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Electrophoretic deposition of tetracycline hydrochloride loaded halloysite nanotubes chitosan/bioactive glass composite coatings for orthopedic implants

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ABSTRACT

Electrophoretic deposition (EPD) was used to apply bioactive multifunctional composite coatings with antibacterial substances on stainless steel AISI 316L (SS). Tetracycline hydrochloride (TCN) loaded halloysite nanotubes were co-deposited with chitosan and bioactive glass (BG) particles to produce composite coatings. SEM/EDX, XRD, and FTIR analyses were performed to characterize the composition and microstructure of coatings. The release of tetracycline hydrochloride (TCN) in phosphate buffered saline (PBS) was investigated by UV spectrometry, and measurements indicated the release of around 54% of the drug within 14 days of immersion in PBS. Furthermore, to determine that the bioactivity of coatings had not been adversely influenced, simulated body fluid (SBF) bioactivity tests were performed. The formation of hydroxyl carbonate apatite on the surface of the coatings was confirmed after 3 days. The ability of coatings to prevent bacterial growth was tested using *E. coli* as gram-negative and *S. aureus* as gram-positive bacteria. Results showed improved bactericidal effect of TCN-containing coatings compared to non-TCN loaded coatings. The corresponding amount of TCN loaded in EPD coatings supported cell viability and proliferation of MG-63 cells for up to 3 days. Fluorescence images of MG-63 cells showed evidence of cell growth in islands on the coated surface. The surface roughness of the coating loaded with halloysite nanotubes supported cell adhesion and proliferation. Additionally, the wettability value of the coatings confirmed a moderately hydrophilic surface, which is suitable for bone regenerative applications. Improved corrosion resistance compared to the pure stainless steel (SS) substrate was confirmed. The adhesion between coatings and substrates was tested by the tape test, and the result showed sufficient adhesion of the coatings to be handled without detachment. In all, the new coating system has potential for applications in orthopedics.

1. Introduction

One convenient method to produce coatings on metallic substrates is electrophoretic deposition (EPD) [1–3]. For attaining a uniform particle packing structure of electrophoretic deposits, adequate stabilization of suspensions is required; this depends on numerous factors including the amount of surfactant, suspension concentration, pH and conductivity [1,4]. EPD is advantageous because it offers the possibility of coating substrates of complex shape, allows accurate control of coating thickness and requires simple equipment [1,5,6]. A distinct group of bioceramics are bioactive glasses (BGs). These are amorphous silicate-based materials, which are classified as osteo-productive, i.e. they can bond to bone and induce new bone growth while dissolving over time when in contact with biological fluids. Bioglass® 45S5 (composition: 45 SiO_2, 24.5 Na_2O, 24.5 CaO, 6 P_2O_5 in wt%) was the first bioactive glass formulation, which was shown to have the ability to bond to the host tissue [7]. The formation of hydroxyapatite (HA) on bioactive glass surfaces upon contact with physiological fluids is responsible for the bone-bonding properties of bioactive glasses [8,9]. These materials stimulate cell attachment [10], and their dissolution products have been shown to control the gene expression in human osteoblastic cells [11,12]. Bioactive

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glasses can be combined with a biopolymer to coat metallic implants, forming organic-inorganic composite coatings for the modification of implants in load-bearing areas [3,13]. Chitosan is an interesting polymer that has been widely used to produce a variety of coatings in combination with electrophoretic deposition (EPD) [1,2,13–15]. Due to its biodegradable behavior [16,17], chitosan has been investigated in different forms in orthopedics, for example, to functionalize metallic implants [6,18], as a bone cement additive and as composite biodegradable scaffolds for drug and cell delivery systems [18]. Halloysite nanotubes (HNTs) are novel natural nanomaterials with predominantly hollow tubular nanostructures. HNTs are a type of aluminosilicate clay of the formula $Al_2Si_2O_5(OH)_4 \cdot nH_2O$, where 'n' equals to 0 or 2, representing HNTs, which are dehydrated and hydrated, respectively [19–22]. HNTs have different morphologies but the tubular structure is the most common [19,22,23] with typical dimensions of 1–30 nm in inner diameter and 30–50 nm in outer diameter with 100–2000 μm in length [19,22,24]. HNTs have high mechanical strength and modulus, which makes them ideal materials for preparing different polymer-based composites [19,25,26]. It has been reported [27–33] that HNTs are biocompatible and potentially used as drug delivery vehicles, therefore HNTs-polymer nanocomposites are promising drug releasing carriers [27,34,35]. Nanocomposite coatings containing a polyelectrolyte and halloysite nanotubes can be fabricated using electrophoretic deposition [27,36,37].

The formation of bacterial biofilms on orthopedic implants is a serious medical problem as such biofilms are extremely resistant to both the immune system and antibiotics [38]. For treatment of such conditions, local delivery of drugs can be effective to achieve the desired results [39]. Coating of the implant with a drug releasing and bioactive coating is one of the promising techniques that has been explored for tackling implant infections [40]. Tetracycline hydrochloride (TCN), a polyketide antibiotic, was used as a model drug in this study to show the feasibility of EPD for obtaining drug loaded composite coatings.

Therefore, we suggest the fabrication of TCN-loaded halloysite nanotubes in chitosan/Bioglass® 45S5 composite coatings using EPD. The aim of this research is to explore the antibacterial functionality of TCN-loaded HNTs incorporated in an organic-inorganic composite coating. The novel coatings differ from similar electrophoretic coatings developed previously (e.g. [1,2]) in the enhanced multifunctionality provided by TCN-loaded halloysite nanotubes. Furthermore, to show the EPD potency for obtaining such chitosan/BG/HNTs/TCN composite coatings, SEM/EDX, XRD and FTIR analyses were performed to characterize the coatings. The release of antibacterial agents in phosphate buffered saline (PBS) was investigated by UV spectrometry. Simulated body fluid (SBF) bioactivity tests were performed to assess the potential suitability of the coatings as bone contacting materials. The ability of coatings in preventing bacterial growth was tested using *E. coli* as gram-negative and *S. aureus* as gram-positive bacteria. The attachment, proliferation and cytotoxicity of MG-63 osteoblast-like cells on the fabricated coatings were explored. The adhesion between coatings and substrates was tested by the tape test and surface roughness, contact angle and corrosion behavior tests were performed on fabricated coatings to assess their suitability for possible orthopedic applications.

2. Experimental procedure

2.1. Materials

Chitosan powder (C) with a deacetylation degree of about 85% (MW = 190 kDa), halloysite nanotubes (HNTs) with a tube diameter of 20–90 nm (Fig. 1) and lengths of 70 μm and acetic acid were purchased from Sigma Aldrich (Germany). HNTs were investigated by transmission electron microscopy (TEM) using a JEOL JEM-2010 ARP microscope (Japan) operating at 200 kV. A thin foil for TEM investigation was prepared by dispersing the powder in ethanol and stirring in order to separate agglomerated particles. Finally, a droplet of the stable suspension was placed on a copper grid covered with carbon film and dried. Bioactive glass powder (45S5 BG) used in this study had a median particle size of 2.0 μm (Schott AG. Germany). Tetracycline hydrochloride (TCN) was purchased from AppliChem GmbH-Darmstadt (Germany). Ethanol was purchased from Merck KGaA (Germany).

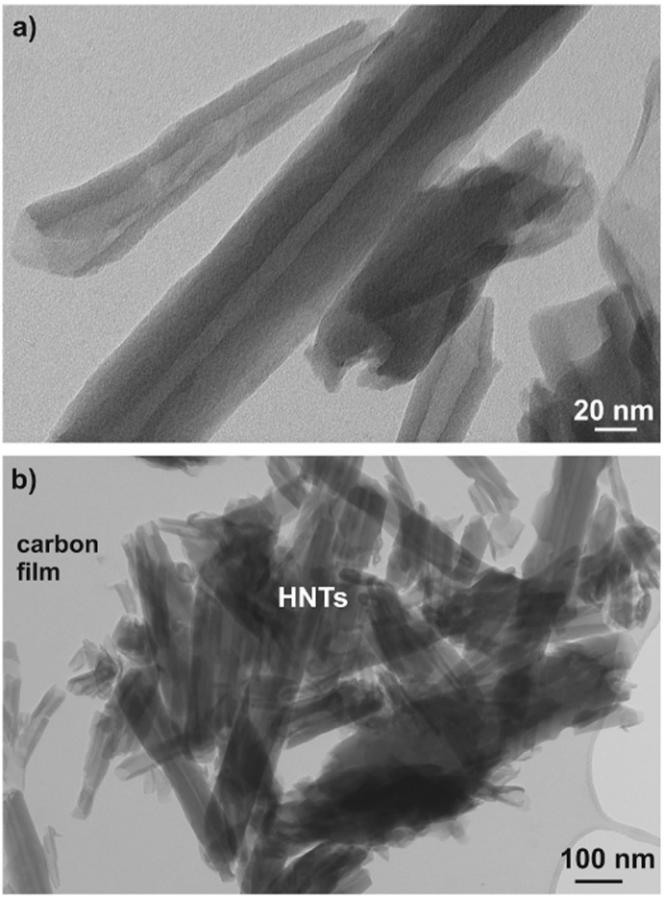

Fig. 1. TEM bright field (BF) micrographs of separate HNTs (a) and HNTs agglomerate (b).

2.2. Loading of HNTs with TCN

The preparation of HNTs loaded with TCN was carried out following a protocol available in the literature [41]: first, TCN (10 mg/L) was dissolved in phosphate buffered saline PBS at 80 °C for 20 min. HNTs (10 mg/L) were then mixed with the drug solution (TCN). The mixture was placed in an ultrasonic bath for 60 min. Vacuum (0.85 bar) was applied for 12 min using a vacuum pump (BRAND GmbH, Germany). The solution was then taken from the vacuum and shaken by hand for 5 min. The application of vacuum was then repeated for 12 min. TCN-loaded HNTs were dried in an oven (Nabertherm, Germany) for 16 h at 50 °C. The dried powders were ground gently using a mortar and pestle. It should be pointed out that in this study the absolute drug loading was not optimized (or considered as a variable) as for each potential application of the coatings a different drug loading may be required. The objective of the experiment was rather to demonstrate the ability to load and release a certain concentration of TCN from the loaded HNTs present in the electrophoretically deposited chitosan based coatings.

2.3. EPD of composite coatings

Chitosan-BG-Halloysite nanotubes with tetracycline (C-BG-HNTs-TCN) composite films were prepared from suspensions containing 2.0 g/L 45S5 BG with 0.5 g/L of chitosan in the blend of HNTs-TCN as