugs inside without loss of drugs. Moreover, it is possible to support and release not only drugs but also nanoparticles. If the nanoparticles supported drugs would be loaded to microparticles, Tandem Drug Delivery System (T-DDS) would be expected to be used for medical and biological application, whose concept is double release of drugs from nano and microparticles. We hope that our technology reported in this article suggests new approach to medical and biological engineering as T-DDS.

2. Materials and methods

2.1. Materials

Oral medicine grade of sodium alginate (Alroid G, Kyosei Pharmaceutical Corporation; Hokkaido, Japan) was used for fabricating microparticles. Two % of calcium chloride solution was purchased from Otsuka Pharmaceutical Corporation (Tokyo, Japan). FITC-labeled nanoparticles with diameters of 25, 100 and 250 nm (micromer®-greenF plain) were purchased from Corefront Corporation (Tokyo, Japan).

2.2. Inkjet printing system

In our laboratory, we have developed 3D Bioprinter system, and succeeded to fabricate microgel beads with uniform size, 2D gel sheets and 3D gel structures with ejecting polymer materials such as alginate or fibrinogen [46–50]. Previously, microgel beads were fabricated by gelling ejected droplets directly. In this time, we used commercial piezoelectric inkjet printing system (Cluster Technology Corporation; Osaka, Japan), which is possible to discharge viscous solution, and developed an original drying equipment. The nozzle head with 15-μm droplet size was consistently used in this article (Fig. 2).

2.3. Preparation of various sized alginate microparticles

Sodium alginate was diluted with ultrapure water at concentration of 0.1, 0.5, 0.8, 1.0, 2.0 and 3.0% (w/v). After these solutions were filtered with 0.2-μm filter, every solution was ejected by inkjet printer at 1000 Hz. Ejected droplets were dried naturally within a drying equipment, and dried microparticles were obtained.

Nanoparticles were dissolved in filtrated alginate solution (0.8%) with final concentration of 1.0 mg/mL. Alginate microgel beads containing nanoparticles were fabricated by being soaking ejected alginate microparticles with 2.0% of $CaCl_2$ solution, respectively.

2.4. Observation of alginate microparticles and microgel beads

Fabricated alginate microparticles were observed with phase contrast microscopy (CKX41 with DP71, Olympus Corporation; Tokyo, Japan), and low-vacuum scanning electron microscopy (Miniscope TM-1000, Hitachi; Tokyo, Japan) without vapor deposition. Size of alginate microparticles was measured from microscopic images with using image analysis software (Vision Builder, National Instruments Japan Corporation; Tokyo, Japan). Fluorescein images were obtained with fluorescent microscopy (Biozero BZ-8000, Keyence; Osaka, Japan).

2.5. Release of nanoparticles from fabricated microparticles

At first, 10 mg of alginate microparticles including nanoparticles were soaked into 100 μL of distilled water, and fluorescent intensity of solutions at 520 nm was measured with fluorescence spectrometer (NanoDrop 3300, Thermo Scientific; MA, USA). And then, 10 mg of alginate microparticles containing nanoparticles were soaked into 100 μL of $CaCl_2$ solution (2.0%) for 1, 2, 3, 5, 8, 12 and 24 h. After soaking for specified time, alginate microgel beads were spun out by centrifugation at 1000 × *g*, and fluorescent intensity of supernatant was measured. The ratios of these fluorescent intensities were calculated as release ratios of nanoparticles.

3. Results and discussion

We have previously shown that uniform alginate microgel beads could be easily manufactured with our original 3D bioprinter [46–50]. In previous report, controlling size of microgel beads required to change nozzle heads. Therefore, we only showed results of two sizes of uniform alginate microgel beads with using two types of nozzle head [47]. In this article, we successfully fabricated uniform alginate microparticles with various sizes, which can be controlled by changing concentration of ejected solution with using a same nozzle head (Fig. 3). On the other hand, sizes of alginate microgel beads, which were directly ejected into $CaCl_2$ solution with same concentration conditions and a same nozzle head, had an almost similar diameter of approximately 13 μm (supplemental data S-1). SEM images showed that fabricated alginate microparticles kept their perfect spherical shapes, and had no damages by drying process (Fig. 4). Moreover, alginate microgel particles were easily obtained by soaking microparticles in calcium chloride solution. Size of microparticles and microgel particles were almost same after being soaked for 24 h (Supplemental data S-2).