



Antibacterial Efficacy of Halloysite Nanotube Minerals Substituted Hydroxyapatite Composite on Titanium Alloy using Electrodeposition Method

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Bacterial infections are the most common and serious complications for titanium based implants in the biomedical industry, and the ideal implant specimens should combine as an excellent bioactivity and good antibacterial ability. Therefore, the antibacterial agents containing composites coated with implant surfaces to prevent the bacterial infections. The present study is an investigation of the effect of hydroxyapatite exclusion on the corrosion resistivity, antibacterial behaviour, and bioactivity of HNT-PEDOT-MHA composite coatings on titanium alloy surfaces. Antibacterial tests showed that pure hydroxyapatite coating did not exhibit any antibacterial activity, whereas the presence of Zn²⁺ in hydroxyapatite composite resulted in a good activity against *S. aureus* and *E. coli*. Potentiodynamic polarization tests were performed on both modified hydroxyapatite (MHA) and nanotube halloysite (HNT) as incorporated with poly(3,4-ethylenedioxythiophene) (PEDOT) coatings shows a better anticorrosion property when compared to uncoated titanium alloy, it also has an excellent biological activity in biomedical applications.

Keywords: Biomedical, Antibacterial agents, Composites, Bacterial infections, Corrosion resistivity.

INTRODUCTION

Titanium and its alloys are of a great importance for the development of prosthetic materials in hard tissue replacements as well as in the biomedical applications because of its excellent fabrication properties, lower cost, broader availability, good fatigue strength, high corrosion resistance, formability, machinability and bio-stability [1-4]. However, they show a poor osteo-integration properties and cannot meet all of the clinical requirements such as inflammation, corrosion, wear, fracture toughness or low fatigue strength and mismatch in elastic modules between the implants and bone interface [5-7]. Therefore, in order to improve the chemical, biological properties, surface modification is often performed. It was also reported that coated materials such as TiO₂, Al₂O₃, ZrO₂ and hydroxyapatite (HA), possess an outstanding corrosion resistance, high chemical stability, mechanical properties when used as coating materials.

In this case the implants are coated with bioactive materials like hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂] (HA) for bone-cells activation and encouraging in-growth of natural bone into the prosthetic

device [8-11]. However, pure hydroxyapatite cannot be used in the load bearing applications for the reason of the lack of antibacterial activity and poor mechanical properties that affects its long term stability and engenders implant failures [12,13]. Strontium containing composite shows excellent biological role, were it increases the activity of bone-forming osteoblasts and decreases the activity of bone-resorbing osteoclasts [14,15]. Strontium(II) can also play an important role to prevent inflammation, improve bone microstructure and strength [16-18]. Zinc is an essential mineral in the biological functions that plays a crucial role in the biochemistry of bone tissue [19,20]. On the other hand, Zn²⁺ is one of the well-known antibacterial agents, which has a high thermal stability and non-toxic to the human cells [21,22]. Recently conducting polymers have been widely studied in promising materials used for several fields because of their chemical and electrical properties that possess conjugated chain structures [23,24].

A poly(3,4-ethylenedioxythiophene) (PEDOT) is one of the most favourable materials and relatively less studied electrically conducting polymer [24,25] and studied with numerous

applications such as for drug delivery system, biosensors, anti-corrosion coating, film forming agent, biomedical applications, because of their high conductivity, ease of synthesis, inherent features, electric, and thermal stability and biocompatibility and potential for copolymerization without compromising the electroactivity favour the choice of PEDOT [26-32]. However, PEDOT has some limitations such as poor bone bonding ability and also the mechanical stability, processing, water up taking and resulting in low stability which needs to be further improved [33]. In order to overcome this problem, preparation of composites with suitable conducting substrates.

The biocompatible properties of halloysite [HNT, $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$] is a naturally available material, which makes it as a prospect for medicinal and household products [34-36]. Halloysites used for encapsulation and controlled release different chemical agents such as antimicrobial agents, drugs, antiseptics, proteins and corrosion inhibitors within protective coatings improves anticorrosive performance and mechanical properties for implant surfaces [37,38]. Halloysites allow different chemical reactions in these tubes interior and external surfaces. Because of its interior and exterior surfaces of halloysites are composed respectively of aluminol (Al-OH) groups and siloxane (Si-O-Si) groups, yielding a positively charged inner lumen and a negatively charged outer surfaces. Halloysites with polymer creates superior adhesion, improves delivery of active ions and enhances both toughness and strength of composite coating [32,39-43]. There are different coating methods available for the development of bioceramic coatings and among the existing methods for the preparation of HNT-PEDOT-MHA coatings. The electrodeposition method has attracted greater interest because of the easy availability and simplicity, due to the ability to produce smooth coatings, good adherence with substrate, relatively low deposition temperature, controls the coating thickness and reduces the formation of surface cracks. As there are no previous reports available on HNT-PEDOT-MHA composite coating on Ti alloy using electrodeposition, an attempt is made to get improved biological properties as well as corrosion protection and mechanical properties of the composite coatings.

EXPERIMENTAL

The chemicals used for modified hydroxyapatite (MHA) electrolyte were commercially available as pure calcium nitrate tetrahydrate, zinc nitrate tetrahydrate, strontium nitrate, diammonium hydrogen phosphate, ammonium hydroxide, hydrochloric acid, ethanol, acetone and solutions were used in this study. The halloysite nanotubes (HNT) and 3,4-ethylenedioxythiophene (EDOP) monomer were used to fabricate the composite coatings. All chemicals were purchased from Sigma-Aldrich. All other reagents were analytical grade and used without any further purification. Deionized water was used throughout the experiment.

Preparation of substrate: Titanium alloy (10 mm × 10 mm × 3 mm) was used as substrate. The substrate was ground with abrasive paper through grit size 120-2000, washed with double distilled water, in acetone for 30 min and finally rinsed in distilled water, dried to be prepared for coating and used for further studies.

Preparation of electrolyte: The electrolyte used for the deposition of HNT-PEDOT-MHA composite was prepared by the mixing of an analytical grade solution of 0.1 M HNT and 3,4-ethylenedioxythiophene (EDOP) in an airtight container. The electrolyte for MHA was prepared by dissolving reagents like 0.4 M ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.05 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.05 M $\text{Sr}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 0.3 M $(\text{NH}_4)_2\text{HPO}_4$ in deionized water to produce the target with (Ca + Zn, Sr)/P ratio of 1.67 and the solutions was continuously monitored [15,21] and separately adjusted to pH 4.5 by adding either a solution of HCl (or) a diluted solution of 0.1 M NH_4OH .

Electrodeposition of composite on Ti alloy: The electrodeposition of HNT-PEDOT-MHA composite coated with titanium alloy substrate was performed in the conventional three electrode cell configuration system using electrochemical workstation (CHI 760 USA). In which platinum electrode was used as the counter electrode, saturated calomel electrode (SCE) and titanium alloy surfaces as the reference and working electrode, respectively. All the potential values in the text are reported with respect to SCE. The electrolyte for HNT-PEDOT-MHA composite coated with titanium alloy in an aqueous solution containing 0.4 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.05 M $\text{Sr}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.3 M $(\text{NH}_4)_2\text{HPO}_4$, 0.1 M halloysite nanotubes (HNT) and 3,4-ethylenedioxythiophene (EDOT) under magnetic stirring at room temperature. The deposition process of HNT-PEDOT-MHA composite was carried out in galvanostatic mode at a current density of 2 mA/cm² for 30 min [36]. The electrolyte solution was stirred at the speed of 300 rpm after the coated sample washed with double distilled water for several times to remove loosely adhering coating and before it was dried in air.

Characterization of HNT-PEDOT-MHA composite coatings: To evaluate the corrosion behaviour of uncoated and coated implant specimen were evaluated by using polarization tests and electrochemical impedance spectroscopy (EIS) test. The corrosion test was carried out in simulated body fluid solution at 37 °C on CH760C Electrochemical Analyzer using a three-electrode cell configuration system with platinum as auxiliary electrode, coated and uncoated implant specimens the working electrode and saturated calomel electrode as a reference electrode. The polarization curves were measured at a potential range between -0.8 to 0.2V vs. SCE at a scan rate of mV s⁻¹ with respect to open-circuit potential (OCP) [36]. The impedance spectra were recorded in the frequency range from 10 MHz to 100 kHz with 5 mV amplitude. All the electrochemical studies were repeated thrice to confirm reproducibility of the obtained results.

Surface characterization: The surface morphologies of the coatings as well as corrosion products were observed using a high resolution scanning electron microscopy (HR-SEM). Before coated with gold sputtering to improve the conductivity, the phase composition of the coating scraped samples were characterized by X-ray diffraction (Rigaku Dymax) using with CuK_α radiation ($\lambda = 1.5406 \text{ \AA}$). The XRD patterns were taken in 2θ range from 10° to 80° with a step size of 0.04°.

Antibacterial activity: The *in vitro* antibacterial activity of HNT-PEDOT-MHA composite coated sample were evaluated against two bacterial strains: Gram-positive *S. aureus* (ATCC 25923) and Gram-negative *E. coli* (ATCC 25922) by the agar

disc diffusion method. The study was examined in Muller-Hinton agar and the diffusion technique was carried out by pouring agar for 4 mm thick layers into the petri dishes and then nutrient medium were evenly inoculated with tested microorganisms and then incubated at 37 °C in tryptic soy broth for 24 h. Discs of 6 mm diameter were prepared from Whatman filter paper, immersed into different (20, 40, 60 and 80 μL) volumes of HNT-PEDOT-MHA composite coated samples. The antibacterial result of the tested samples of inhibition was calculated by measuring the width of inhibited zone.

RESULTS AND DISCUSSION

Morphological results of composite coatings: The surface morphologies of PEDOT-MHA, PEDOT-HNT and PEDOT-HNT-MHA were examined by HR-SEM. The micrographs of polymers showed the excellence of their coating formation ability. From HR-SEM image of PEDOT-MHA possessed smooth and tight surface with flake like structure decorated on the surface (Fig. 1a) and the flake with combined rod like topography was observed on the PEDOT-HNT coating (Fig. 1b). These coating exhibited a homogeneous and compact structure. As regards, conducting polymer of PEDOT with MHA and HNT (Fig. 1c), spherical with chain like structures were observed on PEDOT-HNT-MHA coating. The difference of morphology between PEDOT, MHA, and HNT arises during the process of electrodeposition. The cross-sectional HRSEM morphology of as-formed PEDOT-HNT-MHA composite on Ti alloy is shown in Fig. 1d. The composite coating exhibit a thickness of around $\sim 40 \mu\text{m}$ on Ti alloy. However, PEDOT-HNT-MHA coating showed a uniform and continuous coating without cracks being observed along its length on Ti alloy. Hence, PEDOT-HNT-MHA composite coating can be an effective barrier on Ti alloy and may be more corrosion protected in SBF. This composite coated surface will provide improved physico-chemical properties, biological properties and bonding strength to Ti substrate.

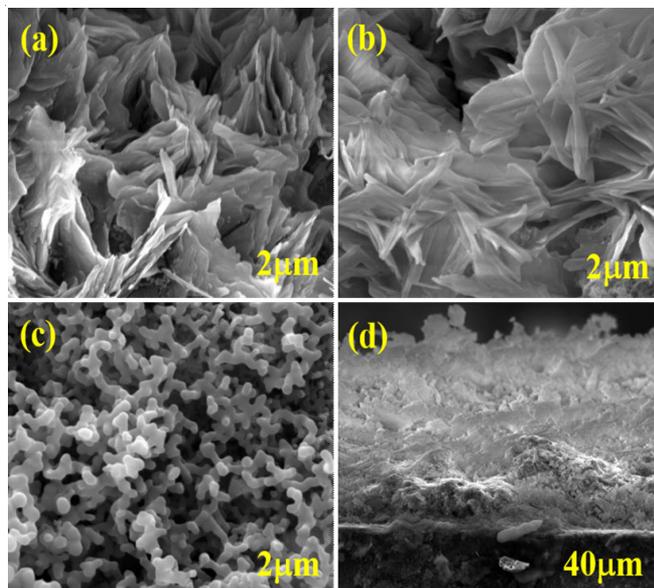


Fig. 1. HRSEM micrographs of (a) PEDOT-MHA composite coated on Ti alloy (b) PEDOT-HNT composite coated on Ti alloy (c) HNT-PEDOT-MHA composite coated on Ti alloy (d) cross section image for HNT-PEDOT-MHA composite coating

X-ray diffraction: The XRD patterns of PEDOT-MHA, PEDOT-HNT and PEDOT-HNT-MHA composite coated Ti alloy specimens are shown in Fig. 2. It can be confirmed that PEDOT was compounded with PEDOT-HNT-MHA matrix. The XRD results demonstrate that MHA peaks can be clearly identified from Fig. 2a at nearby 25°, 28°, 31°, 32°, 40° and 43° at 2θ angles with good agreement with the standard data for ICDD card No. 09-0432. Thus, the XRD analysis provided clear evidence that PEDOT-MHA composite were successfully coated onto the surface of Ti alloys [16,22]. Fig. 2b shows the diffraction peaks of PEDOT-HNT composite coating are located at nearby 2θ values of 20°, 24°, 35°, 36°, 54° and 62°, which are in agreement with ICDD card no. 29-1487. The remaining diffraction 2θ peaks were observed at 12° and 26° of corresponding to PEDOT, respectively. As it can be seen from Fig. 2a-b, the XRD peaks of both PEDOT-MHA and PEDOT-HNT are observed in the diffraction pattern of PEDOT-HNT-MHA nanocomposite coating (Fig. 2c). Thus, all these peaks shown in Fig. 2c demonstrate that composite coating contains both MHA and PEDOT-HNT crystalline phases.

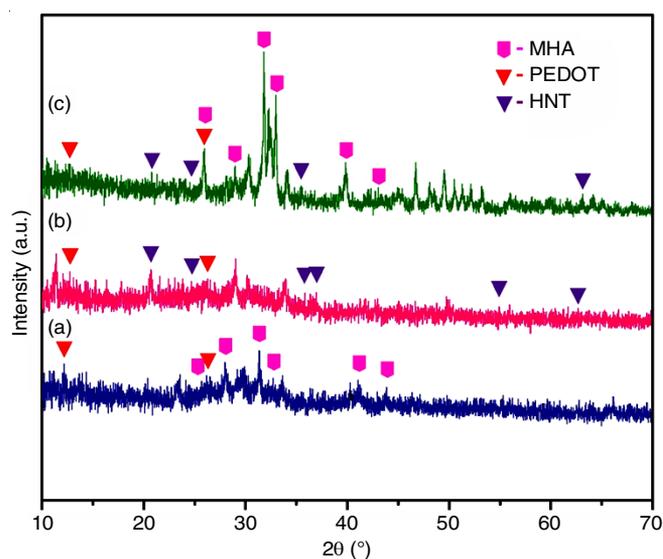


Fig. 2. XRD pattern of (a) PEDOT-MHA composite coated on Ti alloy (b) PEDOT-HNT composite coated on Ti alloy (c) HNT-PEDOT-MHA composite coated on Ti alloy

Electrochemical corrosion test: The corrosion resistance of coated and uncoated implant specimens were examined by the polarization test under contact in SBF solution. From Fig. 3a, it can be seen that developing composite coating on Ti alloy provides nobler illustrations and improve the corrosion resistance of implant surfaces. The polarization curves of PEDOT-HNT-MHA coated Ti alloy are shifted towards higher potentials (E_{corr}) and the lower current density (I_{corr}) with respect to PEDOT-HA and PEDOT-HNT coated Ti alloy which evidences of improvement in corrosion resistance. As it could be seen in Table-1, uncoated Ti alloy possesses higher corrosion current density and lower corrosion potential than the coated samples in SBF solution. The corrosion potential of bare Ti alloy was as low as -0.616 V , while PEDOT-MHA coated dramatically increased its corrosion potential up to -0.582 V . The corrosion current decreased from 1.790×10^{-5} to $4.013 \times 10^{-6} \text{ A cm}^{-2}$, which illustrates that PEDOT-MHA coating can enhance the corrosion

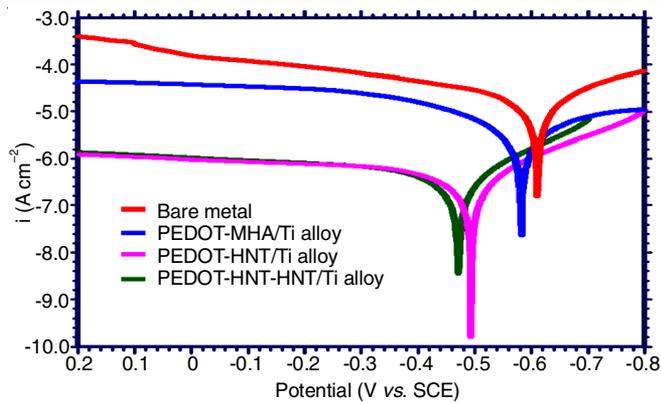


Fig. 3(a). Tafel plots in the SBFs solution for the (a) Uncoated Ti alloy, (b) PEDOT-MHA coated Ti alloy (c) PEDOT-HNT coated Ti alloy (d) PEDOT-HNT-MHA coated Ti alloy

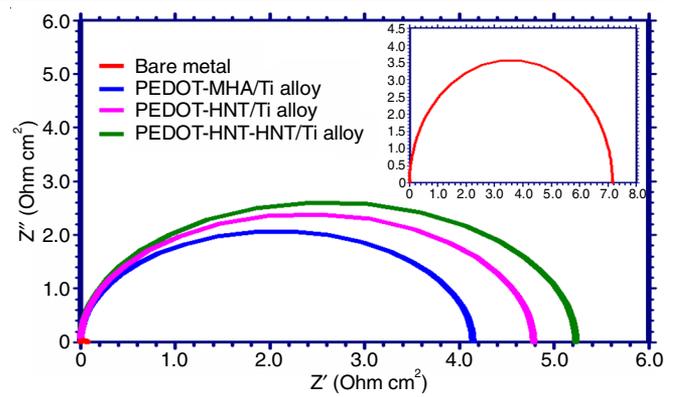


Fig. 3(b). Uncoated Ti alloy, (b) PEDOT-MHA coated Ti alloy (c) PEDOT-HNT coated Ti alloy (d) PEDOT-HNT-MHA coated Ti alloy

TABLE-1

Sample name	E_{corr}	I_{corr}	Impedance
Bare titanium alloy	-0.616	1.790×10^{-5}	7.152×10^4
PEDOT-MHA	-0.582	4.013×10^{-6}	4.140×10^6
PEDOT-HNT	-0.493	2.943×10^{-7}	4.781×10^6
PEDOT-HNT-MHA	-0.471	3.287×10^{-7}	5.228×10^6

resistance of Ti alloy. After the composite coated Ti alloy showed further improvement in terms of corrosion resistance of bare Ti alloy. The PEDOT-HNT-MHA coated samples showed higher E_{corr} and lower I_{corr} values than PEDOT-HNT coated Ti alloy. For the samples coated with PEDOT-HNT-MHA, the E_{corr} values were -0.471 and -0.493V, whereas the corresponding I_{corr} values were 3.287×10^{-7} and 2.943×10^{-7} A cm⁻², respectively. Therefore, it is expected that release of particles or metal ions from the implant surface may effectively be blocked by PEDOT-HNT-MHA composite coating which prevents the inflammation reaction with the surrounding tissues. The use of PEDOT-HNT-MHA formed densely packed and crack-free coating on Ti alloy, thus the embedded composite coating significantly increased the corrosion resistance.

Electrochemical impedance (EIS): The EIS measurements are also conducted to further appeal the protective efficacy of implant coatings. The Nyquist plots drawn from impedance spectra of uncoated and composite coated implant specimens tested in simulated body fluid solution are shown in Fig. 3b and the results are listed in Table-1. For uncoated and PEDOT-HA composite coated Ti alloy specimen, polarization resistance values are obtained as 7.152×10^4 and 4.14×10^6 Ω cm², respectively, which are lesser than those of PEDOT-HNT composite coated Ti alloy specimen.

In comparison, the acquired polarization resistance (R_p) values for PEDOT-HNT-MHA composite coating has higher corrosion resistance as 5.228×10^6 Ω cm² than that obtained for PEDOT-HNT composite coated Ti alloy (4.781×10^6 Ω cm²) surfaces. From these results, it could be well ascertained that PEDOT-HNT-MHA composite coated Ti alloy is more corrosion resistant than the other composite coated and uncoated Ti alloy samples.

Antibacterial activity: The presence of PEDOT-HNT-MHA composite inhibits bacterial growth. The clear zone formation around the disc indicates bacterial inhibition. The antibacterial activities were firstly conducted towards

S. aureus and *E. coli* bacteria because it appears to be most commonly found in orthopedic implant infections. For PEDOT-HNT-MHA composite sample, the inhibition zone for *S. aureus* and *E. coli* are shown in Table-2. It is evident that the highest concentration of PEDOT-HNT-MHA composite exhibited excellent antibacterial activity against *S. aureus* and *E. coli*. When compared to *S. aureus* bacteria, PEDOT-HNT-MHA composite shows higher antibacterial activity for *E. coli*, which exhibits that composite is more reactive against *E. coli*. The reason may be ascribed to the substitution of minerals such as Zn²⁺ in hydroxyapatite and play a very important role in enhancing the activity and hence the surface area of PEDOT-HNT-MHA composite sample increases by forming bonds with *S. aureus* and *E. coli* leading to cell death.

TABLE-2

Name of the bacteria	Zone of inhibition (mm)			
	20 μL	40 μL	60 μL	80 μL
<i>Escherichia coli</i>	1	5	7	8.6
<i>Staphylococcus aureus</i>	1	2.2	5	6.5

Conclusion

Uncoated Ti alloys, through modification of physical microstructure of the surface, had a beneficial effect on the corrosion inhibitor and thus, cell viability to the material. The HRSEM results clearly show that uniform and homogeneous PEDOT-HNT-MHA composite coatings were successfully fabricated onto Ti alloy via electrodeposition method. Further, the XRD results confirm the formation of composite on Ti alloy samples. Electrochemical corrosion protection studies established that PEDOT-HNT-MHA composite coating on Ti alloy showed enhanced corrosion resistance which is ascribed to PEDOT-HNT-MHA coating on Ti alloy. The *in-vitro* antibacterial studies of PEDOT-HNT-MHA composite coating on Ti alloy exhibited excellent antibacterial properties. In summary, PEDOT-HNT-MHA composite coating might be promising for the surface to exhibit good corrosion resistance and surface biocompatibility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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