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Coating a shell on alginate microsphere by liquid phase deposition

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ABSTRACT

A novel core-shell structure of alginate microspheres, was prepared via electrospray and chemical liquid phase deposition successfully. The shell structure could be seen obviously under the optical microscope. The stromal cell-derived factor-1 (SDF-1) was loaded into the microspheres and the homing effect of pre-osteoblasts was tested through a chemotaxis assay with transwell system. This is a promising material for achieving the encapsulation of cytokines and any other hydrophilic drug.

1. Introduction

Microsphere technology is widely applied in the field of drug release and tissue engineering nowadays [1–3]. Alginate, a natural polysaccharide which has similar structure with glycosaminoglycan in the extracellular matrix are receiving considerable attentions for microsphere technology due to its good biocompatibility. By reacting with divalent metal cations such as calcium and copper, alginate can form a gel structure quickly with suitable mechanical properties for biomedical applications. This crosslinking process can happen in a mild condition without adding toxic chemical or treating with high temperature. This is important for the loading of proteins or peptides into the microspheres because of their fragile structures. In addition, the alginate gel formed with calcium has a three-dimensional network porous structure which is beneficial for holding water molecule and providing an aquatic environment for maintaining the activity of biological macromolecular drugs such as proteins. Therefore, alginate based microspheres for proteins loading are prepared by various methods, such as emulsion method [4,5], microfluidics method [6] and electrospray method [7], etc.

In order to protect the drugs better and mitigate drug burst release, core-shell microspheres is a good choice [8]. In this study, a shell was coated on the surface of alginate microspheres to form a core-shell structure by electrospray and chemical liquid phase deposition. The aquatic environment could be kept during preparing process by using this approach. Stromal cell-derived factor-1 (SDF-1) was loaded in the microspheres. Pre-osteoblasts were used to test the homing effect of the microspheres with SDF-1.

2. Materials and methods

2.1. Materials

Alginic acid sodium salt (AS, from brown algae, bioreagent), calcium chloride ($CaCl_2$, anhydrous, bioreagent), tween 80 (premium), disodium hydrogen phosphate (Na_2HPO_4, bioreagent), sodium chloride (NaCl, bioreagent), formalin solution (ACS reagent), crystal violet solution (1% aqueous solution) and acetic acid (ACS reagent, ≥99.7%) were purchased from Sigma-Aldrich. Stromal cell-derived factor-1 (SDF-1) was purchased from GenScript.

2.2. Preparation of core-shell microspheres

The preparation process was shown in Fig. 1. First, tween 80 was dissolved in deionized water with a concentration of 0.1% by stirring for 10 min. Then, AS was added into the above solution with a concentration of 0.015g/mL and stirred for 6 h. The solution was introduced into a syringe with a stainless steel (flat) blunt-ended needle that served as a charged spinneret. A power supper (Tianjin Dongwen High Voltage Power supply Co., Ltd., China) applied 0–30 kV potential.

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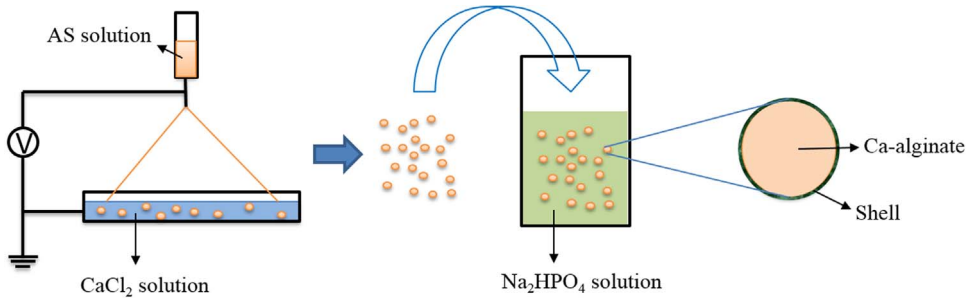

Fig. 1. Schematic of fabrication processes of core-shell alginate microspheres.

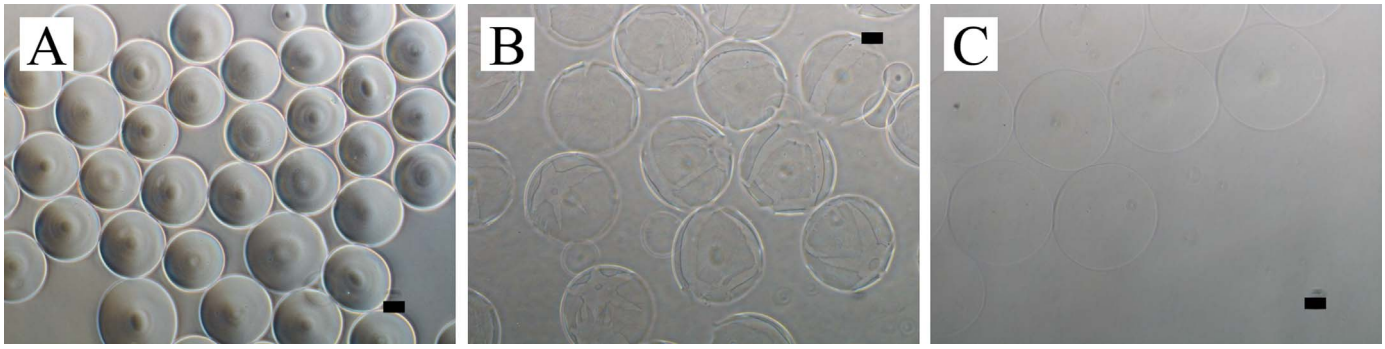

Fig. 2. Optical microscope images of the core-shell microspheres (A), core-shell microspheres treated with Na_2HPO_4 (B) and core-shell microspheres treated with NaCl (C). Scale bar=100 μm.

As a collector, a grounding dish filled by $CaCl_2$ aqueous solution was placed at 10 cm below the nozzle. AS microdroplets would be jetted toward $CaCl_2$ aqueous solution under 29 kV and became microspheres after crosslinking by Ca^{2+}. The apparatus for the electrospray experiment was similar to previous report [9]. After that, the products were collected by centrifugation at 1300 rpm speed for 5 min and washed with water for three time. Products was dispersed in 0.1% Na_2HPO_4 aqueous solution and shaken gently for 5 min. The final products were collect by centrifugation at 1300 rpm speed for 5 min and washed with water for three time.

2.3. Preparation of SDF-1 loaded microspheres

First SDF-1 was dissolved in the AS solution with a concentration of 3.33 μg/mL with stirring for one hour. Then the solution processed the same electrospray and immersed in Na_2HPO_4 solution procedure described above. The products were collected by centrifugation and denoted as SDF-1 loaded core-shell microspheres.

2.4. Chemotaxis assay

Pre-osteoblast cell line, MC3T3-E1 (from ATCC), was used to test the homing effect of the microspheres with transwell system (Corning, pore size:5 μm, insert diameter: 6.5 mm, for 24 well plate) qualitatively. The complete medium was the DMEM (Life Technologies) containing 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% penicillin / streptomycin (Life Technologies). Two groups were established: control group (only medium in basolateral chamber) and experimental group (100 μL suspension of SDF-1 loaded microspheres with complete medium in basolateral chamber). Each group was made in triplicate. All of the apical chambers were added 100 μL cell suspension (39,000 cells/mL). The complete medium would be added into the each basolateral chamber up to 600 μL. The cells were cultured for 24 h (37 °C, 5% CO_2). Then the cells in the apical chamber were expunged by cotton swabs. The cells on the membrane on the basolateral side were washed by PBS three times and fixed by 4% formalin solution for 10 min. The cells were washed by deionized water for three times. The crystal violet solution (1%) was diluted to 0.1% and used to stain the cells for 15 min. The cells were dried at room temperature for 15 min after washing by deionized water for three times and ready to observe. Then 33% acetic acid was used to wash the crystal violet away from cells and was evaluated by a microplate reader under 595 nm. The data were analysed with SPSS 19.0.

3. Results and discussions

3.1. Core-shell structure

$CaHPO_4$ has a small solubility product constant (K_{sp}=1×10^{-7}) so that its solubility in water is quite small. While Ca-Alginate is formed by the relative weak coordinate bond among Ca^{2+}, 5-COO^- group and 2-OH group in alginate. The HPO_4^{2-} ions in the solution would competitively combine with the Ca^{2+} ions in microspheres to form $CaHPO_4$ precipitation on the surface of microspheres when the microspheres were immersed into Na_2HPO_4 aqueous solution. Therefore, a shell should be formed on the surface of microspheres.

Fig. 2 shows the optical microscope images (Eclipse TS100, Nikon) of microspheres. Approximately uniformed microspheres with average diameter of about 300 μm could be observed (Fig. 2A) but the core-shell structure could not be observed obviously. Fig. 2B shows the microspheres after immersing in the Na_2HPO_4 aqueous solution for ten more minutes. The size of microspheres obviously increases and a chapped shell could be observed obviously after the bursting of microspheres. The swelling behaviour is due to the exchanging between Ca^{2+} ions in the microsphere and Na^+ ions in the solution. The shell is from the liquid phase deposition and is too thin to observe in Fig. 2A.

If AS microspheres were immersed in the NaCl aqueous solution which had a same concentration of Na^+ ions, a swelling behaviour can be also observed without the shell structure (Fig. 2C). These results indicated the formation of shell on the surface of the microspheres by liquid phase deposition.