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Bioactive apatite incorporated alginate microspheres with sustained drug-delivery for bone regeneration application

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ABSTRACT

The strontium-substituted hydroxyapatite microspheres (SrHA) incorporated alginate composite microspheres (SrHA/Alginate) were prepared via adding SrHA/alginate suspension dropwise into calcium chloride solution, in which the gel beads were formed by means of crosslinking reaction. The structure, morphology and in vitro bioactivity of the composite microspheres were studied by using XRD, SEM and EDS methods. The biological behaviors were characterized and analyzed through inductively coupled plasma optical emission spectroscopy (ICP-OES), CCK-8, confocal laser microscope and ALP activity evaluations. The experimental results indicated that the synthetic SrHA/Alginate showed similar morphology to the well-known alginate microspheres (Alginate) and both of them possessed a great in vitro bioactivity. Compared with the control Alginate, the SrHA/Alginate enhanced MC3T3-E1 cell proliferation and ALP activity by releasing osteoinductive and osteogenic Sr ions. Furthermore, vancomycin was used as a model drug to investigate the drug release behaviors of the SrHA/Alginate, Alginate and SrHA. The results suggested that the SrHA/Alginate had a highest drug-loading efficiency and best controlled drug release properties. Additionally, the SrHA/Alginate was demonstrated to be pH-sensitive as well. The increase of the pH value in phosphate buffer solution (PBS) accelerated the vancomycin release. Accordingly, the multifunctional SrHA/Alginate can be applied in the field of bioactive drug carriers and bone filling materials.

1. Introduction

Hydroxyapatite (HA) is the main mineral composition of nature bones and teeth, and has been widely used in the biomedical applications due to its favorable bioactivity and biocompatibility. The nano-HA has the advantages of non-immunogenicity, high osteoconductivity and/or osteoinductivity [1], which enables HA to be a promising material for bone repair and bone regeneration. Furthermore, HA also has a bright prospect in drug delivery field attributed to its characteristic pore structure, high surface area and chemical stability, etc. [2]. However, HA lacks the ability to stimulate osteogenetic process and the drug loaded in HA would quick release at the early stage of release process [3,4].

It was reported that HA could be substituted by cations, such as monovalent (Ag^{+}), divalent (Zn^{2+}, Cu^{2+}, Mg^{2+}, Sr^{2+}) and trivalent (Al^{3+}, Fe^{3+}) [5–8]. These ion-doped HA can stimulate osteoblast proliferation and differentiation and bone formation [9]. Especially, the strontium (Sr) is considered as a bone-seeking element that presents a beneficial effect on bone growth [10,11]. The Sr shares similar chemical, physical and biological properties with calcium (Ca), which thus makes Sr be commonly used as a dietary supplement in osteoporosis. Additionally, Sr–Ca coadministration can increase osteogenic gene expression and stimulate new bone formation [12]. Besides, many previous studies have proved that Sr doping can positively impact the drug-release behavior, biological performance, pore structure and surface properties of HA [13–15]. Hence Sr-doped HA may be a better choice than pure HA to be applied as a drug carrier and bone filling material.

In recent years, an increasing number of researchers have focused their attention on the preparation of polymer/inorganic composites, which can be used as drug-release systems to realize the controlled release and reduce the initially released amount of drugs [16–18]. The inorganic materials incorporated into polymers can enhance the drug release behavior, mechanical properties, swelling behavior and drug loading efficiency of the pristine biopolymer matrices [19]. As a bioactive polymer, alginate can form stable gels in the presence of low concentrations of calcium or other divalent cations without the need for organic solvents [20,21]. Calcium alginate is considered as one of the potential materials as a delivery matrix, because calcium alginate gel beads can dissolve under physiological conditions and simultaneously have been demonstrated to be biocompatible [22]. However, calcium alginate microspheres have low drug entrapment efficiency and noncontrollable release kinetics [23]. To improve the properties of pure alginate, some researchers have fabricated HA incorporated alginate composite materials, which owned better biomechanical and bioactive properties

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[24–26]. Meanwhile, the incorporation of HA can also prolong drug-release [19,27].

Compared with powders and block scaffolds, microspheres can be easier to fill irregular bone defects and to reconstruct the shapes of bones completely and possess better drug-delivering properties. Moreover, microspheres also own relatively higher specific surface areas and better biocompatibility than powders [27,28]. In our previous work, Sr-doped HA microspheres (SrHA) have been prepared via hydrothermal method [29]. Compared with pure HA, the SrHA with Sr/(Sr + HA) molar ratio of 0.3 showed the maximum specific surface areas, strongest photoluminescence (PL) intensity and highest drug loading efficiency (DLE). The porous flower-like SrHA in diameter of 2–3 μm was assembled by two-dimensional nano-sheet unit. Additionally, the PL intensity of the drug-loaded SrHA increased with the cumulative release amount of drugs, which may enable the drug release to be possibly tracked by the variation of the PL intensity. Nevertheless, the DLE and the drug release time of the SrHA still remained to be increased. In this paper, we were aimed to fabricate a new type of multifunctional drug carrier and bone filling material with bioactive and osteogenic properties and controlled drug-release capacity. Therefore, we firstly fabricated multifunctional SrHA incorporated alginate microspheres (SrHA/Alginate) by adding SrHA/alginate suspension into $CaCl_2$ solution. Besides, the morphology, DLE, drug release kinetics and the biological behaviors of the SrHA/Alginate were further investigated and characterized. In comparison to the previous papers [19,27], our present work not only investigated the drug release behavior of composite microspheres, but also focused on the osteogenic effect of Sr ions from the SrHA. The results indicated that the SrHA/Alginate was pH-sensitive and possessed controlled drug-release capacity. Furthermore, the SrHA/Alginate showed better osteogenic effect by releasing osteogenic Sr ions, which thus enables it to be a better drug carrier and bone filling material than the alginate composites containing pure HA. Therefore, the as-prepared SrHA/Alginate has promising application prospects as drug carriers and bone fillers.

2. Materials and methods

2.1. Preparation and characterization of drug-loaded microsphere carriers

Firstly, the SrHA with designed Sr / (Ca + Sr) molar ratio of 0.3 was fabricated according to our previous publication [29]. In a typical process, 2.1 mmol $Ca(NO_3)_2$, 0.9 mmol $Sr(NO_3)_2$ and 0.29 g CTAB were dissolved into 40 mL distilled water and the pH of the solution was adjusted to 4.5. Then, 2 mmol $(NH_4)_2HPO_4$ and 6 mmol citric acid trisodium salt dihydrate were dissolved into 20 mL distilled water. Next, the phosphate solution was added into the vigorously stirred nitrate solution. Then the mixing solution was transferred into a teflon bottle (100 mL) held in a autoclave and maintained at 180 °C for 1 d. As the autoclave cooled to room temperature, the SrHA was separated by centrifugation and washed with deionized water and ethanol in sequence. The SrHA/Alginate was prepared according to the following procedures. The SrHA was added into 25 mL deionized water to form homogeneous suspension. Then 16.65 mL alginate aqueous solution with the concentration of 3% (*w*/*v*) was added to the SrHA suspension. The mixing solution was stirred and ultrasonicated for a certain time, in which the mass ratio of SrHA/alginate is 1.67. Next, the mixture was extruded dropwise, with a 0.70 mm diameter needle, into a 0.1 M $CaCl_2$ solution to form spherical particles. The composite microspheres were hardened by immersion in the $CaCl_2$ solution for 1 h, then filtered and dried at 50 °C for 1 d to obtain final products. The pure Alginate was prepared according to the same procedure as above without the addition of the SrHA. The surface morphology and microstructure of the dried microspheres were analyzed by scanning electron microscopy (SEM) and their components were analyzed by Fourier transform infrared spectroscopy (FTIR).

Vancomycin (Aladdin CO., Ltd., Shanghai) was selected for drug loading and release kinetics study due to its wide applications in the treatment of hard tissue inflammation. For the entrapment of vancomycin into Alginate, 25 mg vancomycin was added into the 25 mL deionized water. Then the vancomycin solution was added into alginate aqueous solution. The following procedures were carried out to obtain the drug loaded Alginate as described above. For the entrapment of vancomycin into the SrHA/Alginate, 0.835 g SrHA was added into 25 mL·1 mg/mL vancomycin solution and the mixing solution was stirred for 1 d. Next, 16.65 mL alginate aqueous solution was added to the drug-loaded SrHA suspension. The following steps were the same as the preparation of the drug-unloaded SrHA/Alginate. The process is illustrated in Fig. 1. The $CaCl_2$ solution from the filtering step was collected for later assaying of the residual vancomycin content.

As for the SrHA, drug-loaded microspheres were prepared by adding 0.835 g SrHA to 25 mL vancomycin solution (1 mg/mL). After stirring for 1 d, the drug-loaded SrHA was separated by centrifugation, and dried in vacuum at 50 °C for 1 d. The filtrate was used to analyze the residual vancomycin content.

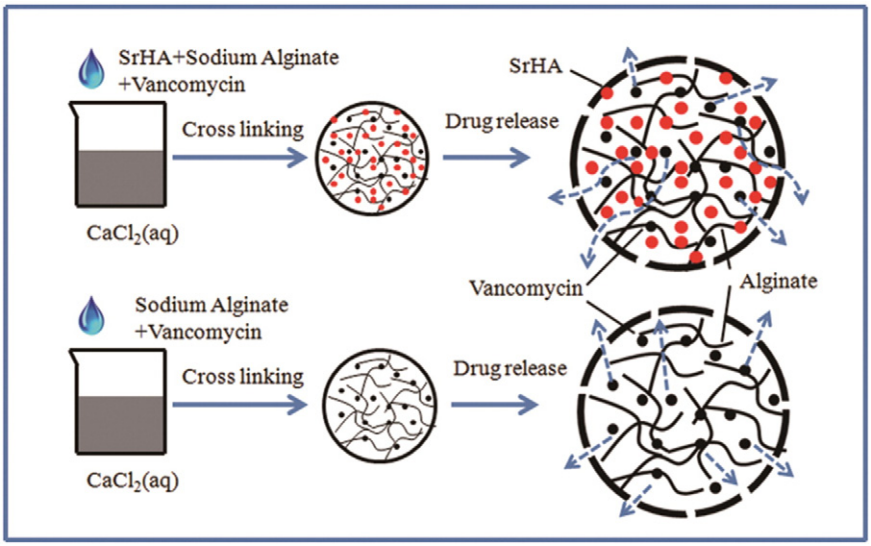

Fig. 1. Schematic illustration of the synthetic process for drug-loaded microspheres.