



Electrochemical and *in vitro* bioactivity of nanocomposite gelatin-forsterite coatings on AISI 316 L stainless steel



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ABSTRACT

AISI 316L stainless steel has been widely considered as implant materials in biomedical applications owing to its low cost and superior strength. However, its weak corrosion resistance due to the release of nickel, chromate and molybdenum ions as well as its inert nature limits its clinical application specifically long-standing performances. The aim of this study was to prepare and characterize gelatin-forsterite (Mg_2SiO_4) nanocomposite coatings consisting of various amounts of forsterite nanopowder (0, 1, 2.5 and 5 wt.%) on AISI 316L substrate in order to improve simultaneously corrosion resistance and *in vitro* bioactivity. Nanocomposite gelatin-forsterite coatings were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and X-ray diffraction. Furthermore, the bioactivity of gelatin-forsterite coated specimens were evaluated via soaking in simulated body fluid (SBF) for 28 days at 37 °C. Results demonstrated the formation of crack-free and homogeneous coatings without any observable defect and pore. The surface roughness and adhesion strength of the coatings enhanced with increasing forsterite content. Moreover, the corrosion evaluation considered by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) demonstrated that compared to unmodified AISI 316L substrate, the corrosion resistance of gelatin-forsterite nanocomposite-coated substrates significantly improved. Moreover, nanocomposite coatings were able to persist severe localized corrosion in physiological solution indicating their long-term biostability. Moreover, the formation of bone-like apatite layer on the nanocomposite-coated samples was observed in SBF, which might be helpful to integrate with host tissue. Overall, it is anticipated that the novel proposed nanocomposite coatings of gelatin-forsterite might be potentially useful for orthopedic implants.

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1. Introduction

AISI 316L stainless steel has been widely applied in medical fields especially as temporary implants owing to its low cost and superior strength [1]. However, AISI 316L has some drawbacks consisting of poor wear resistance to common forms of wear and contact damage [2,3], inability to form directly chemical bonds to natural bone tissue [3] as well as weak resistance to release metal ions such as nickel, chromate and molybdenum leading to less biocompatibility and long-standing performance [4,5]. In order to improve the corrosion resistance and stimulate the bone in growth to AISI 316L implant surface, various surface modification strategies consisting of mechanical and electrochemical polishing [6], thermal treatment [7], ion implantation

[8] and alkali and heat-treatment [9] as well as several ceramic (e.g. calcium phosphate-based material such as hydroxyapatite (HA) [10–12]), polymer (e.g. poly(ϵ -caprolactone) (PCL) [13]) and composite [14,15] coatings have been employed. Between these approaches, composite coatings could simultaneously improve corrosion resistance and bioactivity via the chemical and physical bonding with host tissues making them perfect option for orthopedic implants [16,17]. Compared to pure ceramic coatings, composite coatings revealed reduced brittleness and crack formation providing improved corrosion resistance [18]. Various kinds of bioactive ceramics have been introduced to develop composite coating on metallic substrates consisting of HA [17,18], TiO_2/ZrO_2 [19] and bioactive glass [20] embedded natural and synthetic polymers such as PCL [21] and chitosan [17,22].

Forsterite with chemical formula Mg_2SiO_4 has recently been introduced as a biocompatible and bioactive ceramic with better mechanical properties compared to HA and bioactive glass [23–25]. Results demonstrated that Mg and Si ions could release during soaking in biological environment which stimulate bone in

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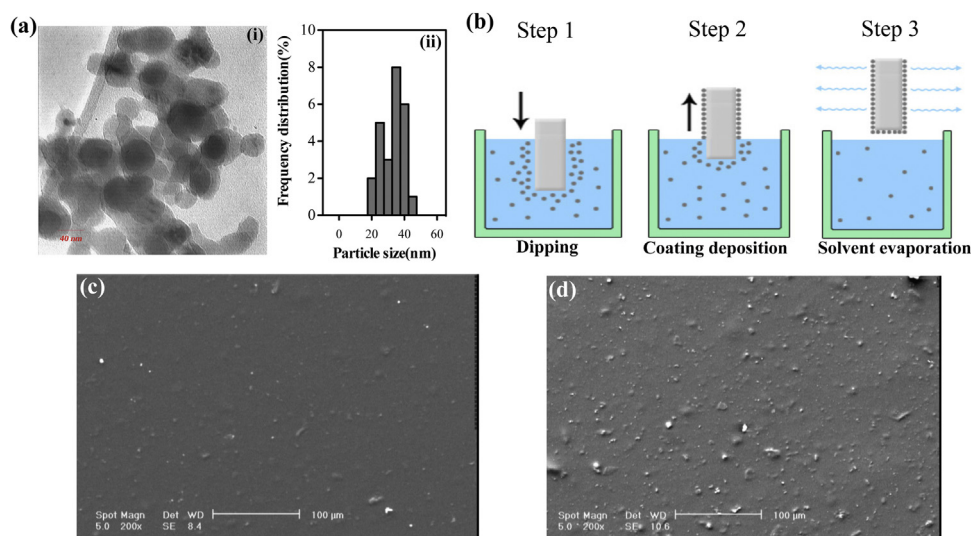


Fig. 1. (a) TEM micrograph (i) and the frequency histogram of particle size (ii) of forsterite nanopowder, (b) The schematic of dip coating process consisting of: step 1) dipping of the substrate into the suspension, step 2) formation of wet layer by withdrawing the substrate, step 3) gelation of the layer by solvent evaporation. SEM image of G-1F coated samples with dipping period of (c) 3 times and (d) 5 times.

growth and regeneration [26]. Forsterite has been widely applied as coatings on various kinds of ceramics and metallic scaffolds and implants [27–30]. Results demonstrated that the incorporation of forsterite nanopowder in the coatings could significantly improve their mechanical properties such as elastic modulus, hardness and fracture toughness as well as corrosion resistance [29]. In other words, gelatin is a natural polymer derived from collagen through an acid (type A-gelatin) or alkali (type B-gelatin) hydrolysis. Thanks to its similarity to the natural bone, relatively low cost, desirable encouragement to bone regeneration and good adhesiveness and hemostatic properties, gelatin has been widely used in tissue engineering [31,32], pharmaceutical applications [33] as well as food and cosmetic products [34]. Moreover, gelatin has recently been applied as coatings on various kinds of implants and revealed the formation of homogeneous and well-adherent coating [35,36]. However, according to our knowledge, the limited studies have focused on the nanocomposite coatings based on gelatin protein. In addition, the bioactivity and corrosion resistance of gelatin based composite coatings have not evaluated yet.

The aim of this study was to prepare nanocomposite coatings consisting of gelatin and various amounts of forsterite nanopowder on AISI 316L substrate using dip coating process. Moreover, the effects of forsterite nanopowder concentrations on the coating roughness, adhesion and morphology were studied. Finally, the bioactivity and corrosion resistance of nanocomposite-coated substrates were evaluated.

2. Materials and methods

2.1. Synthesis of forsterite nanopowder

Forsterite nanopowder with spherical particles (Fig. 1(a–i)) and particle size of 25–45 nm (Fig. 1(a–ii)) was synthesized according to the sol-gel process based on our previous report [37]. Briefly, after preparation of magnesium nitrate hexahydrate ((Mg(NO₃)₂·6H₂O), Merck) aqueous solution, colloidal silica (SiO₂, 34 wt.% solid fraction, Sigma) was added to it in order to provide Mg: Si mole ratio = 2:1. Meanwhile, aqueous solution of sucrose (sucrose-to-metal mole ratio = 4:1, Merck, 99.9% purity) was separately prepared and polyvinyl alcohol (PVA) aqueous solution was added to it (PVA monomer-to-metal molar ratio = 0.8:1, Merck). The amount of sucrose, PVA and sucrose-to-metal mole ratio were

selected based on the previous reports [37,38]. As prepared PVA-sucrose solution was added to Mg-Si solution and pH was adjusted to 1 using nitric acid. PVA and sucrose were applied in the forsterite synthesis procedure due to the different hydrolysis and condensation rates of silica and alkoxides leading to chemical inhomogeneity of the gels and, therefore, unwanted phases. In this process, nitric acid could break sucrose into glucose and fructose. Decomposed products consisted of –OH and –COOH groups which could encourage the binding of Mg²⁺ ions in the homogeneous solution. In other words, PVA could develop polymeric network which could trap colloidal silica nanoparticles leading to their homogenous distribution [38]. After continuous stirring at 90 °C for 2 h, the solution was preserved at room temperature overnight, dried at 200 °C and finally calcined at 900 °C for 2 h.

2.2. Preparation of AISI 316L stainless steel substrates

AISI 316L samples cut into square specimens having dimensions of 10 × 10 × 2 mm³ were applied as substrates. Prior to the coating process, the substrates were mechanically polished using SiC papers with the grit sizes of 80, 120, 240, 320, 600, 800, 1200 and 2000, sequentially, and rinsed with deionized (DI) water. Afterwards, in order to better attachment of coatings to the substrates, they were immersed in solution consisting of 30:70 vol ratio of H₂O₂: HNO₃ for 30 s. Consequently, as prepared substrates were ultrasonically cleaned in acetone to remove residual grease and, then, DI water for 1 h in order to remove surface impurities. Finally, the AISI 316L substrates were air dried at 60 °C overnight.

2.3. Fabrication of gelatin-forsterite coatings

Before coating process, nanocomposite suspensions containing gelatin with different amounts of forsterite nanopowder were prepared. Initially, 8 wt.% gelatin (type A porcine skin, Mw = 50,000–100,000, Sigma) solution in 20 wt.% acetic acid was prepared at room temperature. After complete gelatin dissolution, various amounts of forsterite nanopowder (0, 1, 3, and 5 wt.%) were added to it. To provide uniform dispersion of forsterite nanopowder, the suspensions were magnetically stirred for 24 h at room temperature. Based on the amounts of forsterite nanopowder (0, 1, 3 and 5 wt.%), the suspensions and the final coatings were named as Gelatin, G-1F, G-3F and G-5F, respectively. Since gelatin is water

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