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Improvement of Antibacterial Activity Resistance of NiTi Alloy by Electrophoretic Deposition

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***Abstract**:*

Biomedical NiTi shape memory alloys used in surgery suffer from post-surgery bacterial infection issues and it is importance to prevent bacteria adhesion and growth on the implants. In this paper Electrophoretic deposition (EPD) was used as a surface modification technique to improve the antibacterial activity of NiTi alloy by using organic inorganic coating Nanobiocomposite materials (Hydroxyapatite/Titania) were used as a thin deposition layer on NiTi substrate. EPD technique performed with different parameters used, (20-volt, 4 min, 2%C) for deposit layers of HA, (20 volt, 4 mint, 4%C) for TiO_2 layer and (60 volt, 4 min, 2%C) for composite (HA/TiO_2) layer. Chitosan was used as a binder and good antibacterial material with all coating. The antibacterial activity test performed diffusion methods. Two types of bacteria, E. Coli, and Staphylococcus were investigated on the performance of coatings. The results showed that the coatings revealed good inhibition effect towards E. coli and S. aureus (Staphylococcus) within the incubation period than the substrate NiTi alloy. The effect against Staphylococcus (gram positive) was more than the effect against the E. coli (gram negative).

Keywords: Electrophoretic deposition, NiTi alloy, hydroxyapatite, Titania, Antibacterial activity.

1. Introduction

Biomaterials are natural or synthetic materials which are used to make structures or implants, in order to replace the losing or diseased biological structure to repeat the form and function. Biomaterials are used in various parts of the human body such as stents in blood vessels, bone replacement implants in hips, shoulders, knees and elbows [1].

Ti alloy has been widely employed in the creation of implants for various dentistry and orthopedic applications, but is limited by its inadequate bioactivity [2]. NiTi alloys have been used in different biological applications due to their superior properties such as biocompatibility, high corrosion resistance and good mechanical properties but it have high Ni content undergo a series of corrosion reactions in human body fluid , The products of these reactions are released metallic ions, which are toxic in high dosages [3]. To resolve this issue, researchers have proposed depositing coatings, such as hydroxyapatite (HA), metallic oxides (e.g., TiO_2, Al_2O_3), etc., on implant substrates in order to enhance bone/implant interaction while covering the substrate from corrosion [4].

Electrophoretic deposition (EPD) was an attractive technique that used deposit the nanostructured materials from colloidal solution. It was a two-steps process for depositing coatings which was with suspension particles and polymer molecule. EPD requires the homogeneous dispersion of charged particles in a suspension medium. The suspension should be stable over the deposition period to prevent uncontrolled microstructure of the coatings [5-6].

Sintering at high temperature is an important step in EPD in order obtain dense coating layers with good mechanical strength for coating layer. Sintering step leads to significant oxidation, thermal stresses of the substrates, chemical reactions between HA and the substrates and shrinkage [7]. To solve this problem, biopolymer (chitosan) was used as a binder among coating layers. Chitosan is one of the most promising natural biopolymers for tissue engineering it has an Important properties chitosan like: chemical stability biocompatibility, good antimicrobial activity [8].

In this study, nanobiocomposite coatings of bio ceramics hydroxyapatite (HA) and titanium oxide have been deposited on (TiO_2) on NiTi shape memory alloy substrate with chitosan was developed using electrophoretic deposition (EPD) [9-10].

2. Experimental Procedures

NiTi shape memory alloy has the chemical composition (54.7%,45.6). Hydroxyapatite (30 to100nm, purity of 99%) purchased from Sigma Aldrich was used to deposit it as a coating layer and Titania (99.9% ,30 nm). The Chitosan (medium molecular weight with a degree of deacetylation of about 85% soluble in 1% acetic acid), it was used as binder material to the ceramic material. Acetic acid with purity (> 98%) and Solvents of ethanol absolute (99.9%). The (cathode) was NiTi and (anode) 316L stainless steel. EPD was performed at 20, 40, and 60 volts using a laboratory DC-Power supply. EPD was performed at different deposit times from 2,4 and 6 min. HA, TiO_2 and composite solutions were deposited on NiTi alloy by EPD at room temperature. The different coating layer deposited on five samples by used this parameter. The first sample was coated by HA layer, the second samples coated with TiO_2 layer, the third sample coated with composite (HA+TiO_2) layer, the fourth sample coated with three layers (TiO_2+Composite+HA) and the fifth sample coated with three layers (Acamprosate + TiO_2). scanning electron microscopies were used to characterize the surface morphology and the cross section of the coatings. Assessment of antibacterial (diffusion method) against two types of bacteria (E. coli grams negative and Staphylococcus aureus gram positive). The diffusion method known as qualitative technique since this method will only give an idea of the presence or absence of substances with antimicrobial activity. Two types of bacteria were prepared Staphylococcus bacteria and E. coli bacteria. Two groups contain from (single, multi-layer composites coating samples and sample uncoating) are used. The first group immersed in Staphylococcus bacteria and the second group immersed in E. coli bacteria. The two groups of samples were dispersed in 9 ml of normally saline, and then added of 1 ml of the diluted cell suspensions. Then the mixture was incubated at 37 °C in a shaking incubator (160 rpm) for 24 h. Over a period (overnight), the suspension was serially diluted 10-fold in normal saline (100ml spread out on a solid nutrient agar medium) using spread plate technique. The dishes were incubated overnight at 37 °C, after that a number of organism colonies in the dishes were determined by multiplication a number of colonies with the factors dilution and then compared with a controlling sample, represented bacterial suspension. According to the following equation the percentage of reduction was calculated based on the firstly counted [11].

Colonies forming unit [(CFM)/ml] = (Number of colonies for each dilution) * sample volume/(dilution 10^2 factor)

3. Results and discussions

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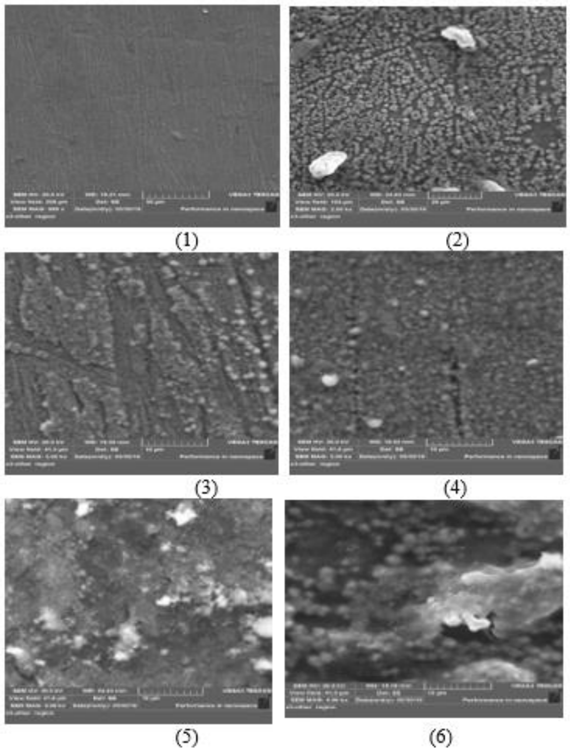
(SEM) is a powerful magnification tool that utilizes focused beams of electrons to obtain information. The high-resolution, three-dimensional images produced by SEMs provide topographical, morphological and compositional. Fig.1 shows the surface morphology and cross section of NiTi alloy before treated to observe homogeneous of coating layers and measure the thickness. The thickness in SEM of HA (50 µm), TiO_2 (33 µm), composite (41 µm), Three layer (TiO_2+Composite+HA) (58 µm) and the three layers (HA+ Composite+ TiO_2) (62 µm). Table 1 shown the Thickness of coating layers by optical microscope an SEM .EDX results in Fig.1 indicates that there are no phases present other than titanium and nickel and HA in sample (1) , titanium and nickel and TiO_2 in sample (2) , titanium , nickel , HA and TiO_2 in composite sample (3) and titanium , nickel , HA and TiO_2 in the multi-layer coating samples (4,5). Its rustle identical with optical microscope examination of the uniformity and thickness of coating layer. This due to good stability of solutions it has been confirmed by zeta potential values. Author Name(s) and Affiliation(s).

Table 1. Thickness of coating layers at optimal condition optical microscope and SEM

Type of sample	Thickness(µm) by SEM
One-layer HA	50
One-layer TiO_2	33
One-layer composite	45
Three layers (Tio_2+ Composite +HA)	58
Three layers (HA+ Composite+ Tio_2)	62



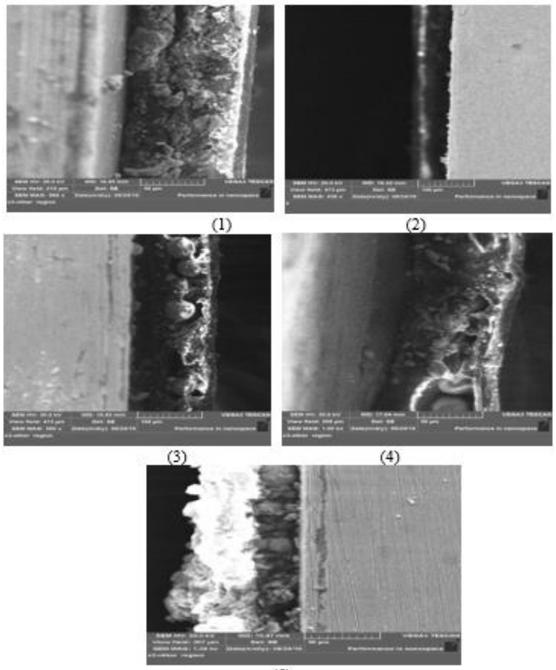


Fig 1. SEM images of topography and cross section for (1) one-layer HA, (2) one-layer TiO_2, (3) one-layer Composite, (4) Three-layer (TiO_2+Composite +HA) and (5) Three-layer (HA+ composite + TiO2) coating samples. 10X

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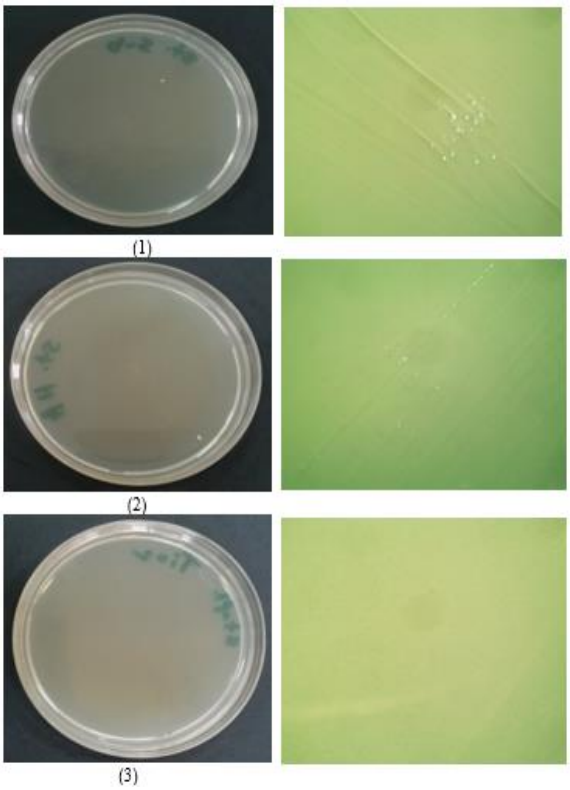
The rustle of immersion in Staph bacteria for samples (HA, TiO_2, Composite, three layer (TiO_2+Composite +HA) and three layer (HA+ composite + TiO_2) and uncoating NiTi substrate) (98%, 98.6%, 100%, 95%, 100%, 99.9% respectively). The rustle of immersion in E. coli bacteria for samples (HA, TiO_2, Composite, three layer (TiO_2+Composite +HA) and three (HA+ composite + TiO_2) and uncoating NiTi substrate) (80%, 100%, 100%, 100%, 100%, 99.9% respectively). Therefore, the presence of different concentration of (HA + TiO_2) nanoparticle inhibits the bacterial growth this material used are considered as a good antimicrobial material due to their antimicrobial activity. Fig. 2 has shown image of the samples for Staphylococcus aureus (gram positive), while Fig 3 shows the result of E. coli (gram negative) the clear zone formation around the disc indicates the bacterial inhibition. Table 2 shows the Percentage of killing two types of bacteria Staphylococcus aurous and E. coli.

The variation in antimicrobial activity of the (HA + TiO_2) nanoparticles due to the variation in surface charges and also due to the Van der Waals interaction between the nanocomposite and the bacterial surface. The surface roughness has a better chance for bacterial adhesion on surface of the composite coatings. Mostly rough surfaces promote greater adhesion of bacterial than smooth surface, roughness of surface have effect on initial adhesion of bacteria, when the bacteria adherent on surface coatings, they were killed because of the effect of antimicrobial of materials. These results agreed with observation of Sigrid et al [12].

Table2 Percentage of killing two types of bacteria Staphylococcus aureus and E. coli

Sample	Type of bacteria	Death%	Type of bacteria	Death
Substrate	Staph	98 %	E.coli	%80
One layer HA	Staph	98.6 %	E.coli	%100
One layer TiO_2	Staph	100 %	E.coli	%100
One layer composite (HA+TiO_2)	Staph	95 %	E.coli	%100
Three layer(TiO_2+Composite +HA)	Staph	100 %	E.coli	%100
Three layer (HA+composite + TiO_2)	Staph	99.9 %	E.coli	%99.9



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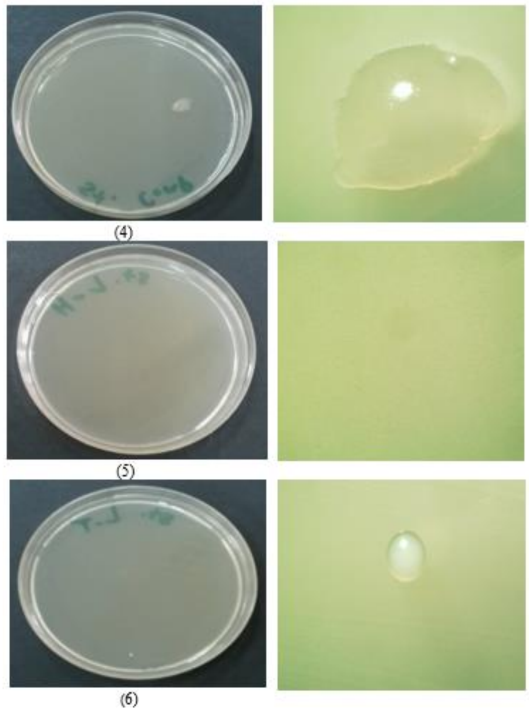

Fig 2 Shows images for Staph bacteria by diffusion method (1) substrate (2) one-layer HA, (3) one-layer TiO_2, (4) one-layer Composite, (5) Three-layer (TiO_2+Composite +HA) and (6) Three-layer (HA+ composite + TiO_2) coating samples

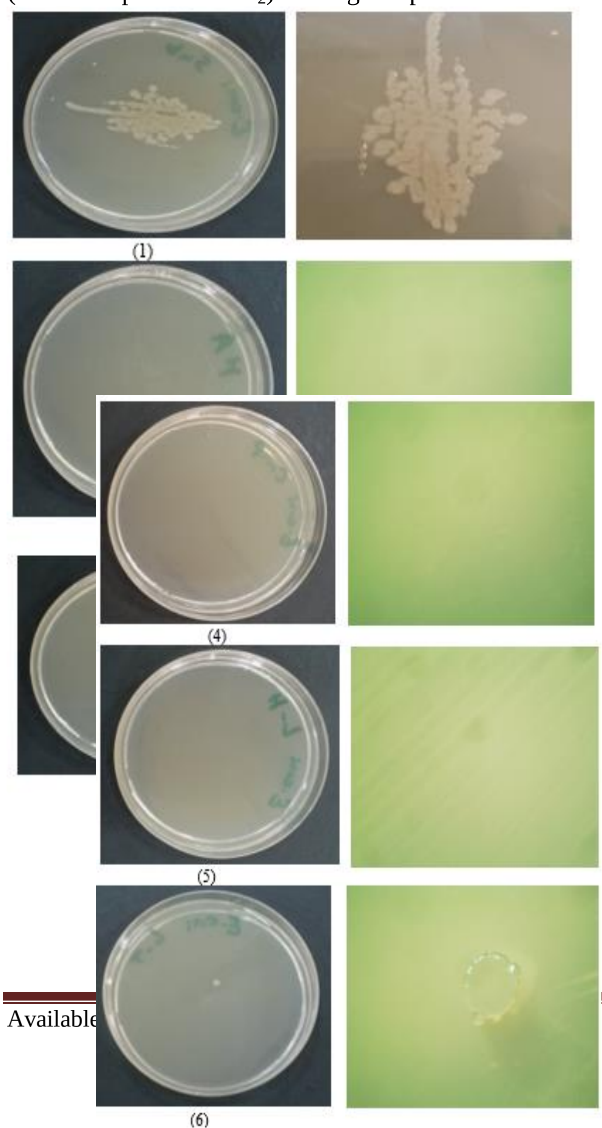

Fig 3 Shows images for E. coli bacteria by diffusion method (1) substrate (2) one-layer HA, (3) one-layer TiO_2, (4) one-layer Composite, (5) Three-layer (TiO_2+Composite +HA) and (6) Three-layer (HA+ composite + TiO_2) coating samples

4. Conclusion

1- The Thickness the important role in electrophoretic deposition the high thickness obtains by SEM in one-layer coating in HA coating (50 µm) because of the high particle size of HA (100 nm) and the TiO_2 give lower thickness (33 µm) because of low particle size of TiO_2 (25 nm). The higher thickness in Multi-Layer (HA+ Composite + TiO_2) with thickness (68 µm).

2- Antibacterial test showed excellent antibacterial activity against gram negative E.coli 100% killing and excellent antibacterial activity against gram positive Staphylococcus aureus (S. aureus) bacteria 100% killing for composite and multi - coating layer.

3- The using of chitosan enhancement substrate inhibit bacterial activity.

4- EPD technique was good method to modify the surface of NiTi alloy.

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