

# Enhancement of antimicrobial and long-term biostability of the zinc-incorporated hydroxyapatite coated 316L stainless steel implant for biomedical application

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## Abstract

Antimicrobial hydroxyapatite (HAp) nanoparticles with different concentrations (0, 3, and 6 mol%) of zinc were prepared by the ultrasonication process. The prepared nanoparticles and chitosan (CTS) composite were coated on 316L stainless steel implant by spin coating technique. The powder samples were characterised by particle size analyser, X-ray fluorescence, and X-ray diffraction studies. The morphology of the coating was investigated by scanning electron microscopy. The diameter of the particle size decreased with increase in the concentration of zinc in HAp structure. The structure of the coated implant was found to be uniform without any cracks and pores. Antimicrobial activity of the composites against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi* and *Pseudomonas aeruginosa* was analysed. The results showed that the increase in the concentration of zinc enhances the antimicrobial properties of 316L stainless steel implant. The stability of the implant in physiological environment was characterised by electrochemical impedance spectroscopy and polarisation analysis. The higher concentration of the ZnHAp/CTS composite shows higher corrosion resistance than that of the HAp/CTS-coated implant. This study shows that the coating provides corrosion resistance to the stainless steel substrate in simulated body fluid (SBF). The *in vitro* bioactivity study of the coated samples immersed in SBF solution confirms the formation of bone-like apatite layer on the surface of the implant. Thus, highly biocompatible ZnHAp/CTS-coated materials could be very useful in the long-term stability of the biomedical applications.

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## 1. Introduction

316L stainless steel (SS) is used for restoration of anatomical structure because of its high mechanical strength, good biocompatibility and cost effective. It is widely used in the orthopaedic surgeries such as joint replacement and fracture fixation [1]. The corrosion rate of metallic implant in the human body must be negligible. Over the past two decades, the ion release and corrosion properties of metallic implant materials under physiological conditions have been extensively studied under physiological conditions [2]. The release of iron, nickel and

chromium from the implant leads to unwanted reaction around the implanted area, which may cause permanent implant failure. Thus, the corrosion-resistant coating and surface modification of the implant are required [3,4]. Coating of bioactive materials using hydroxyapatite (HAp) in the metallic implant has many advantages, including improved corrosion resistance of implant surface and enhanced biointeraction with the surrounding tissues [5]. HAp is one of the naturally available biocompatible and bioactive materials that show the ability to interact with surrounding bone [6]. Low tensile strength, low mechanical properties and high dissolution rate of HAp limit its role in biomedical applications [7]. HAp contains trace amount of cations (Mn, Mg, Zn and Sr) and anions ( $\text{SiO}_4$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{CO}_3$ ), which influence its physical properties [8]. The apatite structure has great flexibility

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in accepting substitution. The introduction of small quantities of ions to HAp structure improves the quality of the clinical applications [9].

Majority of the infections after orthopaedic implantation occur mainly due to the microorganisms present in the implanted materials, leading to permanent implant failure [10]. Coating of several antimicrobial ions such as copper, zinc and silver minimises bacterial load on the implant surface. The small amount of zinc is essential for metabolic processes and DNA replication, whereas its increased concentration is potentially toxic to the living organisms [11]. Zinc substitution on HAp has important implications because natural bone and teeth enamel are composed of trace amount of zinc in HAp structure [12]. The incorporation of small quantities of zinc in HAp lattice promotes bone formation around the implant and enhances mechanical strength [13]. Owing to extremely low solubility of HAp, the release of Zn ion is slow in the ZnHAp and this enhances the bone formation (osteoblast activity) around the implant [14].

To develop a homogeneous coating with high mechanical properties, we prepared an organic–inorganic composite by dispersing ceramic nanoparticles in polymer matrix [15,16]. Chitosan (CTS) is one of the highly biocompatible natural polymers that provide temporary mechanical strength to the cell in-growth [17]. Thus, its addition improves osteoblast mineralisation. Owing to this unique property, CTS has attracted great attention in the field of orthopaedic surgery. The coating of HAp ceramics with CTS at low temperature imparts chemical stability, biocompatibility and antimicrobial properties without degrading the mechanical properties of the SS substrate [18].

The aim of this study was to prepare HAp and ZnHAp nanoparticles to enhance antimicrobial properties by the ultrasonication method. The developed HAp and ZnHAp nanoparticles were mixed with CTS and coated on the 316L SS substrate by spin coating technique. Elemental composition, particle size and the functional group of the ZnHAp were determined. The corrosion stability of the HAp and ZnHAp/CTS composites coated on the SS plate in prepared simulated body fluid (SBF) was examined. The antimicrobial activity of the ZnHAp/CTS composites against bacterial strains was also analysed. In addition, using SBF, the Zn-coated samples were further analysed for its *in vitro* bioactivity.

## 2. Experimental procedure

### 2.1. Materials

Calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ ; 99.9%; Merck AR), diammonium hydrogen phosphate ( $(\text{NH}_4)_2\text{HPO}_4$ ; 99.9%; Merck AR), ammonium hydroxide (25%; Merck GR), and zinc nitrate (99.8%; Merck GR) were used as precursors. All the chemicals were used as such without any further purification. Ultrapure water (Arium 611

Ultrafilter; Sartorius AG, Germany) was used throughout the experiment.

### 2.2. HAp and ZnHAp nanocomposite preparation

HAp nanocomposites doped with different concentrations (0, 3 and 6 mol%) of zinc with a stoichiometric ratio of 1.67 were prepared through the ultrasonication process using a sonochemical reactor (VC 505; Sonics, USA). In this process,  $\text{Ca}(\text{NO}_3)_2$  was dissolved in ultrapure water at room temperature under sonication at 30 kHz. After 15 min, zinc nitrate with different concentrations was added to the solution. To achieve the stoichiometric ratio,  $(\text{NH}_4)_2\text{HPO}_4$  solution was added dropwise into  $\text{Ca}(\text{NO}_3)_2$  solution. The pH of the solution was adjusted to 10.2 using ammonium hydroxide solution and the ultrasonication process was continued for 1 h. The HAp nanocomposites containing different mole percentages of zinc (i.e., 0, 3 and 6 mol%; hereafter termed HAp, Zn3HAp and Zn6HAp, respectively) were then filtered, washed and dried at 353 K for 12 h. Once dried, the precipitates of pure and zinc-doped HAp samples of all compositions were ground into a fine powder.

### 2.3. ZnHAp/CTS composite coating on the 316L SS substrate

Prepared HAp and ZnHAp powders (1 g) were individually mixed with 5% acetic acid solution containing 1 wt% CTS. The composites were ultrasonicated for 30 min at 30 kHz for uniform and complete dispersion. The dispersed polymer composites were coated on the 316L SS plate by spin coating technique. Before the coating,  $10 \times 7 \text{ mm}^2$  SS specimens were mechanically polished using series silicon carbide papers (50–1600 grits). Then, the specimens were washed three times with acetone followed by distilled water. Two to three drops of the uniformly dispersed polymer composite solution containing ZnHAp were placed on the prepared 316L SS plate, which was then spin coated at 2500 rpm. The coated plate was then dried in a hot-air oven at 353 K. The coating and drying processes were repeated thrice for a better, thick and uniform coating. Finally, the coated plate was dried at 353 K for 12 h.

### 2.4. Characterisation

#### 2.4.1. Size and composition measurement

The diameter of the HAp and ZnHAp samples was measured using dynamic light scattering (DLS; Nanophox; Sympatec, Germany) in the range of 1–500 nm with the scattering angle of  $90^\circ$ . The light source used was HeNe laser with a maximum intensity of 10 mW. Elemental composition of the prepared HAp and ZnHAp samples was determined using an X-ray fluorescence (XRF) spectrometer (EDX-720; Shimadzu, Japan). The Ca/P/Zn ratio in the HAp structure was measured.

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