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In vitro degradability, bioactivity and cell responses to mesoporous magnesium silicate for the induction of bone regeneration

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ABSTRACT

Mesoporous magnesium silicate (m-MS) was synthesized, and the in vitro degradability, bioactivity and primary cell responses to m-MS were investigated. The results suggested that the m-MS with mesoporous channels of approximately 5 nm possessed the high specific surface area of 451.0 m^2/g and a large specific pore volume of 0.41 cm^3/g compared with magnesium silicate (MS) without mesopores of 75 m^2/g and 0.21 cm^3/g, respectively. The m-MS was able to absorb a large number of water, with water absorption of 74% compared with 26% for MS. The m-MS was also degradable in a Tris–HCl solution, with a weight loss ratio of 40 wt% after a 70-day immersion period. The m-MS exhibited good in vitro bioactivity, inducing apatite formation on its surfaces after soaking in simulated body fluid (SBF) at a faster rate than observed for MS. The m-MS surface clearly promoted the proliferation and differentiation of MC3T3-E1 cells, and their normal cell morphology indicated excellent cytocompatibility. This study suggested that mesoporous magnesium silicate with a high specific surface area and pore volume had suitable degradability and good bioactivity and biocompatibility, making it an excellent candidate biomaterial for the induction of bone regeneration.

1. Introduction

Magnesium (Mg) is the fourth most abundant cations in the human body [1,2]. The adult body contains a total of approximately 24 g (1 mol) of Mg per 70 kg, and approximately 50–65 wt% of the total Mg is located in bone [3]. Mg may improve bone mineral density and affect bone fragility, and a growing body of evidence suggests that Mg plays another key role in the development of bony tissue by promoting the adhesion and growth of osteoblastic cells [4,5]. Previous studies revealed that the attachment and spreading of cultured human bone-derived cells was significantly enhanced on Mg-coated aluminum oxide (Al_2O_3) bioceramics compared with the uncoated Al_2O_3 [6]. Moreover, the Mg ion embedded hydroxyapatite (HA) coating on titanium alloy significantly improved bone-bonding properties compared with the ordinary HA coating [7].

Magnesium-based biomaterials have attracted increasing attention as potential bone repair materials [8,9]. Several magnesium-based bone repair materials have been reported, including magnesium-containing bioactive glasses, biodegradable magnesium alloys, β-tricalcium phosphate (β-TCP) coated porous Mg and Mg-substituted calcium phosphate bone cements, and these are excellent candidate biomaterials for the induction of bone regeneration [10,11].Therefore, the development of new magnesium-based biomaterials for bone repair and substitution is an important pursuit.

Over the past few years, a great deal of research effort has been dedicated to enhancing the bioactivity of the bone-implanted materials [12]. Bioactive biomaterials (such as bioglass, calcium phosphate and calcium silicate-based biomaterials) have drawn significant attention due to its excellent bioactivity and their ability to promote the development of bone tissue when in contact with physiological fluids [13,14]. The in vitro bioactivity (apatite formation on the material surface) of a biomaterial has been shown to

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depend not only on its chemical composition but also on its surface morphology and microstructure [15]. For example, bioactive materials with high surface area have been reported to be effective in inducing apatite formation, with the micropores on the material surface acting as apatite nucleation sites, shortening the time to reach the supersaturation required for apatite precipitation [16].

Mesoporous materials with pore sizes in the range of 2 to 50 nm have attracted significant attention due to their structural characteristics including their uniform pore size distribution, high specific surface area, high pore volume and tunable pore size, encompassing the main characteristics required for application as bioactive materials [17,18]. In addition, supramolecular chemistry has allowed for the design and synthesis of mesoporous materials with fascinating textural and structural features that open many paths for the research on bioactive materials for bone tissue regeneration [19]. Increasing the specific surface area and pore volume of m-MS was reported to greatly accelerate the kinetics deposition, thus enhancing the bone-forming bioactivity of the m-MS [20]. Mesoporous materials (such as mesoporous bioactive glass and mesoporous silicate) are promising candidate bioactive materials that have been investigated for bone repair [21,22]. To the best of our knowledge, no previous studies have reported the preparation of mesoporous magnesium silicate (m-MS) for application as a bone regeneration material. We expected that m-MS with high specific surface area and pore volume will have a greater bioactivity than m-MS. Therefore, m-MS was synthesized and characterized, and the water adsorption, in vitro degradability, bioactivity and primary cell responses to m-MS were investigated.

2. Materials and methods

2.1. Preparation of m-MS

A P123 (EO20PO70EO20, 5800, Sigma Aldrich) solution was prepared by stirring 4 g of P123 in 30 mL of H_2O and 120 mL of 2.0 M HCl (Shanghai Lingfeng Chemical Reagent Co., Ltd.) in a water bath for 1 h. Then, 9.6 g of magnesium nitrate hexahydrate (Sinopharm Chemical Reagent Co., Ltd.) was added to the solution, followed by the dropwise addition of 9.12 mL of tetraethyl orthosililate (TEOS, Shanghai Lingfeng Chemical Reagent Co., Ltd.) was magnetic stirring at 50 °C for 5 h. The white suspension obtained was placed under a fume hood in air at room temperature for 24 h to induce precipitation. Then, the precipitate was isolated by centrifugation, washed thoroughly with deionized water, and dried at 60 °C under vacuum to obtain the powder. Disc-shaped samples ($\Phi 12 \times 2$ mm) of the as-synthesized powder (0.3 g) were prepared by uniaxial and isostatic pressure (2 MPa). The samples were calcinated in air heated to 600 °C at a heating rate of 1 °C/min and held for to remove the template (P123) and obtain the mesoporous magnesium silicate (m-MS). Non-mesoporous magnesium silicate (MS) for use as a control material was synthesized in the same way without adding P123.

2.2. Characterization of m-MS

The morphology and mesoporous structure of m-MS was observed by using scanning electron microscopy (SEM; S-3400N, Hitachi, Japan) and transmission electron microscope (TEM; JEM2010, JEOL, Japan). The phase composition of m-MS was characterized by X-ray diffraction (XRD; Geigerflex, Rigaku Co. Ltd., Japan). Nitrogen adsorption–desorption isotherms of m-MS were collected on a Micromeritics porosimeter (Tristar 3000, Micrometrics Instrument Corp., Norcross, GA, USA) at 77 K under a continuous adsorption conditions. The specific surface area and pore size were calculated by the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively.

2.3. Water absorption of m-MS

m-MS samples and MS control samples with sizes of $\Phi 12 \times 2$ mm were weighed and then immersed in water for 0.5, 6, 12, 24 and 48 h. At the predetermined time points, the specimens were removed from solution, the water on the surface was wiped off, and the weights of the samples were measured. The water absorption ratio was defined as weight increase/original weight (%). The reported results represent the mean ± standard deviation (SD) of three samples for each time point.

2.4. Degradation of m-MS in Tris–HCl solution

The in vitro degradation behavior of $\Phi 12 \times 2$ mm m-MS and MS samples were determined by testing the weight loss ratio after soaking the samples in Tris–HCl in sealed polyethylene bottles for different periods of time. A 100 mL buffer solution of Tris(hydroxymethyl) aminomethane–HCl buffer(Tris–HCl, pH = 7.4) was prepared by mixing 50 mL 0.1 mol/L Tris solution with 42 mL 0.1 mol/L HCl solution and 8 mL water. The bottles were placed in a temperature and humidity chamber at a constant temperature of 37 °C with a solid/liquid mass ratio of 0.1 g/20 mL, and the solution was refreshed once every three days. At the selected time points, the specimens were removed from the solution, carefully washed with distilled water, and then dried in an oven at 100 °C for 6 h. The degradation ratio was expressed as the weight loss ratio at different time points, which was calculated according to the following equation:

$$\text{weight loss}(\%) = \frac{(W_t - W_0)}{(W_0)} \times 100\%$$

where W_0 is the initial dry weight and W_t is the dry weight at time t.

2.5. Bioactivity of m-MS in SBF

The in vitro bioactivity of $\Phi 12 \times 2$ mm m-MS and MS samples was tested by soaking the samples in simulated body fluid (SBF) for different time period. The SBF was prepared and buffered at pH 7.4 with tris(hydroxymethyl)aminomethane [$(CH_2OH)_3CNH_2$] and hydrochloric acid (HCl). The m-MS specimens were immersed in SBF at a solid/liquid mass ratio of 0.1 g/20 mL without changing the SBF solution. The system was placed in a shaking bath at 37 °C.

At each soaking time (6, 12, 24, 72, and 120 h), samples were removed and the concentrations of Mg, Si, Ca and P in the SBF solution were tested by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; IRIS 1000, Thermo Elemental, USA). The pH values of the soaking solutions were also determined with a pH meter (PHS-2C, JingkeLeici, Shanghai, China). The results represent the mean ± standard deviation (SD) of three samples for each time point. The samples removed from the solution were rinsed with deionized water and dried in an oven at 37 °C for 24 h. The phase composition and morphology of the sample surfaces after soaking were characterized by energy dispersive spectroscopy (EDS, Falcon, USA) and scanning electron microscopy (SEM; S-3400N, Hitachi, Japan), respectively.

2.6. Cell proliferation and alkaline phosphatase activity

The MTT assay was used to assess cell proliferation assay at 1, 4 and 7 days after MC3T3-E1 cells were seeded on the $\Phi 12 \times 2$ mm m-MS specimens in 24-well plates, with the MS specimens and the tissue culture plate (TCP) used as controls. The seeded cells were