RESEARCH PAPER

Improving the mechanical and bioactivity of hydroxyapatite porous scaffold ceramic with diopside/forstrite ceramic coating

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ABSTRACT

Objective(s): Scaffolds are considered as biological substitutes in bone defects which improve and accelerate the healing process of surrounding tissue. In recent years a major challenge in biomaterials is to produce porous materials with properties similar to bone tissue. In this study, the natural bioactive hydroxyapatite scaffolds with nano Diopside /Forstrite coating was successfully synthesized to be used in tissue engineering applications.

Materials and Methods: The spongy part of bovine bone was cut and the subsequent sintering temperature was applied for fabrication of natural hydroxyapatite. Then the scaffolds were coated with 30 wt% nano-Diopside/Forstrite composite slurry. The scaffolds were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and energy-dispersive spectroscope (EDS).

Results: In the present study, the mechanical properties of natural HA scaffold were improved when coated with a composite nmaed Diopside/Forstrite ceramic. The optimum properties were evaluated for the scaffolds containing 30 wt% composite ceramic coating. The pore size of the obtained scaffold was measured to be in the range of 300-400 nm. Compressive strength and porosity of the composite scaffold were approximately 1.5±0.2 MPa and 93±1.1 MPa, respectively.

Conclusion: Based on the mechanical and bioactivity result, the natural bioactive hydroxyapatite scaffolds with nano Diopside /Forstrite coating showed improved mechanical properties, pore size, porosity content and apatite formation ability whcih can be a promising candidate for bone tissue engineering applications.

Keywords: *Coating, Diopside, Forstrite, Hydroxyapatite, Porous materials*

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INTRODUCTION

The bone tissue engineering (TE) scaffolds have been suggested as a template in bone regeneration that allows cells to attach, proliferate, differentiate and organize into normal, healthy bone as the scaffold is degraded [1]. One of the significant challenges in this approach is the fabrication of highly porous scaffolds and interconnected pores with appropriate mechanical properties [2, 3]. Various materials including metals, polymers and ceramics have been used in bone tissue engineering, but ceramics have been widely employed due to their excellent mechanical and biological properties such as high compressive strength and modulus, wear and corrosion resistant, bioactivity and biocompatibility [4, 5].

In recent years many of researchers have shown that bio-ceramics based on Mg, Ca and Si have higher mechanical strength in comparison to hydroxyapatite (HA); furthermore release of Mg, Si and Ca ions from these materials in many cases have positive effect on cell proliferation, differentiation, and adhesion [6]. Diopside ($CaMgSi_2O_6$) is a member of pyrosilicate minerals. Diopside, in comparison to HA, exhibits fairly high mechanical properties such as 3.5 $MPa{\cdot}m^{1/2}$ fracture toughness and 170 GPa young's modulus [6-7].

Forsterite (Mg_2SiO_4) is a member of the olivine family of crystals in the $MgO–SiO_2$ system. Previous studies have shown that Forsterite has

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better mechanical properties compared to HA [8-9]. Some researchers have shown that nano-Forsterite ceramic powder has fracture toughness and micro hardness of about 3.61±0.1 MPa.$m^{1/2}$ and 940±10 HV, respectively which are high in comparison to HA with 0.75 - 1.2 MPa·$m^{1/2}$ fracture toughness and 700 HV micro-hardness [6].

Previous studies have shown that Diopside and Forstrite have good apatite-formation ability on their surface. This layer formed on these ceramics is similar to natural cortical bone and also dentine [10-11].

These ceramics are capable of inducing osteogenesis in biological fluids and are good bioactive materials for hard tissue engineering while they are not cytotoxic [8]. Several methods have been used to improve the mechanical properties of ceramics scaffolds [12]. The aim of this study is to improve the mechanical properties and apatite formation ability of natural HA scaffold by nano-composite Diopside/Forstrite coating on the surface of HA scaffold.

MATERIALS AND METHODS

Preparation of Nano-Diopside/Forstrite composite powder

Diopside/Forstrite powder was prepared by mechanical alloying method.

Talc ($Mg_3Si_4H_2O_{12}$) (98% purity, Merck), magnesium carbonate ($MgCO_3$) (98% purity, Aldrich) and calcium carbonate ($CaCO_3$) (98% purity, Merck) powders were used as starting materials. In order to produce the powder, talc, $MgCO_3$ and $CaCO_3$ powders with molar ratio of 1:2:1 were mixed in a high energy ball mill with zirconia vial and balls with 2 cm diameter. The ball/powder mass ratio was 10:1 and the rotational speed of disc was set at 445 rpm. The time of MA was chosen 10 hr and samples were sintered at 1200 °C for 1 hr.

Natural HA scaffold preparation and scaffold coating

The spongy part of bovine bone was cut into rectangular specimens with 6×6×12 mm in size. The subsequent sintering temperature was set to 800 °C for 3 hr. Then the scaffolds were coated with 30 wt% nano-Diopside/Forstrite composite slurry. The recipe of prepared slurry was shown in Fig 1. Briefly, polyvinyl alcohol polymer (3% w/w) was dissolved in 50 ml water at 70 °C. Then 30 wt% nano-Diopside/Forstrite composite powder was added to the solution and stirred for 3 hr. The prepared scaffold was immersed in this slurry for

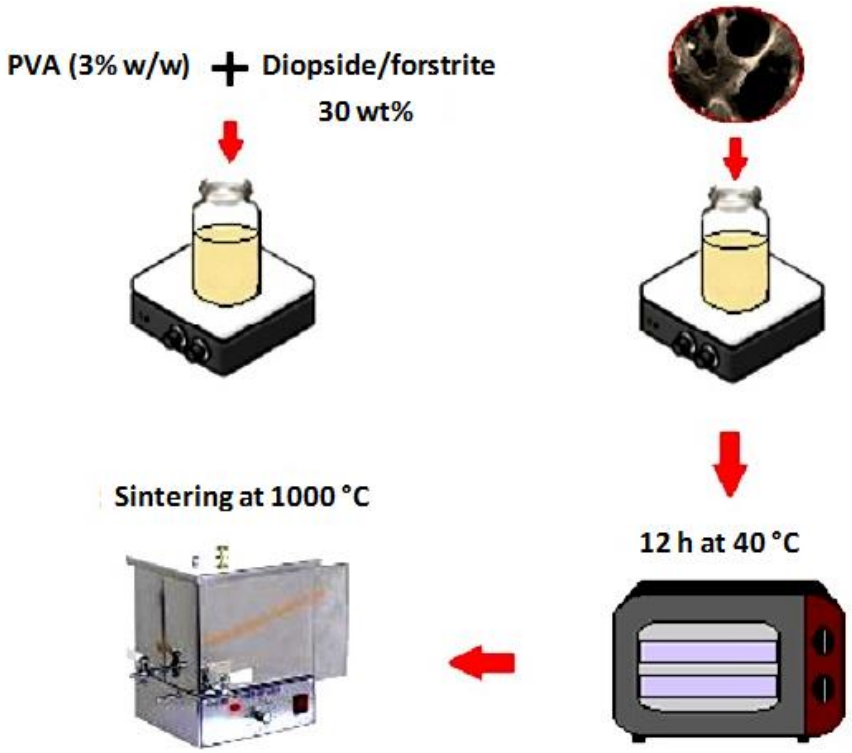

Fig 1. Fabrication procedure of the HA- nano- Diopside/Forstrite composite scaffold

20 min. The coated scaffold was dried in an oven for 12 hr at 40 °C followed by siteration at 1000 °C for 3 hr.

Powder and scaffold characterization

Crystallite size and phase transformation assessment during MA were evaluated using X-ray diffraction (XRD) with Cu Kα radiation (λ = 0.154 nm at 20 kV and 30 mA). The XRD patterns were recorded in the 2θ range of 20°-70° (step size 0.05° and time per step 1 s). The crystallite sizes were measured by Williamson- Hall method [13].

$$\beta \cos\theta = \frac{0.9\lambda}{D} + 2\varepsilon \sin\theta$$

where θ is the Bragg diffraction angle, ε internal strain, D the crystallite size, λ the wavelength of the radiation, β the diffraction peak width at half maximum intensity, and 0.9 the Scherer constant. The morphology of powder particles and architecture structure and analysis of scaffolds were determined by scanning electron microscopy (SEM) in a Philips XL30 at an acceleration voltage of 30 kV and energy-dispersive spectroscope (EDS). The morphology and size of powder was investigated by TEM images.

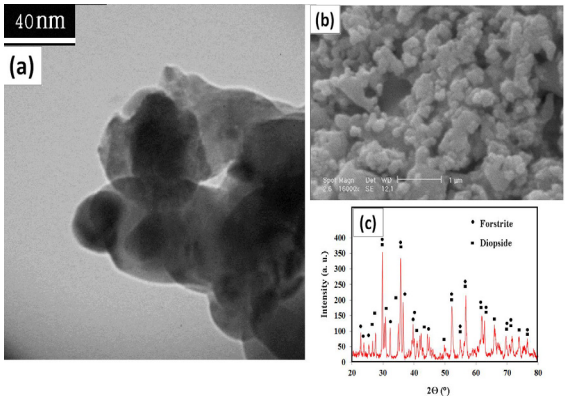

Fig 2. a (TEM micrograph, b) SEM micrograph and c) XRD pattern of nano-Diopside/Forstrite composite powder

The compressive strength and elastic modulus were measured by Hounsfield (H25KS). In this study, the porosity of scaffolds was evaluated by Archimedes technique. In order to evaluate the bioactivity of scaffolds, the modified and unmodified samples were soaked in a simulated body fluid (SBF) [36] with pH=7.38 for 1, 7, 14, and 21 days. All samples were kept in water bath at the temperature of 37 °C. After soaking, the scaffolds were dried at 70 °C for 5 hr. Scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS) were utilized to evaluate the formation of bone-like apatite.

RESULTS AND DISCUSSION

The TEM micrograph (Fig 2a) was taken to investigate the morphology and grain size of pure Diopside/Forstrite composite after 10 h milling which showed spherical morphology and low degree of agglomeration. The grain size, measured by Image J, was found to be 28±2 nm. Fig 2b shows the SEM micrographs of nano-Diopside/Forstrite composite powder after 10 h milling followed by sintering at 1200 °C for 1 hr. Fine agglomerated particles with spherical shapes were formed. Size distribution of particles was observed in the SEM icrograph. Fig 2c shows the XRD pattern of sintered powder with 10 h MA. The XRD pattern only shows the characteristic peaks of Diopside/ Forstrite. The crystallite sizes of prepared powder could be measured by broadening of peaks of diffraction patterns. The crystallite sizes of Diopside/Forstrite composite powders were determined by Williamson-Hall equation [13]. The crystallite size of the sintered powders after 10 hr MA was measured ro be about 73±4 nm.

The SEM micrographs of scaffold coated with nano-Diopside/Forstrite composite was demonstrated in Fig 3(a-c). As can be seen, the scaffold is consisted of interconnected porosities. The size of pores was in the range of 300-400 µm. It is ideal to produce bone scaffolds with macro pores in the range of 150-500 µm to provide an appropriate environment for vascularization, cell migration, bone ingrowth and diffusion of nutrients [14-15]. In order to confirm the presence of nano-Diopside/Forstrite composite coating on the surface of scaffold, the EDS analysis was carried out as shown in Figure 3d. The EDS spectrum of sample after coating by nano-Diopside/ Forstrite composite confirmed the presence of nano- Diopside/Forstrite on the surface of HA scaffold. The compressive strength and porosity of HA and HA- nano-Diopside/Forstrite composite scaffolds were measured to be 0.46±0.1 MPa, 1.5±0.2 MPa and 97±1%, 93±1%, respectively. The mechanical property of HA- nano-Diopside/ Forstrite composite scaffold which was produced in this atudy is similar to spongy bone (0.2-4 MPa) [16-17]. As can be seen, the compressive strength of HA- nano-Diopside/Forstrite composite (1.5 MPa) is higher than that of HA with compressive strength of 0.46 MPa. In order to prevent stress

shielding phenomenon, use of biomaterials with similar mechanical properties to human bone is suggested [18]. As a result, the modified HA scaffold by nano-Diopside/Forstrite composite coating can be considered as a potential scaffold in low-load bearing applications due to their similar compressive strength to the lower limit of the compressive strength of spongy bone.

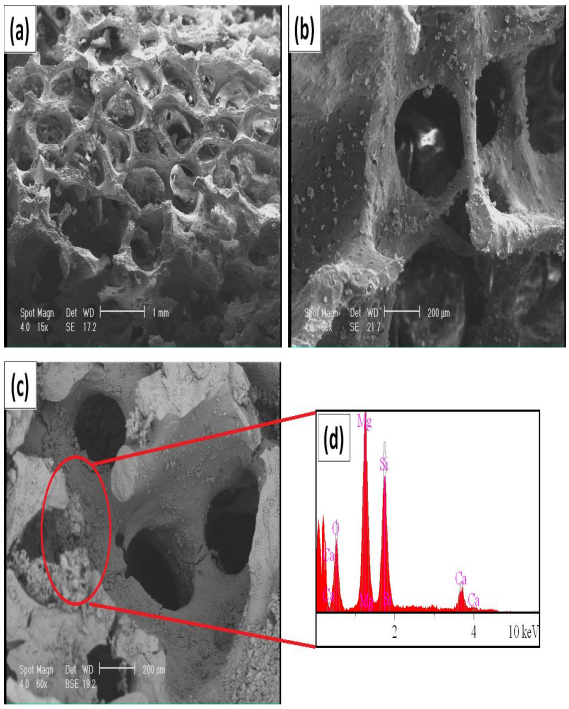

Fig 3. a-c(SEM micrograph and d) EDS spectrum of HA- nano-Diopside/Forstrite composite scaffold

In order to evaluate the bioactivity of scaffolds before and after modification, the samples were immersed in SBF for up to 21 days.

Figure 4 shows SEM micrographs of various samples. Some fine spherical white particles was observed on the surface of HA (Fig 4a), and HA-30 wt% nano-Diopside/Forstrite (Fig 4b).

EDS results of the particles on the surface of scaffolds confirmed the formation of apatite after soaking the scaffolds in SBF for 21 days (Fig 4c). Applying the nano-Diopside/Forstrite coating on the surface of HA led to an increase in the formation and deposition of apatite. Based on the previous studies [19-21], the major disadvantages of hydroxyapatite ceramic in comparison to bioactive glasses and other bioceramics is its low reactivity with bone. The bioactivity index (IB) of HA is 3.1 while the bioactivity of 45S5 and 55S4 bio-glasses is 12.5 and 33.7, respectively [22-23]. Therefore, improving the bioactivity of HA can increase its demand in the biomedical applications. In this study, we developed another strategy to improve the biactivity index of ceramic by applying the nano-Diopside/Forstrite ceramic coating on the surface of HA. Thus higher exchange rate of ions with surrounding environment improved the apatite formation ability of HA-30 wt% nano-Diopside/Forstrite scaffolds.

The size of apatite particles was measured to be in the range of about 2 µm.

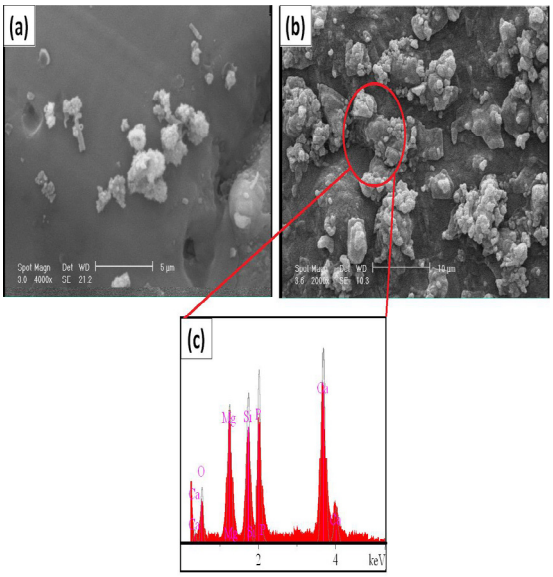

Fig 4. SEM micrograph of a(HA, b) HA- nano- Diopside/ Forstrite composite scaffold and c) EDS spectrum of HA- nano-Diopside/Forstrite composite scaffold after 21 days soaking in SBF

CONCLUSION

Natural HA- nano-Diopside/Forstrite composite scaffold with a highly porous structure, interconnected porosity and highly mechanical properties was successfully fabricated by applying coating on ceramic surface. The pores size, total porosity, and compressive strength of the produced scaffold were 500-600 µm, 93±1.1%, 1.5±0.2 MPa, respectively. Considering the biological and mechanical properties as well as highly porous structure with desired pore size, natural HA-nano-diopside/forstrite composite scaffold can be introduced as an excellent bioactive ceramic scaffold for bone tissue engineering applications.

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