Materials Science & Engineering C 81 (2017) 571–579

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Characterization and degradation study of chitosan-siloxane hybrid microspheres synthesized using a microfluidic approach

Susana Cruz-Neves[a], Yuki Shirosaki[b,*], Toshiki Miyazaki[a], Satoshi Hayakawa[c]

[a] Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0196, Japan
[b] Graduate School of Engineering, Kyushu Institute of Technology, 1-1 Sensui-cho, Tobata-ku, Kitakyushu, Fukuoka 804-8550, Japan
[c] Graduate School of Natural Science and Engineering, Okayama University, 3-1-1 Tsushima, Kita-ku, Okayama 700-8530, Japan

ARTICLE INFO

Keywords:
Chitosan-siloxane hybrid
Microspheres
Microfluidic system
Degradation

ABSTRACT

Chitosan microspheres can address challenges associated with poor bioavailability or unsustained drug release when used as drug delivery systems thanks to their mucoadhesiveness, which allows the drug dosage to be retained in the gastrointestinal track for extended periods. Chitosan-3-glycidoxypropyltrimethoxysilane-β-glycerophosphate (chitosan-GPTMS-β-GP) hybrid microspheres were synthetized through sol-gel processing using a microfluidic approach. Microspheres with uniform spherical shapes and sizes of approximately 650 μm were obtained. The microstructures of the microspheres consisted of four different siloxane structures. The degradation behaviors of the hybrid microspheres were examined under acidic pH conditions mimicking those found in the gastrointestinal track. Microspheres with different GPTMS molar ratios were incubated under several pH conditions for 2 weeks. The microspheres incubated at pH 7.4 extended the lowest weight loss (27%–32%), whereas those incubated at pH 1.7 and pH 5.4 showed greater weight losses of 43–59% and 69–77%, respectively. The inhibition of the degradation at low pH was dependent on the siloxane network in the chitosan matrix. Phosphate was mostly released in early stages, and the released amount of silicon was dependent on the composition. GPTMS was released with a chitosan chain via the hydrolysis of a chitosan molecule. The pelargonidin was incorporated in the microspheres and the slow releasing was observed at acidic condition. The resistance of these hybrid microspheres to low-pH conditions for longer than a full digestion cycle is promising for gastrointestinal drug delivery applications.

1. Introduction

Biodegradable microspheres have presented several advantages compared with conventional delivery systems. For instance, they offer a more sustained and controlled release of drugs over time, thereby reducing the need for multiple doses and allowing the release of insoluble drugs [1,2]. Moreover, both synthetic and natural polymers are normally cleaved into biocompatible byproducts, resulting in no bodily harm [3]. Even though microspheres are small, they have large surface area to volume ratios [1,2]. The synthesis of microspheres with good size uniformity has been reported when using a microfluidic approach [4,5]. This method is simple and has the advantage of using small solution volumes.

Organic-inorganic hybrids that involve natural biodegradable polymers such as chitosan, are studied by researchers in the form of scaffolds, films, hydrogels and spherical particles. In particular, the organic-inorganic hybrid hydrogels are suitable candidates as drug carriers for the controlled releasing [6–8]. Chitosan is a natural polymer that consists of polysaccharide chains. The biocompatible, bioresorbable, mucoadhesive, non-toxic and non-antigenic [9–11] properties of chitosan make it a suitable candidate for medical applications. Additionally, chitosan-mediated systems can considerably improve the bioavailability across the epithelial layer of the oral cavity [12] and gastrointestinal track [13]. However, this polymer lacks mechanical endurance and a controlled degradation rate [1]. To overcome these disadvantages, non-toxic crosslinking agents are required. Shirosaki et al. assessed the cytotoxicity of several crosslinking agents for chitosan, 3-glycidoxypropyltrimethoxysilane (GPTMS) monomer proved to be less cytotoxic than glutaraldehyde for MG63 human osteosarcoma cells [14]. The chitosan-GPTMS hybrids are degradable with a controllable degradation rate because the crosslinking ratio and formation of a Si—O—Si network. The grafting of Si—OH groups or Si—O—Si networks into the polymer also induces bioactive properties. However, this precursor requires pH neutralization for application in the human body. β-glycerophosphate (β-GP) is a weak base and one of the osteogenic supplements used for the cultures of human bone marrow mesenchymal

* Corresponding author.
E-mail address: yukis@che.kyutech.ac.jp (Y. Shirosaki).

http://dx.doi.org/10.1016/j.msec.2017.08.035
Received 17 May 2017; Received in revised form 20 July 2017; Accepted 10 August 2017
Available online 12 August 2017

stem cells. Chenite et al. [15] prepared injectable hydrogels using chitosan–β-GP and reported their good cartilage tissue regeneration. Shirosaki et al. [16] reported that MG63 cells exhibited good cytocompatibility on chitosan-GPTMS-β-GP hydrogels compared with the chitosan-β-GP system.

Assessment of the degradation behavior of a biomaterial is essential. The human body presents a diverse range of pH levels. The normal range pH reference of arterial blood is 7.35 to 7.45 [17–19]. In contrast, the pH in the stomach of healthy people in the second and third postprandial period varied from 1.7 to 4.3 [20]. Furthermore, the duodenal pH decreased from 6.1 to 5.4 when a meal is ingested [21].

In the present study, a microfluidic approach was used to synthesize chitosan-siloxane-β-GP hybrid microspheres. Their structural composition was analyzed and the degradation behaviors were examined under several pH conditions; in addition, their stability and elemental release from the microspheres matrix were evaluated. The microspheres also incorporated pelargonidin as a drug model and their releasing behavior was tested. Pelargonidin is one of the anthocyanins, which have a role on the protection against a myriad of human diseases (prevention of cardiovascular and neuronal diseases, diabetes, etc.) due to their antioxidant properties [22,23].

2. Materials and methods

2.1. Preparation of chitosan-siloxane hybrid microspheres

Chitosan (high molecular weight, DA > 75%, Sigma-Aldrich®) was dissolved in 0.1 M hydrochloric acid to attain a concentration of 2% (w/v). The chitosan solution was mixed in a planetary centrifugal mixer (ARE-310, Thinky, USA) at room temperature, followed by autoclaving at 121 °C for 20 min and filtering (polyethersulfone, 0.22 μm pore) to obtain a homogeneous solution. An appropriate amount of GPTMS (97%, Alfa Aesar) was added to the chitosan solution, and the mixture was stirred at room temperature for 2 h. For 10 mL of chitosan-GPTMS, 3.25 mL of 2.5 M β-GP (pH 9.6, Sigma) was added at 0 °C for 10 min to neutralize the precursor sol pH to 7. The microfluidic system consisted of two syringe pumps (YMC KeyChem), one containing the hybrid solution and the other containing the oil solution. Both syringes were connected to a Y-shaped microchannel (Fig. 1). The precursor sol was used as the dispersed phase, and an oily solution of 4% (w/v) span/squalene (Sigma-Aldrich®) was used as the continuous phase. When both solutions fused in the Y microchannel, the microspheres were formed. The optimized conditions used in the microfluidic system are listed in Table 1. Then the microdrops were gelated at 60 °C for 1 h in 4% (w/v) span/squalene oil solution to obtain the wet microspheres. The wet microspheres were rinsed with a series of graded ethanol dilutions (100%, 90%, 80%, 70%, 60%, 50% and 25%) to remove the oil. These microspheres were intended to be used for future biological characterization, therefore the microspheres were sterilized by autoclaving at 121 °C for 20 min in distilled water. To incorporate pelargonidin, pelargonidin chloride (Sigma-Aldrich®) was dissolved into 0.1 M HCl and then added into chitosan-GPTMS solution to achieve the concentration of 1 mg/mL. After mixing for 20 min at room temperature, the microspheres with pelargonidin were prepared following the above procedure.

Table 1
Starting compositions of the hybrid microspheres and optimized parameters used in the microfluidic system.

Sample	Molar ratio		Flow rate (mL/min)		Channel (mm)		Outlet (mm)
	Chitosan	GPTMS	Oil	Sol	Depth	Width	
ChG10	1.0	1.0	0.100	0.005	1.0	0.8	1.0
ChG15	1.0	1.5	0.100	0.005	1.0	0.8	1.0

2.2. Structural characterization of the hybrid microspheres

The size and shapes of the microspheres were examined under a bright-field microscope (IX73, Olympus, Japan). ImageJ v1.48 software (National Institutes of Health, USA) was utilized to measure the diameter of > 100 microspheres. The surface morphology of the microspheres was examined using SEM (JMS-6010 PLUS/LA, JEOL, Japan) equipped with an energy dispersive X-ray spectrometry (EDS) to detect the elements present in the microspheres at 15 kV operating voltage and a working distance of 10 mm. The samples were coated with a 15 nm thick layer of Pt/Pd using a magnetron-sputter coater (MSP-1S Magnetron Sputter, Vacuum Device Inc., Japan). The surface charge of the microspheres in phosphate buffered saline (PBS, pH 7.4, Gibco) and in distilled water were measured via Zeta Potential (ELS-Z, Photal Otsuka Electronics, Japan) using the rectangular cell to determine the surface charge and potential stability. Solid-State ^{13}C, ^{29}Si, ^{31}P, and ^{1}H NMR measurements were performed on an Agilent DDS 500 MHz NMR spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA) operating at 11.7 Tesla. A zirconia rotor with a diameter of 3.2 mm was used with an Agilent HXY T3-MAS probe. The rotor spinning frequency for magic angle spinning (MAS) was controlled to be 15 kHz. $^{1}H \rightarrow {}^{13}C$ cross-polarization (CP)-MAS NMR experiments were performed with contact time of 500 μs and recycle delay of 10 s, where the signals of 3700 and 5400 pulses were accumulated for ChG15 and ChG10, respectively, with adamantane ($C_{10}H_{16}$) as the external reference (38.52 ppm vs. 0 ppm TMS). In addition, ^{1}H MAS NMR spectra were taken at 499.8 MHz with a 1.15 μs pulse length (pulse angle, π/4) and 5 second recycle delays, where the signals of 8 pulses were accumulated with adamantane ($C_{10}H_{16}$) as the external reference (1.91 ppm vs. 0 ppm TMS). Furthermore, $^{1}H \rightarrow {}^{29}Si$ CP-MAS NMR experiments were performed with contact time of 5 ms and recycle delay of 5 s, where the signals of 40,580 and 79,460 pulses were accumulated for ChG15 and ChG10, respectively, with polydimethylsilane (PDMS) as the external reference (− 34.44 ppm vs. 0 ppm TMS). Direct polarization ^{31}P MAS NMR spectra were taken at 202.3 MHz where a 1.4 μs pulse length (π/4-pulse angle) and 120 s recycle delays using $NH_4H_2PO_4$ as the external

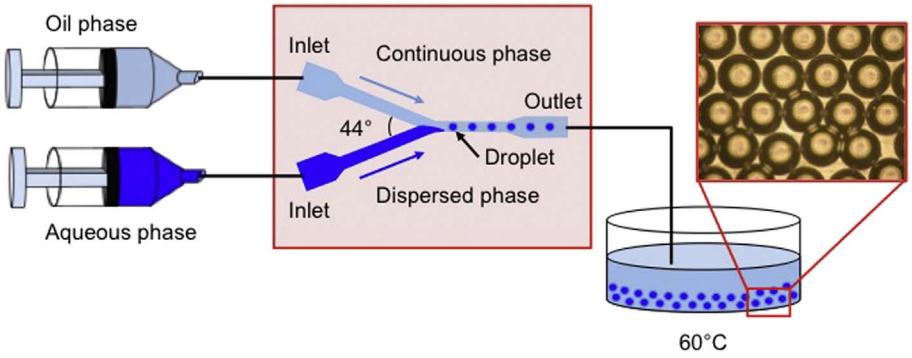

Fig. 1. Schematic representation of the shape and dimensions of the microchannel and microspheres before the gelation.