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Porous diopside ($CaMgSi_2O_6$) scaffold: A promising bioactive material for bone tissue engineering

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ABSTRACT

Diopside ($CaMgSi_2O_6$) powders and dense ceramics have been shown to be bioactive biomaterials for bone repair. The aim of this study is to prepare bioactive diopside scaffolds and examine their physicochemical and biological properties. X-ray diffraction, scanning electron microscopy (SEM), micro-computerized tomography and energy-dispersive spectrometry were used to analyse the composition, microstructure, pore size and interconnectivity of the diopside scaffolds. The mechanical strength and stability as well as the degradation of the scaffolds were investigated by testing the compressive strength, modulus and silicon ions released, respectively. Results showed that highly porous diopside scaffolds with varying porosity and high interconnectivity of 97% were successfully prepared with improved compressive strength and mechanical stability, compared to the bioglass and $CaSiO_3$ scaffolds. The bioactivity of the diopside scaffolds was assessed using apatite-forming ability in simulated body fluids (SBF) and by their support for human osteoblastic-like cell (HOB) attachment, proliferation and differentiation using SEM, and MTS and alkaline phosphatase activity assays, respectively. Results showed that diopside scaffolds possessed apatite-forming ability in SBF and supported HOB attachment proliferation and differentiation. Bioactive diopside scaffolds were prepared with excellent pore/structure art, and improved mechanical strength and mechanical stability, suggesting their possible applications for bone tissue engineering regeneration.

1. Introduction

Silicate biomaterials, including bioglass, $CaSiO_3$ and Ca–Si–M (M = Mg, Zn, Ti, Zr) ceramics, have become a hot topic of research for bone tissue repair applications [1–7]. A significant characteristic of silicate biomaterials is their ability to release Si ions at a concentration that stimulates osteoblast growth and differentiation [8,9]. Recently, Chang and his colleagues reported on the superiority of porous $CaSiO_3$ and akermanite ($Ca_2MgSi_2O_7$) ceramic scaffolds in terms of material degradation and in inducing in vivo bone formation, compared to β-tricalcium phosphate (β-TCP) ceramic scaffolds, suggesting that silicate ceramics have potential application in bone tissue regeneration [3,10]. The chemical composition of diopside ($CaMgSi_2O_6$) is similar to that of $CaSiO_3$ and akermanite, but it has a relatively slower degradation rate [11]. Dense diopside bulk ceramics were found to have the ability to induce in vitro apatite formation in simulated body fluids (SBF) and in vivo bone formation [12,13]. Further in vitro and in vivo studies by Nonami and Miake et al. confirmed that dense diopside bulks possessed good bioactivity and excellent bending strength and fracture toughness [14,15]. However, to the best of our knowledge, most studies related to diopside biomaterials have focused on diopside powders and dense diopside ceramic bulks. Bioactive scaffolds with the appropriate mechanical strength and degradation rates are needed for bone regeneration [16]. Presently, bioactive Ca–P ceramics, such as hydroxyapatite (HAp) and β-TCP ceramics lack adequate mechanical strength, which limits them to only non-load-bearing applications. Although bioactive Ca–Si ceramics, such as bioglass and $CaSiO_3$, have excellent bioactivity, they have a relatively quick degradation rate and their mechanical stability is compromised [17]. The aim of this study is to prepare porous diopside scaffolds, evaluate their physical (pore structure mechanical strength and mechanical stability), chemical (degradation and apatite formation) and biological (cell morphology, proliferation and differentiation) properties and explore their potential application in bone tissue engineering.

2. Materials and methods

2.1. Preparation of porous diopside ceramic scaffolds

Diopside powders were synthesized by the coprecipitation process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), magnesium

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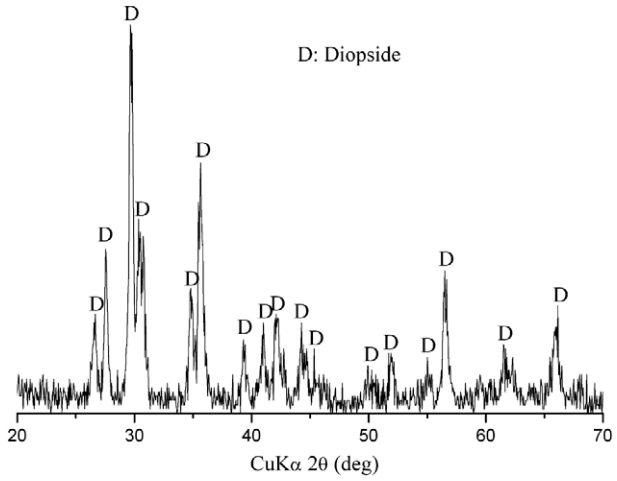

Fig. 1. X-ray diffraction pattern of diopside scaffolds sintered at 1300 °C.

nitrate hexahydrate ($Mg(NO_3)_2{\cdot}6H_2O$) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) as raw materials according to our previous publication [11]. Diopside powders were suspended in polyvinyl alcohol aqueous solution (6 wt.%) and stirred in a glass beaker to obtain well-dispersed slurry. Diopside scaffolds were prepared using the polymer sponge template method. Polyurethane foam template (density 25 ppi) was cut into the desired shape and size to replicate a porous scaffold. Then, the prepared sponge was immersed in the glass beaker containing diopside slurry and compressed with a glass stick to force the diopside slurry to migrate into the pores of the foams. The struts of the foams were uniformly coated with ceramic slurry while keeping the pores open. The porosity of the scaffolds could be controlled by adjusting the thickness of the slurry coat on the foams. The impregnated sponge was then dried at 60 °C for 1 day. After drying, the impregnated sponge was sintered at 1300 °C for 3 h.

HAp powders were synthesized by a chemical precipitation method. Briefly, 0.1 M $Ca(NO_3)_2$ water solution (100 ml) was dropped into 0.06 M $(NH_4)_2HPO_4$ solution (100 ml) under stirring for 12 h and maintained at pH >10. The resulting powders were filtered and washed three times in water and once in ethanol, then dried and calcined at 800 °C for 2 h. HAp porous scaffolds with a porosity of 80% were prepared using the same method to serve as cell culture controls.

2.2. Phase composition, microstructure, porosity and interconnectivity of diopside scaffolds

The phase composition, pore morphology and microstructure of the sintered scaffolds were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

The porosity of the scaffolds was measured according to Archimedes' principle. Scaffolds 6 mm × 6 mm × 6 mm in size were used for the measurement, and water was used as liquid medium. The porosity (P) was calculated according to the following formula: $P = (W_2 - W_1)/(W_2 - W_3) \times 100\%$, where W_1 is the weight of scaffolds in air, W_2 is the weight of scaffolds with water, and W_3 is the weight of scaffolds suspended in water.

The inner structure and interconnectivity were evaluated by micro-computerized tomography (μCT) (M-CT, The Netherlands). The interconnectivity (I) was calculated according to the equation: $I = \{[(V_{\text{total pore}}) - (V_{\text{disconnected pore}})]/V_{\text{total pore}})\} \times 100\%$. $V_{\text{total pore}}$ stands for the total pore volume and $V_{\text{disconnected pore}}$ stands for the disconnected pore volume.

2.3. Mechanical properties of diopside scaffolds

To evaluate the effect of porosity of diopside scaffolds on their mechanical properties, the compressive strength and compressive modulus of the sintered scaffolds (10 mm × 10 mm × 10 mm) with different porosities were tested using a computer-controlled universal testing machine (Instron) at a crosshead speed of 0.5 mm min^{-1}. Four scaffolds were used for the testing.

To evaluate their mechanical stability, diopside scaffolds were soaked in the SBF for 1, 3, 7, 14 and 28 days (see Section 2.4). The compressive strength and compressive modulus of diopside scaffolds soaked in SBF were tested.

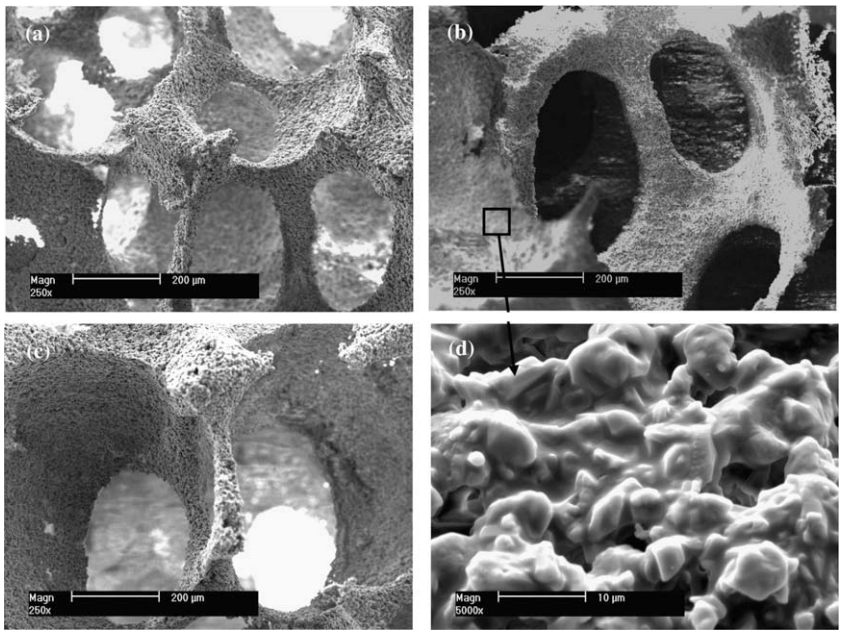

Fig. 2. Scanning electron microscopy of diopside scaffolds with different porosities: (a) 90%, (b) 80% and (c) 75%. High-magnification SEM shows that the diopside particles have been sintered (d).

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