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The calcium silicate/alginate composite: Preparation and evaluation of its behavior as bioactive injectable hydrogels

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ABSTRACT

In this study, an injectable calcium silicate (CS)/sodium alginate (SA) hybrid hydrogel was prepared using a novel material composition design. CS was incorporated into an alginate solution and internal in situ gelling was induced by the calcium ions directly released from CS with the addition of D-gluconic acid δ-lactone (GDL). The gelling time could be controlled, from about 30 s to 10 min, by varying the amounts of CS and GDL added. The mechanical properties of the hydrogels with different amounts of CS and GDL were systematically analyzed. The compressive strength of 5% CS/SA hydrogels was higher than that of 10% CS/SA for the same amount of GDL. The swelling behaviors of 5% CS/SA hydrogels with different contents of GDL were therefore investigated. The swelling ratios of the hydrogels decreased with increasing GDL, and 5% CS/SA hydrogel with 1% GDL swelled by only less than 5%. Scanning electron microscopy (SEM) observation of the scaffolds showed an optimal interconnected porous structure, with the pore size ranging between 50 and 200 μm. Fourier transform infrared spectroscopy and SEM showed that the CS/SA composite hydrogel induced the formation of hydroxyapatite on the surface of the materials in simulated body fluid. In addition, rat bone mesenchymal stem cells (rtBMSCs) cultured in the presence of hydrogels and their ionic extracts were able to maintain the viability and proliferation. Furthermore, the CS/SA composite hydrogel and its ionic extracts stimulated rtBMSCs to produce alkaline phosphatase, and its ionic extracts could also promote angiogenesis of human umbilical vein endothelial cells. Overall, all these results indicate that the CS/SA composite hydrogel efficiently supported the adhesion, proliferation and differentiation of osteogenic and angiogenic cells. Together with its porous three-dimensional structure and injectable properties, CS/SA composite hydrogel possesses great potential for bone regeneration and tissue engineering applications.

1. Introduction

A number of biomaterials have been used in bone regeneration and bone tissue engineering [1,2], ranging from synthetically derived materials to naturally occurring biopolymers [1,3]. Biomaterial scaffolds can be divided into preformed and injectable, according to the application. The simplest and most convenient approach for clinical applications is to inject the repair material–cell–drug system into the damaged site. Injectable systems offer specific advantages over preformed scaffolds, including ease of application, confined delivery for a site-specific action, and improved patient compliance and comfort [4].

Calcium silicate (CS) ceramics have received much attention as potential scaffolds for bone regeneration and bone tissue engineering, and have been proved to be bioactive, degradable and hydrophilic [5–9]. Recent studies have shown that CS bioceramics possessed excellent bone regeneration ability, biodegradability and the ability to induce angiogenesis [10–16]. However, common drawbacks of CS are brittleness and difficulty in shaping. Pure CS also usually exhibits rapid ionic dissolution of calcium and silicon ions when it is in contact with fluid, which leads to a local high pH environment and may result in adverse cellular response [17–19]. In order to make use of the advantages but avoid the disadvantages of both CS and polymers to obtain an improved scaffold for bone tissue regeneration and tissue engineering, composite scaffolds containing CS and a number of biopolymers, such as poly(3-hydroxybutyrate-hydroxyvalerate) [20], poly(lactic-co-glycolic acid) (PLGA) [21] and poly-DL-lactic acid [19], have been constructed. For example, it is reported that the CS/PLGA composite scaffold shows better performance than the pure CS or PLGA with

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regard to degradation rate and biocompatibility [21]. However, these CS/polymer scaffolds are preformed, and are not injectable.

Some recent studies have indicated that hydrogels, especially those derived from natural proteins and polysaccharide, are ideal scaffolds for tissue engineering, since they not only offer several advantages over synthetic polymers and inorganic scaffolds but also provide a three-dimensional (3-D) environment and morphology similar to the extracellular matrix (ECM) of native tissues [22,23]. Sodium alginate (SA) has been widely investigated as a 3-D tissue culture substrate [22,24,25]. SA has the distinctive ability to form hydrogels via ionotropic crosslinking in the presence of divalent cations such as calcium and barium ions [26]. Hydrogels based on calcium-crosslinked alginate have been widely investigated for various drug delivery purposes [26–30]. Their gentle property has also led to their wide use as cell transplantation vehicles for the growth of new tissue and as wound dressing materials [31–33]. Calcium chloride is widely usually used to form calcium alginate gels [34]. However, Ca^{2+} reacts with alginate rapidly, and it is difficult to form calcium alginate gel homogeneously. Additionally, the liquification of gelled alginate due to the loss of Ca^{2+} [35] usually results in a high calcium concentration, which has been reported to inhibit the growth of cells in culture [36]. Besides the external addition of calcium to crosslink the alginate hydrogel, it has been recently reported that SA can form an injectable system with Ca^{2+} slowly and partially released from calcium-containing materials such as $CaCO_3$, $CaSO_4$ and hydroxyapatite (HA) in the presence of D-gluconic acid δ-lactone (GDL) [37–39]. However, alginate inherently lacks adhesivity to mammalian cells and bioactivity to stimulate cell differentiation, which hampers the application of alginate for cell culture and tissue engineering [39,40]. In order to further improve the cell compatibility of alginate hydrogels, bioceramics such as HA have been used to prepare bioceramic/aginate composite hydrogels, which are conducive to the adhesion and growth of cells [38].

Since CS has been reported to be biodegradable and is able to release calcium ions in a physiological environment, it is reasonable to assume that the addition of CS to alginate could lead to an in situ-forming and injectable CS/SA composite hydrogel, which could combine the advantages of SA and CS, especially maintaining the bioactivity of CS and the injectability and porous structure of the SA hydrogel. Therefore, in this study, CS/SA composite hydrogels were prepared by self-crosslinking of alginate with Ca^{2+} slowly released from CS in the presence of GDL without employing any extrageneous crosslinking agents. The gelling time and swelling ratios of the hydrogel system were investigated with different CS and GDL contents. The in vitro bioactivity of CS/SA hydrogels was assessed by soaking the hydrogels in simulated body fluid (SBF). In addition, the abilities of composite hydrogels to stimulate proliferation and osteogenic differentiation of rat bone mesenchymal stem cells (rtBMSCs) and to induce angiogenesis of human umbilical vein endothelial cells (HUVECs) were evaluated by culturing the cells in the presence of the composite hydrogel and its ionic extracts.

2. Materials and methods

2.1. Materials

Sodium alginate (low viscosity) and GDL were obtained from Sigma–Aldrich. $CaCl_2$ was acquired from Sinopharm Chemical Reagent Co., Ltd. As previously reported, the powders of CS bioceramics were synthesized by a chemical coprecipitation method [20,41]. In brief, continuous mixing of an aqueous solution of Na_2SiO_3 (1 mol l^{-1}) with $Ca(NO_3)_2$ (1 mol l^{-1}) in equal volumes at room temperature was carried out overnight. The stirring was then stopped and the resulting CS precipitate was filtered and washed, first with deionized water and subsequently with ethanol. After being dried at 80 °C overnight and calcined at 800 °C for 2 h, the CS powders were obtained. Calcium silicate particles between 100 and 150 μm were obtained for future use after being sieved.

2.2. Preparation of the injectable hydrogel

CS/SA composite hydrogels were prepared by adding different amounts of CS powders into aqueous alginate solution 2% (w/v) in the presence of GDL. Briefly, CS powders were dispersed homogeneously in a stirred aqueous alginate solution, followed by the addition of GDL in order to release calcium ions from the CS. The gelling solution was pipetted up and down with a syringe, and the obtained homogeneous solution was casted in a mold (h = 15 mm, Φ = 15 mm), with the shell made of Teflon and the central pillar made of silicone, for 24 h at room temperature to allow complete gelification (Fig. 1). For the control experiments, pure alginate gels (CS-free) with the same amount of GDL as the corresponding counterparts were polymerized by adding the crosslinking agent $CaCl_2$ at a concentration of 0.1 M [32]. With regard to sterilizing the pure SA and CS/SA composite hydrogels for the biological experiments, 2% SA solution was first sterilized through a filter (Millipore, 220 nm), and the CS and GDL powders were sterilized under ultraviolet light for 5 min. In addition, to encapsulate cells in the gels, the cells were first homogeneously suspended in the gelling solution, then the obtained cell suspension solution was injected into culture plates, at 40 μl per well for 96-well plates and 120 μl per well for 12-well plates.

2.3. Gelling time determination

To determine the gelling time, 1 ml of CS/SA mixture was reacted in water with a certain amount of GDL in a 10 ml vial at 37 °C. The vial was tilted every 15 s and inverted every 30 s until the CS/SA composite did not change its position relative to the vial axis. The relevant period of time was recorded as the gelling time. Values presented are the average of more than 10 determinations ± standard deviation (SD).

2.4. Mechanical properties

Cylindrical specimens of the gels prepared with different contents of CS and GDL were made in the 10 mm height and 15 mm diameter molds. The compressive strength of the specimens after gelling for 24 h was determined by an Electronic Universal Testing Machine (Zwick T1-FR020. A50), for which the compressive speed was set at 1 mm min^{-1} and no preload was applied. Three replicates were averaged for each sample.

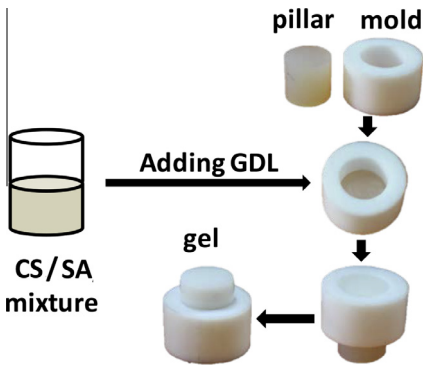

Fig. 1. The flowchart of the preparation of injectable CS/SA composite hydrogels.

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