Antibacterial activity of functionally graded coating HAP/YSZ deposited on 316L SS by electrophoretic deposition

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Abstract

Antibacterial activity was evaluated in vitro on functionally graded coating (FGM) hydroxyapatite/yttria stabilized zirconia coatings prepared by electrophoretic deposition on substrate of 316L stainless steel. Chitosan was used as a thin film on substrate and binder for coating layers. In this study two cases of functionally graded coatings were analyzed. Different processing parameter were employed (Voltage, time, concentration, and temperature) for deposition.

The antibacterial activity test performed using N. agar and diffusion methods. Two types of bacteria, *E. Coli, and Staphylococcus were investigated on the performance of FGM*. The results showed that the FGM coatings revealed good inhibition effect towards *E. coli* and *S. aureus* (*Staphylococcus*) within the incubation period. The effect against *Staphylococcus* (gram positive) was more than the effect against the *E. coli* (gram negative). The high percentage of killed bacteria colonies for two kinds (*E. coli and S. aureus*) are high. Shape and dimensions of bacteria were investigated by optical microscope (OM) and scanning electron microscopy (SEM). It was found that the cell shape of *E. coli* gram negative bacteria in living state is rod shape with length of about 2- 3 µm. The cell shape of gram positive Staphylococcus is circular with diameter about 1.84µm

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. Austenitic stainless steel, especially the type of medical grade 316L stainless steel was used for implant fabrication in orthopedic applications. Bioceramics materials were used to improve its biological and antimicrobial properties.

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.

In order to obtain coatings layer, collect between the biological and good mechanical properties, in this study two types of Bioceramics were used. Bioactive material (HA) and bio inert material (YSZ) by improving coating technique with functionalize these materials.

Methods and Materials

316L stainless steel, Hydroxyapatite and Zirconia–Yttria (ZrO_2 – 3molY_2O_3, 50 nm), which were used to deposit it as a coating layers and to prepare (FGM). Chitosan (medium molecular weight with a degree of deacetylation of about 85%) which is used as a substrate and binder with ceramic materials. Sand blast surface was used to deposit coating layers with sample dimensions of (20 x 10 x 2) mm.

The important step in EPD is to prepare the aqueous suspensions. So the first step was dissolve 0.5g/L of chitosan by using 1% acetic acid in mixture of (94% ethanol+5%deionized water) to prepare thin chitosan layer on 316LSS. The parameters have been used to perform EPD process for deposition layers are (voltage, time and temperature and concentration of powder). For chitosan is (90V, 9 min, 40°C and 0.5g/L chitosan) , for HA is (50V, 5min, 30°C and 3%HA) and for YSZ is (30v, 6min, 30C° and 3%YSZ). Two cases of FGM coating layers were prepared with four layers that deposit functionally graded on thin film of chitosan. Ethanol was used and pH value was performed and fitted at 4.

For case (1) of FGM layers, the first layer is (100%), second layer (70%HA+30% YSZ), third layer (30%HA+70% YSZ) and forth layer is (100%YSZ). While preparation of case (2) of FGM is: First layer is (100%YSZ), second layer (70%YSZ+30% HA), third layer (30%YSZ+70% HA) and forth layer consist of (100%HA). These layers were deposited on thin layer of chitosan in addition to use 0.5g/L chitosan as a binder with all layers. All suspensions were de agglomerated by a magnetically stirred, then are followed by a high-energy sonicate. Two methods of biological test were used for assessment antibacterial activity: antibacterial activity test by zone inhibition method and antibacterial Activity test by diffusion method with two types of bacteria.

Results

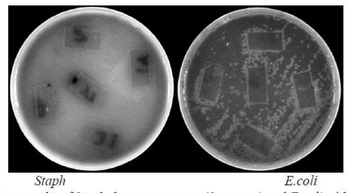
The optical images by using inhibition zone method were shown in Fig. 1, Results revealed that all coatings samples exhibited good inhibition effect towards E. coli and S. aureus within the incubation period and the effect against S. aureus (gram positive) was more than the effect against the E. coli (gram negative). The tests samples are divided into two parts, first part was uncoated (316L stainless steel) and the second part was coated with 1cm2. From the results, it was shown that the uncoated area (316L stainless steel) doesn't inhibit bacteria growth. There are many bacteria colonies visible after all the test periods, while the coated area exhibits good inhibition especially pure chitosan sample which showed a very good antibacterial behavior. The chitosan itself is antibacterial and since it was found in all samples as a binder therefore, a strong antibacterial effect of the composite coatings was expected. Some of the colonies accumulated together and formed into large sizes, indicating early stage of biofilm formation. It proposes that the coatings were effective in minimizing bacterial adhesion on the surface and in turn, preventing bacteria growth as well as the formation of biofilm. Additionally, the surface roughness has a better chance for bacterial adhesion on surface of the composite coatings. In order to confirm these results second method (diffusion method) was used to estimate the ant-bacterial activity at colonies of viable organisms have been counted on the plates. Fig. 2 has been showed optical images of the samples for *Staphylococcus aureus* (gram positive) while Fig. 3 showed the result of *E. coli* (gram negative). So the percentage for killed results of this method were showed in Table 1.

Table 1. percentage of killed two types of bacteria *Staphylococcus aureus* and *E.coli*

Staphylococcus aureus			E. coli	
Samples	Bacterial rate %		Samples	Bacterial rate %
Chitosan	100		Chitosan	75
100% HA	99.7		100% HA	70
100% YSZ	100		100% YSZ	82
Case (1) FGM	99.6		Case (1) FGM	80
Case (2) FGM	99.6		Case (2) FGM	72

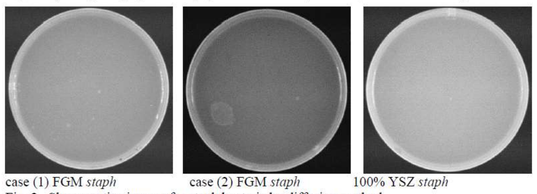
It has been found that all coatings (single and composites) have excellent antibacterial activity to gram positive bacteria (Staphylococcus) since the ratio of bactericidal ratio was 99.6% - 100% in samples, while for the gram negative type (*E. coli*) it was also the coatings exhibited good antibacterial activity the coatings layers showed between (70% - 82%) killing ability so that it was an effective ratio. The samples of chitosan and (YSZ) showed the higher value of bacterial rate than the samples with (HA). These results could be explained by there is a truth that the coatings layers have different characterizes roughness which gave a better adhesion of bacterial on surface. In addition, (YSZ was a bio-inert material.

the SEM images of S. *aureus* bacteria shape in Fig. 4 and incubated samples for both *E. coli* and are shown in Fig. 5 which approved that the cell shape of *E. coli* gram negative bacteria in living state is rod shape with length about (2.1 - 3.11) µm, this is on stainless steel without coating, and when it was interacting with coated materials the cell was flat and lost the cellular integrity and vitality and then the cell damaged. While the cell shape of gram positive Staphylococcus is circular with diameter about 1.84µm.



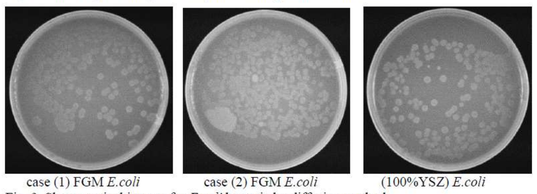
Staph *E.coli*

Fig. 1. Optical photographs of Staphylococcus aureus (S. aureus) and *E.coli* with agar dish method



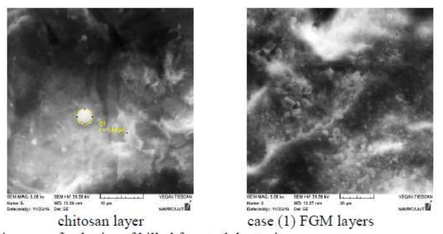
case (1) FGM *staph* case (2) FGM *staph* 100% YSZ *staph*

Fig. 2. Shows optica images for staph bacteria by diffusion method



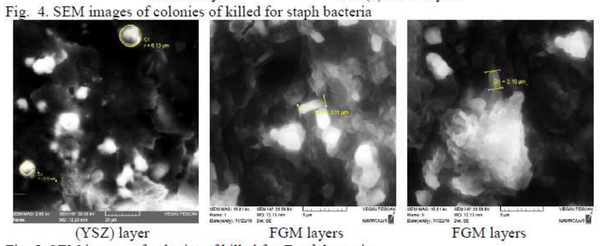
case (1) FGM *E.coli* case (2) FGM *E.coli* (100%YSZ) *E.coli*

Fig. 3. Shows optical images for *E.coli* bacteria by diffusion method



chitosan layer case (1) FGM layers

Fig. 4. SEM images of colonies of killed for staph bacteria



(YSZ) layer FGM layers FGM layers

Fig. 5. SEM images of colonies of killed for *E.coli* bacteria

Conclusions

Single and functionally graded layers were exhibited good antibacterial activity against gram negative *E.coli* and excellent antibacterial activity against gram positive *Staphylococcus aureus* (S. aureus) bacteria. Surface roughness has been given a better chance for killed bacterial. Nanoparticles of chitosan and YSZ have positive surface charge improve for their antibacterial activity because of the interaction with negatively charged biological membranes and site-specific
targeting in vivo.

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