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Facile development of a hollow composite microsphere with porous surface for cell delivery

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ABSTRACT

Hollow polymer microspheres have attracted great attention in tissue regeneration as cell carrier. In this study, hollow poly(lactic-co-glycolic acid)/hydroxyapatite/calcium carbonate (PLGA/HA/CC) microspheres with porous surface were successfully fabricated using bioactive minerals as in situ pore-forming agents. No templates were introduced in this facile approach. The capability of HA to hold water contributed to the formation of the porous surface and the hollow structure. Furthermore, the gas-releasing effect originating from CC decomposition facilitated the generation of opened superficial macropores. Porcine mesenchymal stem cells (PMSCs) were cultured on the microspheres and the results highlighted a better proliferation on microspheres with opened macropores. Therefore, this subtly designed microsphere shows a potential application in cell delivery.

1. Introduction

Porous structured polymer microspheres have received much attention in cell culture and delivery for tissue regeneration [1]. One important category is hollow microsphere, which offers inherent advantages such as low density, internal encapsulation etc [2,3]. When applied as the bone defect filler, the structural strength and stability of the microspheres are demanded. Therefore, hollow microsphere composed of synthetic polymer is preferred, given that synthetic polymers are mechanically advantageous over natural ones [4]. However, the capability of synthetic polymers to aid bone repair is usually limited due to issues like hydrophobicity and lack of osteoconductivity. It is well known that bioactive minerals have excellent osteoconductivity and can promote bone repair [5]. Hence, incorporating bioactive minerals into synthetic polymer is believed to be an effective approach to improve the outcome of bone repair.

So far, hollow microspheres composed of synthetic polymers are mainly fabricated through various template techniques [6]. Template technique often requires extra later procedure to remove the template since most of the templates are biologically incompatible. Although several studies have exploited other techniques to obtain hollow microspheres, either the adopted devices or procedures are somehow complicated [7,8]. In addition, the produced microspheres generally have smooth surface, which is not suitable for hosting a large number of cells. In terms of composition, the very majority of hollow microspheres consist of just synthetic polymer component, which, as mentioned above, is not preferred in bone repair.

Here we tried to combine PLGA polymer with HA and CC minerals to develop a hollow composite microsphere with a porous surface. PLGA is an FDA-approved synthetic polymer having good biocompatibility and biodegradability [9]. Bioactive minerals of HA and CC have been well proven to be osteoconductive and can promote in vivo bone regeneration [10,11]. In this work, HA and CC were expected to act as the in situ pore-forming agents, which contributed to the formation of the superficially porous and internally hollow structure. This approach circumvented the use of any additional template. The formation mechanism of this microsphere was explored in detail. Finally, PMSCs were cultured on the microsphere to investigate the effect of the surface macropores on the potential of the microsphere as cell carrier.

2. Materials and methods

A facile approach based on emulsion solvent evaporation technique was adopted to prepare hollow composite microspheres. Poly(lactic-co-glycolic acid) (PLGA, 50/50, M_w=31 kDa, Daigang Biomaterials, China) was first dissolved in dichloromethane (10%, w/v), followed by the addition of HA (1.2 μm) and CC (23.4 μm) particles. The mixture was stirred completely to form a well dispersed slurry. Subsequently, the slurry was dropped into 500 mL of 0.6% (w/v) poly(vinyl alcohol) (PVA, Aladdin Chemistry, China) solution containing gluconic acid lactone (GDL, Aladdin

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Chemistry, China) and stirred at 300 rpm for 12 h to evaporate the organic solvent. The solidified PLGA/HA/CC microspheres were collected, washed and lyophilized.

The morphology of microspheres was observed by scanning electron microscopy (SEM, Quanta 200, FEI, Netherland). To view the internal structure, microspheres were suspended in a solution of 20% gelatin and 5% glycerol at 37 °C for 3 h. Then the suspension was frozen and sectioned at −20 °C. The intact and cross-sectioned samples were sputter-coated with gold before observation.

Porcine synovium-derived mesenchymal stem cells (PMSCs) were isolated and propagated in high glucose DMEM supplemented with 10% (v/v) FBS. Microspheres were placed into 24-well plates that were preliminarily coated with 1.5% (w/v) agarose. The microspheres were sterilized by soaking in 75% ethanol for 1 h, rinsed with PBS for three times and pre-wetted in culture medium for another 12 h. Then 1000 μL of cell suspension (1×10^5 cells/mL) was seeded into each well and incubated under a humidified atmosphere of 5% CO_2 at 37 °C. Cell proliferation was evaluated by Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan) according to the protocol provided by the manufacturer. The spectrophotometric absorbance at 450 nm was recorded by Thermo 3001 microplate reader (Thermo, America) ($n=4$).

3. Results and discussion

The morphologies of PLGA/HA/CC microsphere were shown in Fig. 1. It was noted that there were numerous opened macropores within the size of 1–10 μm distributed around the surface (Fig. 1a and b). The cross-section image showed a hollow structure and imbedded mineral particles (Fig. 1c). Nanoscale HA has been proven to be able to hold water and form cone-like pores on the surface of PLGA/HA microsphere in double emulsion preparation [12]. CC particles were also reported to produce macropores in PLGA microspheres [13,14]. Here, the microscale HA particles located near the surface were supposed to attract water from aqueous phase and form a water/solid/oil mixed zone. As the organic solvent evaporated and oil droplets solidified, the water was retained and left the macropores after drying. GDL tends to hydrolyze in water and produce acid, reducing the pH value. So the presence of GDL in aqueous phase would cause the decomposition of exposed CC particles in oil phase and the subsequent release of CO_2 (Fig. 2). The gas-releasing effect of CC was believed to benefit the formation of opened superficial macropores. To confirm this, PLGA/HA microsphere without CC was prepared. Although there were still quantities of macropores existing on the surface, the majority of the macropores were closed (Fig. 1d). It was worth mentioning that no exposed HA particles were observed on the surface (Fig. 1d), suggesting that GDL might also cause the eventual dissolution of exposed HA Fig. 2.

It was speculated that the hollow structure of the PLGA/HA/CC microsphere was also derived from the water attracted by HA. In the emulsification process, the oil phase was first deformed and broken into smaller droplets by shear force resulting from stirring (Fig. 3a) [15]. The nascent droplets would cause Laplace pressure due to the difference in pressure between the convex and the concave of droplet [16]. Water might be encapsulated when the droplets transformed into a spherical shape to obtain a thermodynamically stable state (Fig. 3b and c). The water attracted in the initial phase of emulsion might coagulate with each other and formed an inner aqueous phase, which eventually became the hollow structure after drying (Fig. 3b–d). The fact that PLGA/HA microsphere also had the hollow structure further confirmed this speculation (Fig. 1e). PLGA/CC microsphere was also prepared to verify if CC particles played a part in the formation of hollow structure. It was seen that PLGA/CC microsphere was solid with CC particles embedded inside (Fig. 1f). This meant that CC, either as water-attracting or gas-releasing source, was not involved in the formation of hollow structure.

PMSCs were cultured on microspheres to evaluate the potential of the PLGA/HA/CC in cell delivery and more importantly, investigate the role of opened superficial macropores. Cell proliferation on PLGA/HA/CC and PLGA/HA (as control) microsphere was analyzed by CCK-8 after 1, 4 and 6 days of culture. As shown in Fig. 4, the absorbance of the 4th and 6th day in both microspheres was obviously higher than

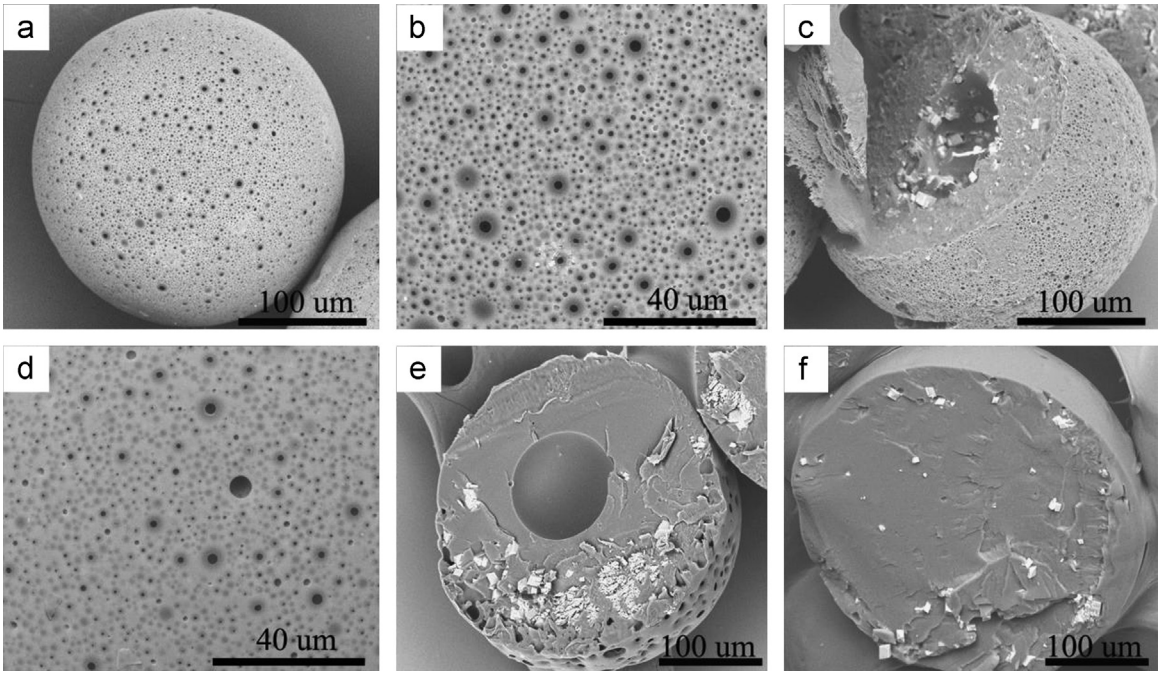

Fig. 1. SEM images of studied microspheres. Morphologies (a and b) and cross-section (c) of PLGA/HA/CC microsphere; morphology (d) and cross-section (e) of PLGA/HA microsphere; cross-section (f) of PLGA/CC microsphere.