Materials Science and Engineering C 55 (2015) 137–144

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Electrophoretic deposition of ZnO/alginate and ZnO-bioactive glass/alginate composite coatings for antimicrobial applications

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ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form 20 February 2015
Accepted 8 May 2015
Available online 15 May 2015

Keywords:
ZnO nanoparticles
Alginate
Bioactive glass
Coating
Electrophoretic deposition (EPD)

ABSTRACT

Two organic/inorganic composite coatings based on alginate, as organic matrix, and zinc oxide nanoparticles (n-ZnO) with and without bioactive glass (BG), as inorganic components, intended for biomedical applications, were developed by electrophoretic deposition (EPD). Different n-ZnO (1–10 g/L) and BG (1–1.5 g/L) contents were studied for a fixed alginate concentration (2 g/L). The presence of n-ZnO was confirmed to impart antibacterial properties to the coatings against gram-negative bacteria *Escherichia coli*, while the BG induced the formation of hydroxyapatite on coating surfaces thereby imparting bioactivity, making the coating suitable for bone replacement applications. Coating composition was analyzed by thermogravimetric analysis (TG), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDS) analyses. Scanning electron microscopy (SEM) was employed to study both the surface and the cross section morphology of the coatings. Polarization curves of the coated substrates made in cell culture media at 37 °C confirmed the corrosion protection function of the novel organic/inorganic composite coatings.

1. Introduction

Around 1.5 million bone replacement surgical procedures per year are performed worldwide, with a cost of around US$10 billion [1]. Infection of orthopedic implants occurs in 5% of the cases for a total amount of 100,000 cases per year just in the USA [1]. This problem is originated by bacterial colonization of the implant surface where bacteria form a biofilm causing infection of the bone and surrounding tissues [2]. Bacteria can come from a variety sources: deficient hygienic standards in hospital [3], and also from the patient's own skin and/or mucosa, etc. [4,5]. These microorganisms attach to the implant surface in an irreversible way. After implantation bacteria can produce a relatively thick extracellular matrix layer on the implant surface leading to the formation of an adherent biofilm [4–6]. This biofilm makes difficult the penetration of antibacterial agents (e.g., immune cells or antibiotics) being thus extremely resistant and adhesive [5]. A chronic infection adjacent to the implant can lead to osteomyelitis, acute sepsis, and even death [7]. To tackle this problem, a solution being proposed is the incorporation of antibacterial coatings on the implant surface to prevent the biofilm formation.

Another common problem observed for metallic implants is its encapsulation by fibrous tissue [8], which leads to micromovements of the implant, migration and possible loosening [8,9]. To solve these problems a bioactive material can be used to coat the implant and in order to induce its osteointegration. Bioactive glasses are well-known biocompatible materials with osteoinductive properties that are being increasingly used in the orthopedic field to promote bone repair and regeneration [10–13]. Bioactive glasses form a hydroxyapatite surface layer where osteogenic cells can attach and differentiate [13–15] thereby improving the bone–implant contact and promoting bone in-growth. Moreover, bioactive silicate glasses show antibacterial, anti-inflammatory and angiogenic effects [16–18].

ZnO has been used in the production of solar cells, photovoltaic devices, batteries and biosensors mainly due its semiconducting properties [19–21]. This material has also been used to produce biomimetic membranes able to immobilize proteins due to its rapid transfer of electrons, which represents an application of ZnO in the field of biomaterials [22]. Antibacterial properties of ZnO have been reported [23–25], which opens possible applications of this material in the production of coatings with antibacterial activity on metallic implants.

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http://dx.doi.org/10.1016/j.msec.2015.05.034
0928-4931/

Combining ZnO with a bioactive glass (e.g., 45S5 Bioglass®) [10], a new composite material can be developed that tackles simultaneously the two main challenges of traditional orthopedic implants: probability of infections and lack of osteointegration.

Spray plasma coating is a technology widely used to produce bioactive coatings on metallic implants, mainly based on calcium phosphates, e.g., hydroxyapatite [26]. However, due to the high temperatures reached during the process, a morphological change of the bioactive material is induced which may reduce its bioactivity. To solve this inconvenience, a new family of organic/inorganic composite coatings made by room temperature electrophoretic deposition (EPD) is emerging, where a biocompatible polymer and a bioactive glass (or ceramic) material are combined [27–32]. EPD has been used to produce pure bioactive glass coatings [33] and composite coatings of bioactive glass with different polymers [34,35] for orthopedic and dental applications. With this room temperature processing method possible degradation and microstructural damage of the coating and substrate, e.g., phase changes and microcracking due to thermal expansion mismatch, are avoided. A growing family of this type of organic–inorganic composite coatings is being produced, as reviewed elsewhere [27].

An interesting polymer for fabrication of organic/inorganic composite coatings with potential biomedical applications is alginate [36], which has been used only to a limited extent in combination with EPD to produce bioactive coatings [28,31,36,37]. Alginate is a natural polysaccharide which, due to its low toxicity and biocompatibility [38–40], has been studied for different applications, e.g., biosensors, drug delivery systems and tissue engineering. This polymer presents a potential binding effect with proteins, growth factors and bone-forming cells, being thus also attractive to develop coatings for bone contacting materials.

EPD appears as a versatile, simple and low cost technique to create highly homogeneous coatings with clear advantages, like the possibility to obtain homogeneous coatings on 3D structures of complex shape as well as on porous substrates [27,41,42]. Moreover, EPD enables production of a wide variety of coatings due to the possibility of depositing different types of materials and combination of materials, e.g., inorganic, polymeric and composite materials with high microstructural homogeneity and tailored thickness [27,41–43]. The EPD process is based on the application of an electric field between two conductive electrodes immersed in a colloidal suspension [43]. The electric field imparts electrophoretic motion to charged particles in suspension causing their movement to the oppositely charged electrode, where they deposit forming a coherent coating over it.

The aim of this research was to develop a new group of electrophoretic alginate-based coatings on stainless steel substrates incorporating ZnO nanoparticles and bioactive glass microparticles as inorganic phases. The deposition conditions (i.e., suspension concentration, electric field and deposition time) as well as the colloidal stability of the starting suspensions were investigated. Coating compositions were studied using XRD, FTIR and TG techniques. The electrochemical behavior was also evaluated by obtaining polarization curves of coated substrates to assess the protective effect of the coatings on the corrosion behavior of the stainless steel substrates. Antibacterial test against gram-negative bacteria was performed to evaluate the potential antibacterial properties of the coatings. Gram-negative bacteria were chosen for the tests because even if ZnO nanoparticles have been frequently tested against this type of bacteria, the effect of the presence of the alginate matrix and bioactive glass particles on antibacterial activity is unknown.

2. Materials and methods

2.1. Suspension preparation

Sodium alginate (Sigma Aldrich, Germany), zinc oxide nanoparticles (n-ZnO, Intrinsiq Materials, UK), bioactive glass (BG) microparticles (5–25 μm particle size) of 45S5 composition [10], deionized water and ethanol were used to prepare the composite coatings. A 2 g/L alginate solution was used in all experiments while the ceramic content was varied from 1 to 10 g/L. At the same time, different n-ZnO/BG ratios were chosen, varying the n-ZnO content from 25 to 100 wt.%. Samples were labeled ZA (100 wt.% ZnO), 50-ZBA (50 wt.% ZnO and 50 wt.% BG) and 25-ZBA (25 wt.% ZnO and 75 wt.% BG). In order to avoid hydrogen evolution formation during the EPD process (due to water electrolysis) a mixture of 40 vol.% ethanol–60 vol.% water was used [31,37]. To achieve an adequate dispersion of the components, the suspensions were magnetically stirred for 10 min followed by 60 min of ultrasonication (using an ultrasonic bath, Bandelin Sonorex, Germany). Zeta-potential measurements were carried out in order to analyze the colloidal stability of the suspensions. These measurements were done by Laser Doppler Velocimetry (LDV) technique using a Zetasizer nano ZS equipment (Malvern Instruments, UK). The solid content of all suspensions was adjusted to 0.1 g/L in order to ensure reliable measurements.

2.2. Electrophoretic deposition

Stainless steel AISI 316 L electrodes (foils of 2.25 cm^2 deposition area and 0.2 mm thickness) were used to deposit the coatings via constant voltage-EPD. The distance between the electrodes in the EPD cell was kept constant at 10 mm. Deposition voltages and times in the ranges 5–40 V and 5–35 s, respectively, were studied. The deposition yield was evaluated using an analytical balance (precision 0.0001 g). Coated substrates were dried during 24 h in normal air at room temperature prior to mass determination.

2.3. Characterization

In order to characterize the coatings, XRD (D8 Philips X'PERT PW 3040 MPD), FTIR (Bruker Instruments, Germany) and thermogravimetric (TG) (TGA/SDTA 851e, Mettler Toledo) tests in air (heating rate: 10 °C/min) were performed. The microstructure of the ZnO nanoparticles was characterized with TEM (JEOL 2100, 200 kV). The surface microstructure and composition of the coatings were analyzed by SEM (Hitachi S4800) and energy-dispersive X-ray spectroscopy (EDX), respectively. To determine the coating thickness, cross section of the samples were cut using and ion mill (Hitachi IM4000) and further observed by SEM. Bending tests were also performed in order to qualitatively evaluate the deformation ability of the coatings and the adhesion between the substrate and the coating.

2.4. Electrochemical behavior and corrosion

The electrochemical behavior of the coatings was studied in order to test their possible corrosion protective properties. Potentiodynamic polarization curves were obtained using a potentiostat/galvanostat (Autolab PGSTAT 30). The samples were immersed in 100 mL of Dulbecco's MEM (DMEM, Biochrom) at 37 °C. A conventional three electrode system was used, where a platinum foil served as counter electrode and Ag/AgCl (3 M KCl) was used as reference electrode. The analysis was carried out using an O-ring cell with an exposed sample area of 0.38 cm^2 with a potential sweep rate of 1 mV/s.

2.5. Bioactivity in-vitro assessment

The bioactivity of the coatings was determined through immersion in simulated body fluid (SBF) using Kokubo's protocol [44]. The samples with an area of 2.25 cm^2 were immersed in 50 mL SBF (pH = 7.4) for 7 days at 37 °C. XRD was used to evaluate the formation of hydroxyapatite (HA) on the coatings.