J Nanostruct 12(4): 870-881, Autumn 2022

RESEARCH PAPER

Investigation on the Corrosion Resistance and Release of Gentamicin Drug from Titanium Implant Reinforced by Polyethylene Glycol / Polyvinyl Alcohol Based Polymer Composite Coating

Neda Aryan [1], Mohsen Behpour [2], Ali Benvidi [1], Fereshteh Jookar Kashi [3], Mostafa Azimzadeh [4], and Hamid Reza Zare [1]*

[1] *Department of Analytical Chemistry, Faculty of Chemistry, University of Yazd, Yazd, Iran*

[2] *Department of Analytical Chemistry, Faculty of Chemistry, University of Kashan, Kashan, Iran*

[3] *Department of Cell and Molecular Biology, Faculty of Chemistry, University of Kashan, Kashan, Iran*

[4] *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

ARTICLE INFO

Article History:
Received 05 August 2022
Accepted 26 September 2022
Published 01 October 2022

Keywords:
Corrosion
Local drug delivery
Titanium dioxide nanotubes
Titanium implants

ABSTRACT

The main reasons for the failure of titanium implants are insignificant infection with cataracts and ossoeintegration. In this paper, the production of titanium dioxide nanotubes on a titanium substrate by electrochemical anodization method is a suitable substrate for nanocomposite coatings. Polyethylene glycol (PEG) polymer coating on the surface of titanium dioxide nanotubes increases biocompatibility and controls long-term drug release kinetics. Polyvinyl alcohol (PVA) polymer coating is a biodegradable polymer that controls drug release. Polymer coatings on the surface of titanium dioxide nanotubes also increase the corrosion resistance of titanium dioxide nanotubes. Using gentamicin (gen) as an antibiotic increased the antimicrobial susceptibility of the implant. Electrochemical results show that the simultaneous coating of two polymers of polyethylene glycol and polyvinyl alcohol increased the corrosion resistance of the implant, and its corrosion current (1.6843×10^{-6} A/cm^2) decreased. Microbial results showed that the sample of titanium dioxide nanotube coated with gentamicin had the highest antimicrobial properties and the lowest optical density (0.5). Because when titanium dioxide nanotubes are co-layered with polymer in addition to gentamicin, it causes the drug to show less antimicrobial properties. The cytotoxicity results show that the sample of titanium dioxide nanotubes coated with polyethylene glycol and the drug has the highest cell viability percentage (99.5%) because gentamicin has high antimicrobial properties for the cell and polyethylene glycol polymer has low antimicrobial properties for cells.

How to cite this article
Aryan N, BehpourM, Benvidi A, Jookar Kashi F, Azimzadeh M, Zare H . Investigation on the Corrosion Resistance and Release of Gentamicin Drug from Titanium Implant Reinforced by Polyethylene Glycol / Polyvinyl Alcohol Based Polymer Composite Coating. J Nanostruct, 2022; 12(4):870-881. DOI: 10.22052/JNS.2022.04.009

* *Corresponding Author Email: m.behpour@kashanu.ac.ir*

INTRODUCTION

Dental diseases, caries, and partial fractures caused materials that could replace teeth [1]. To make dental implants, most research has focused on titanium and its alloys, which are widely used to make hard tissue implants. Titanium is a neutral metal widely used for drug delivery applications due to its excellent biocompatibility, high strength and corrosion resistance during the presence of implants in the human body [2-4]. Implants are prone to various problems such as infection, non-integration, inflammation and complete rejection by the host body. Bacterial infections are a major cause of implant failure. Infections are usually caused by adhesion, colonization, and biofilm formation by bacterial colonies on the implant surface after implantation. One of the most promising methods to overcome these limitations is to produce a layer of titanium dioxide nanotubes on the implant surface by electrochemical anodizing. This electrochemical method is based on a simple, scalable, cost-effective industrial process that can be applied to medical implants based on current titanium and titanium alloys. Recently, titanium dioxide nanotubes as implants have attracted increasing attention in biomedical applications [5]. Titanium dioxide has many applications, including photocatalytic properties of TiO_2 are used to destroy cancer cells, bacteria, viruses and algae under UV light [6]. Another application of titanium dioxide is the ability to protect against corrosion of modified copper in 2-[((Z)-1-{6- [(2-sulfanylphenyl)ethanimidoyl]-2-pyridyl}ethylidene) amino]-1- benzenthiol contains TiO2 nanoparticles in 3.5% sodium chloride solution [7] and another application of titanium dioxide that can mention is the synergistic effect of nano TiO_2 stabilized on activated carbon, zeolite Y and ZSM-5 to remove styrene vapours in polluted air [8]. Modifying the surface of titanium dioxide nanotubes with polymers improves drug release kinetics and improves antibacterial properties and ossification [9]. When polyethylene glycol is attached to the surface of the nanotube, it increases its biocompatibility [10-11]. Deposition of polyethylene glycol (PEG) coating to cover the open pores of drug-loaded titanium dioxide nanotubes, with reduced explosion release and long-term release kinetics [12]. Polyvinyl alcohol (PVA), made from polyvinyl acetate by hydrolysis, is easily degradable. It is used in the industrial, commercial, medical, and food sectors. PVA is one of the most widely used polymer groups in biomaterials for medical implants [13]. A polymer is biodegradable, and its degradability is increased by hydrolysis due to the presence of hydroxyl groups. In addition, it is water soluble and water-friendly in nature [14]. Gentamicin (gen) is an antibiotic sold to the general public under the brand name Garamycin and is used to treat various types of bacterial infections. Gentamicin is an aminoglycoside that works to make proteins by disrupting the ability of bacteria to normally kill bacteria [15].

In this paper, after converting titanium to titanium dioxide nanotubes by electrochemical anodizing method, polyethylene glycol was layered by hydrothermal method in TNT and then polyvinyl alcohol by hydrothermal method on TNT. Finally, gentamicin was coated by immersion on TNT using a deep coating. Samples of TNT + PEG + gen, TNT + PVA + gen and TNT + PEG + PVA + gen were placed in a body buffer simulation solution (PBS), and their absorbance was recorded on different days using a spectrophotometer and compared. Took Corrosion resistance, antibacterial properties and cytotoxicity of the samples were also compared.

MATERIALS AND METHODS

Preparation of TiO_2 nanotubes on Ti substrate

The titanium sheet was cut to a size of 1 x 5 cm^2. The titanium sheet was then polished with various grades of silicon carbide sandpaper. Then put in ethanol-acetone solution (20-20 ml) and in ultrasonic (3 times and 5 minutes each time) to clean the titanium sheet. Finally, the anodizing process was performed. The electrolyte solution for the anodizing process consists of 0.3 g of ammonium fluoride, 3 ml and 97 ml of deionized water. Aluminum foil as the opposite electrode and titanium foil as the working electrode was immersed in the electrolyte solution. The titanium sheet was anodized at 60 volts at 18-20 °C for 45 minutes. Finally, the anodized sample was placed in a furnace at 450 °C for one hour to crystallize the amorphous titanium dioxide nanotube into the anatase crystalline phase.

Coating of polyethylene glycol and polyvinyl alcohol on titanium dioxide nanotube

Polyethylene glycol 1000 was loaded onto titanium dioxide nanotubes by hydrothermal method. The polymer solution was made of polyethylene glycol (0.25 g of polyethylene glycol

+ 25 ml of deionized water) at room temperature and on a stirrer. Then, titanium dioxide nanotubes were placed in 4 ml of polyethylene glycol polymer solution and an oven at 85 °C for 3 hours. After finishing the coating layer, titanium dioxide nanotubes were washed with deionized water.

Coating of polyvinyl alcohol on titanium dioxide nanotube

Polyvinyl alcohol was loaded onto titanium dioxide nanotubes by hydrothermal method. The polymer solution was made of polyvinyl alcohol (0.25 g of polyvinyl alcohol + 25 ml of deionized water) at room temperature and on a stirrer. Then, titanium dioxide nanotubes were placed in an autoclave in 4 ml of polyvinyl alcohol polymer solution and placed in an oven at 150 °C for 3 hours. After finishing the coating layer, titanium dioxide nanotubes were washed with deionized water.

Loading of gentamicin drug in titanium dioxide nanotube

Dilute 2 ml of gentamicin (80 mg/2 ml) with 4 ml of deionized water and then titanium dioxide nanotubes, a certain number of times and in a certain amount of time (5 times and each time for 1 minute) into the gentamicin solution by a dip-coater device. Between each immersion, the metal surface should be dried at room temperature. The sample was washed with deionized water after finishing the coating layer.

Measurement of absorption of gentamicin released in buffer solution

Titanium dioxide nanotubes coated with

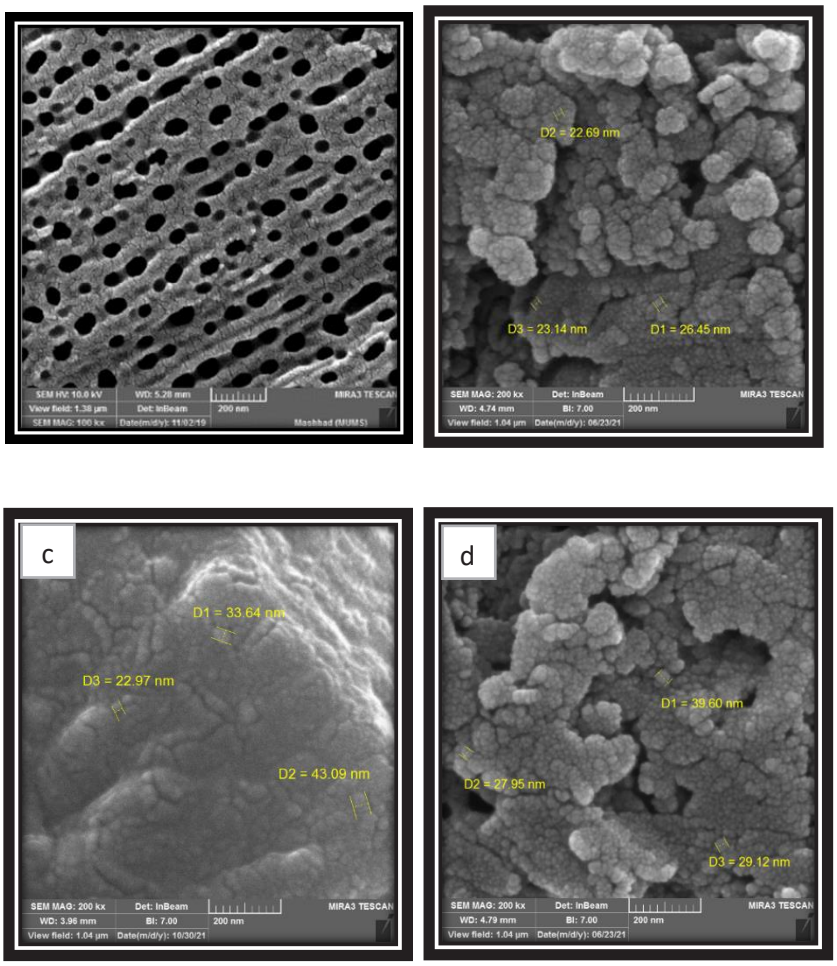


Fig. 1. FE-SEM images of a) TNT b) TNT + PEG + gen c) TNT + PVA + gen d) TNT + PEG + PVA + gen

polyethylene glycol, drug/polyvinyl alcohol, drug/polyethylene glycol, polyvinyl alcohol and drug were immersed in 4 ml of PBS buffer solution at 37 °C and its absorption was measured using UV-Visible spectroscopy at 254 nm.

Measurement of Electrochemical

The workstation obtained the electrochemical corrosion behavior of uncoated and coated titanium dioxide nanotube samples. A standard three-electrode system was set up, including an opposite electrode (platinum), a reference electrode (Ag/AgCl (3 M KCl)) and a working electrode (sample). Open circuit potential (OCP) was obtained by immersing the samples in PBS electrolyte. Electrochemical impedance spectroscopy and polarization data were expressed as Nyquist and TOEFL curves, respectively.

Antimicrobial activity test and Cell viability assay

Staphylococcus aureus ATCC 29737 was considered to evaluate the antibacterial activity of coatings. The broth nutrient medium was used as the bacterial culture medium. To achieve a 0.5 McFarland turbidity, the bacteria were inoculated into the culture medium and incubated at 37 °C. Take the number of Erlenmeyer and control samples, pour 20 ml of half McFarland suspension into the jar, and then place the specimens (implants) in the Erlenmeyer flask and the Erlenmeyer as control; it was without a sample. The Erlenmeyer flakes were then heated in an incubator shaker for 24 hours at 37 °C. To measure the number of living microorganisms, the amount of optical density (OD) was read using an ultraviolet spectrophotometer at a maximum length of 600 nm after 24 hours. To determine the adhesion of *S. aureus*, the samples were washed three times with physiological saline to separate non-adherent bacteria. Each sample in a test tube containing 10 mL of sterile saline was placed in an ultrasonic bath (5 times, each for one minute) to remove bacteria attached to the implant. Then 50 µl of serum containing *S. aureus* was inoculated in nutrient agar plates and placed at 37 °C. Finally, after 24 hours, the bacterial colonies were counted.

The ISO 10993-1 international standard protocol of US food and medicine was used to measure cell survival. Described for minor changes in the reference [16].

RESULTS AND DISCUSSION

Surface characterization

SEM analysis to determine the surface morphology assessed by Fe -SEM for uncoated and

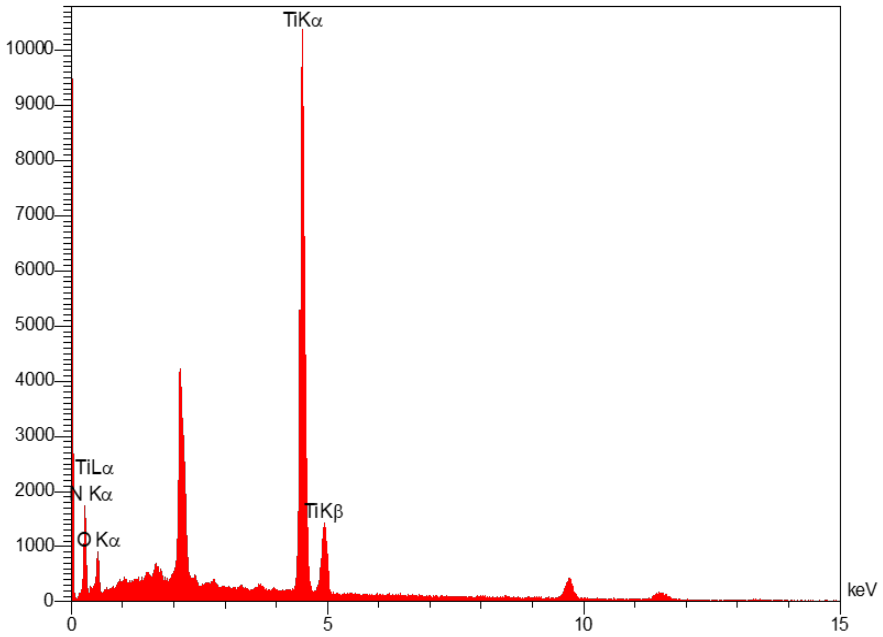


Fig. 2. EDX image of TNT + PEG + PVA + gen

coated TNT samples. Fig. 1(a) shows the titanium dioxide nanotube. Fig. 1(b) shows a titanium dioxide nanotube coated with polyethylene glycol and drug. Fig. 1(c) shows a layer of titanium dioxide nanotubes coated with polyvinyl alcohol and drug. Fig. 1(d) shows titanium dioxide nanotubes coated with polyethylene glycol, polyvinyl alcohol and drug. Fig. 1(a) confirms the growth of nanotubes on the surface of the titanium. The textured surface of polymer coatings has a nanoscale roughness, and their surface roughness is much less than uncoated titanium dioxide nanotubes. In Fig. 1(d), due to having two polymer layers (polyethylene glycol, polyvinyl alcohol) on the surface of the titanium dioxide nanotube, the surface of the nanotube has become denser, and its thickness has increased.

EDX, shown in Fig. 2, is used to analyze elements. Ti, O, C and N atoms are present at the surface of Ti samples, as shown in the spectrum. Elements of C and O confirm the presence of two polymers (PEG and PVA), and element N confirm the presence of gentamicin drug. The unnamed peak is related to gold because the gold coating was used for EDX analysis. EDX map, shown in Fig. 3, confirms the uniform distribution of elements at the Ti surface.

The XRD image in Fig. 4 is for a TNT + PEG + PVA + gen sample. The JCPDF number equal to 01-073-1764 is corresponds to titanium dioxide in the anatase phase. The JCPDF number equal to 00-044-1294 is related to titanium, and the JCPDF number equal to 00-050-2158 is related to polyethylene glycol. The peak at 19 <2θ <20 is related to polyvinyl alcohol, according to the reference [17], which has a low peak intensity. Gentamicin was not observed to peak due to its low concentration.

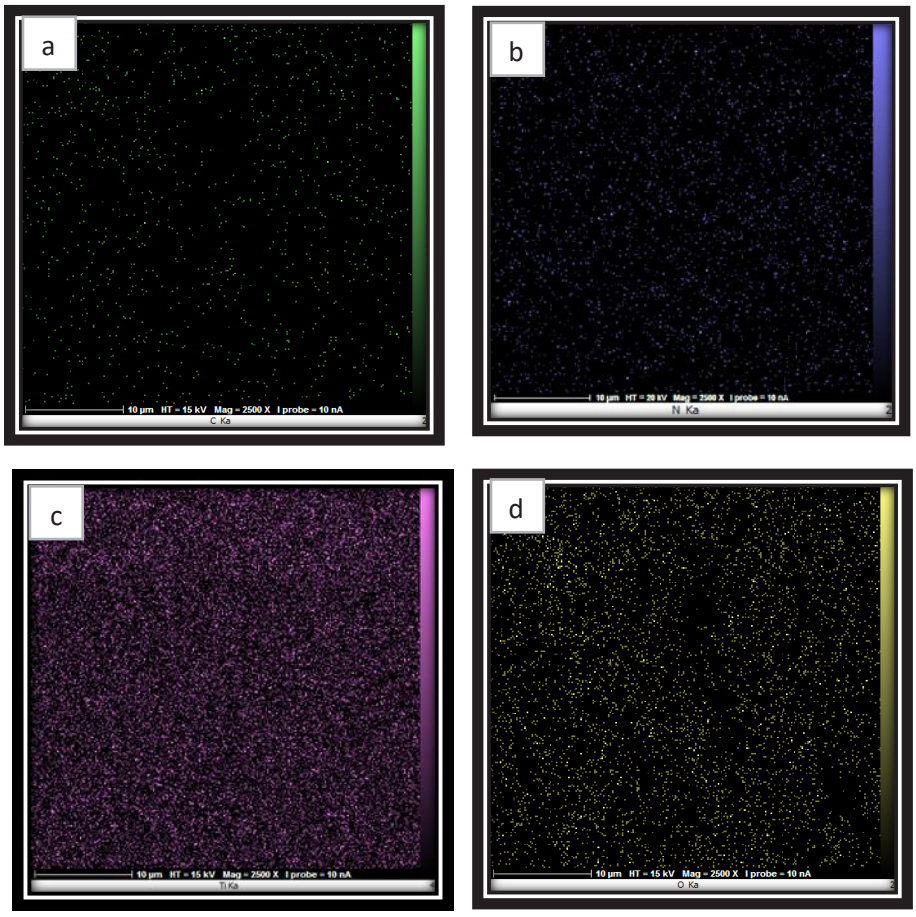


Fig. 3. EDX mapping spectrum of a) C b) N c) Ti d) O elements

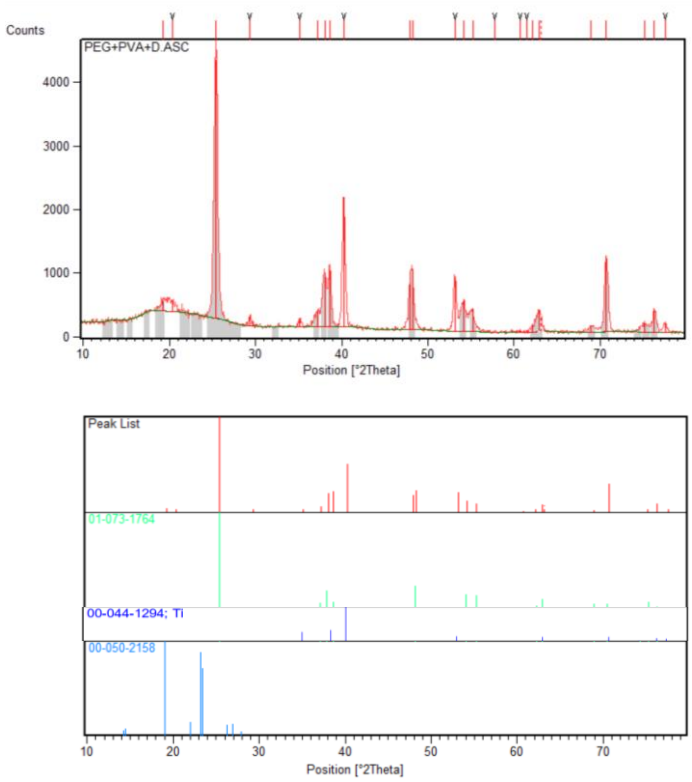


Fig. 4. XRD pattern of TNT + PEG + PVA + gen

Antimicrobial activity

Fig. 5 shows optical density in the control, TNT, PEG +gen, PVA + en, PEG + PVA + gen and gen. The optical density of the PVA + gen sample is lower than TNT the presence of gentamicin. However, gentamicin is an antibiotic with antimicrobial properties. The optical density, in the PEG + PVA + gen sample is lower than PVA + gen due to the presence of PEG. However, PEG has a small number of antimicrobial properties. In the PEG + gen sample, optical density is lower than PEG + PVA + gen sample because in PEG + PVA + gen sample, due to the simultaneous presence of PVA and PEG causes the gen to show less antimicrobial properties. The gen sample has the highest antimicrobial properties and the lowest optical density because gentamicin is an antibiotic. The optical density in the PEG + gen sample is higher than in the gen sample because PEG has caused gentamicin to show less antimicrobial properties.

Electrochemical studies

To create OCP for all samples, a time frame of 1 hour in PBS solution was given. The potentiodynamic polarization curve for all samples of TNT, PEG + gen, PVA + gen and PEG + PVA + gen is shown in Fig. 6. The TNT sample has the highest corrosion current (0.00017572 A/cm^2) because TNT has no coating layer, so corrosive ions penetrate the surface of the titanium dioxide nanotube and cause corrosion. Samples of titanium dioxide nanotubes coated with PEG + gent and PVA + gen has a lower corrosion current (corrosion current of PEG + gen and PVA + gen samples are 4.4542 × 10^{-6}, 2.5252 × 10^{-6} A/cm^2 respectively) is than TNT due to the layered surface of the nanotubes and cause less corrosive ions to penetrate the surface of the nanotubes. PEG + PVA + gen sample has the lowest corrosion current (1.6843 × 10^{-6} A/cm^2) because both PEG and PVA polymer is deposited on the surface (Table 1). Therefore, these polymers interact with corrosive ions and cause less corrosive ions to penetrate the surface of the nanotube.

The Electrochemical impedance curve for all samples of TNT, PEG + gen, PVA + gen and PEG + PVA + gen is shown in Fig. 7. The TNT sample has the least polarization resistance because TNT

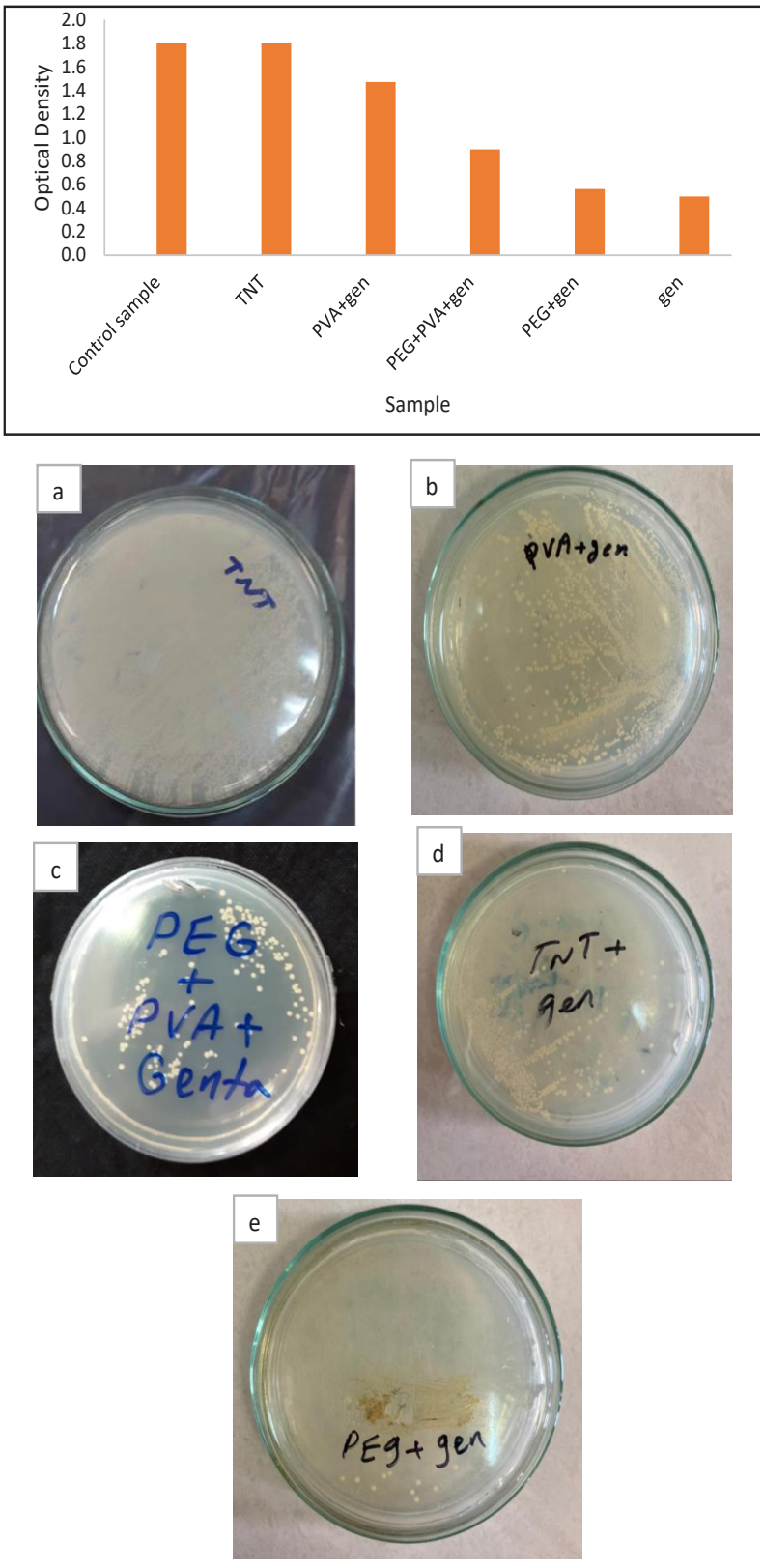


Fig. 5. Bacterial density of *S. aureus* diagram after 24 h

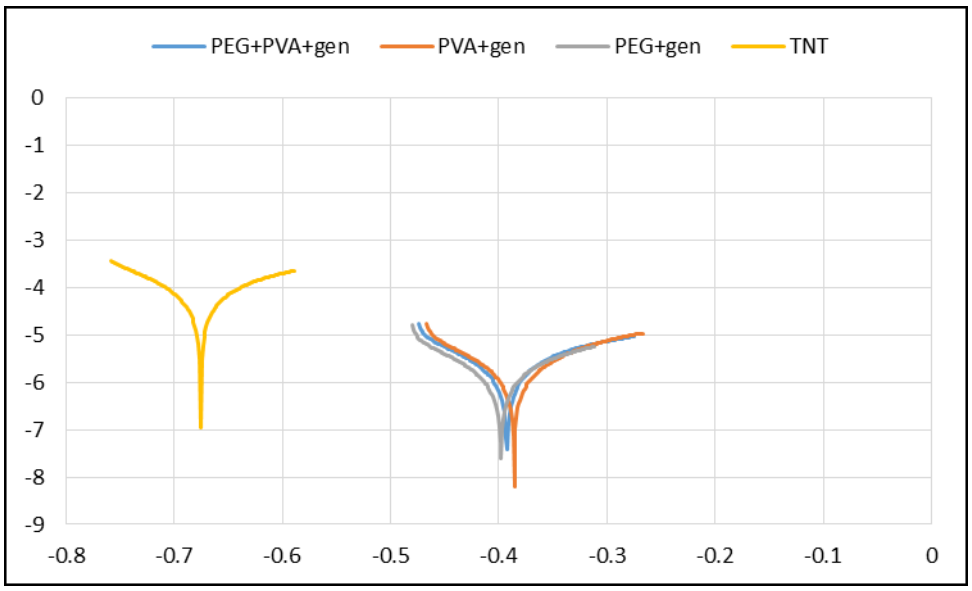


Fig. 6. The polarization curves for TNT, TNT + PEG + gen, TNT + PVA + gen, TNT + PEG + PVA + gen

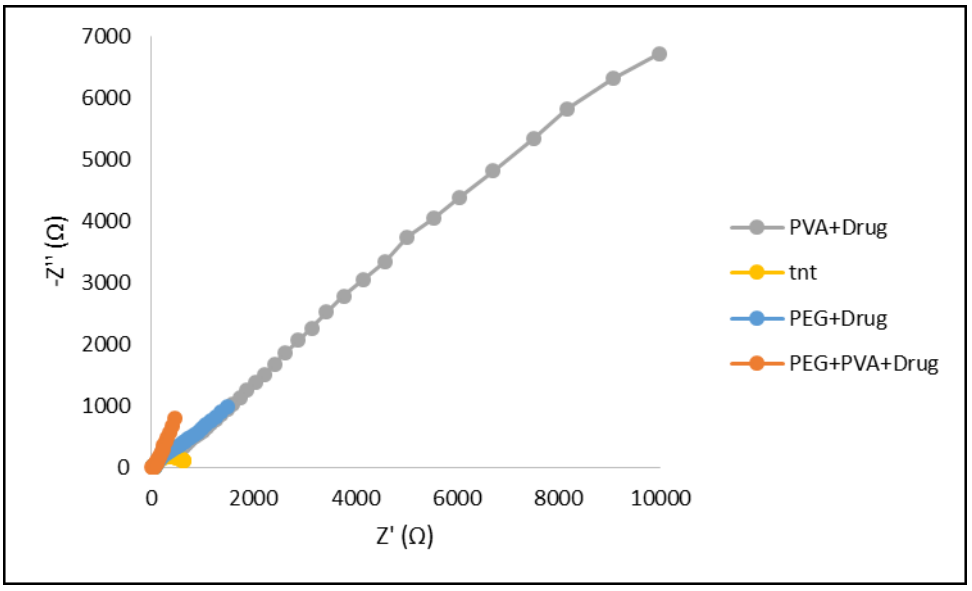
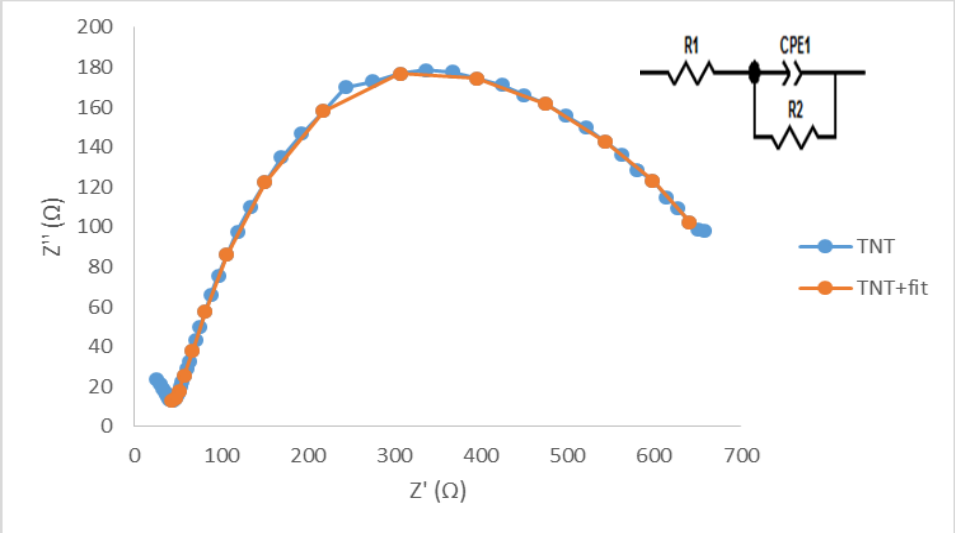


Fig. 7. EIS image of TNT, TNT + PEG + gen, TNT + PVA + gen, TNT + PEG + PVA + gen

has no coating layer, so corrosive ions penetrate the surface of the titanium dioxide nanotube and cause corrosion. Samples of titanium dioxide nanotubes coated with PEG + gen and PVA + gen have a higher polarization resistance than TNT due to the layered surface of the nanotubes and cause less corrosive ions to penetrate the surface of the nanotubes. PEG + PVA + gen sample has the highest polarization resistance because PEG and PVA polymer is deposited on the surface. Therefore, these polymers interact with corrosive ions and cause less corrosive ions to penetrate the surface of the nanotube. Fig. 8 shows the equivalent circuit for the Nyquist curves. The

Table 1. Corrosion current of TNT + PEG + PVA + gen, TNT + PVA + gen, TNT + PEG +gen and TNT samples

Sample	TNT + PEG + PVA + gen	TNT + PVA + gen	TNT + PEG +gen	TNT
i_{corr} (A/cm^2)	1.6843 ×10^{-6}	2.5252 × 10^{-6}	4.4542 × 10^{-6}	0.00017572



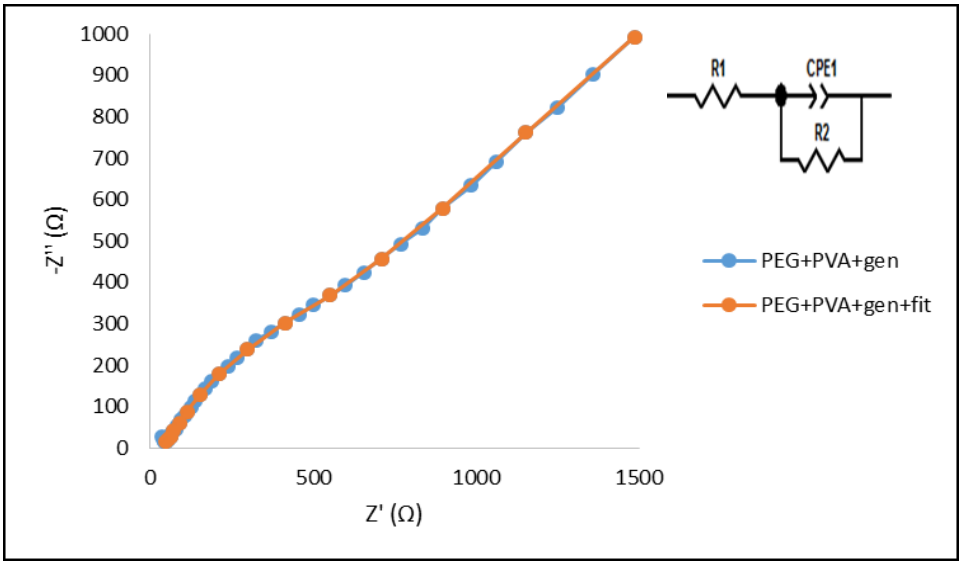


Fig. 8. Equivalent circuit for the Nyquist curves of TNT, TNT + PEG + gen, TNT + PVA + gen, TNT + PEG + PVA + gen

equivalent circuit for the Nyquist curve of TNT, PEG + gen, PVA + gen and PEG + PVA + gen samples is $R_1CPE_1R_2$, that R_1 is the solution resistance, R_2 is the coating resistance, and CPE is the capacitance

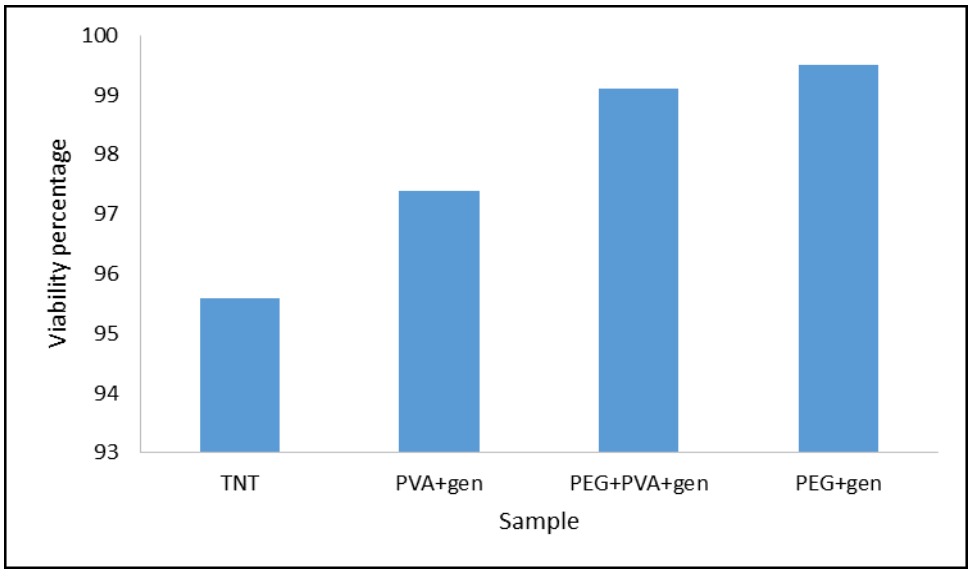


Fig. 9. Cell viability assay in samples of TNT, TNT + PEG + gen, TNT + PVA + gen, TNT + PEG + PVA + gen

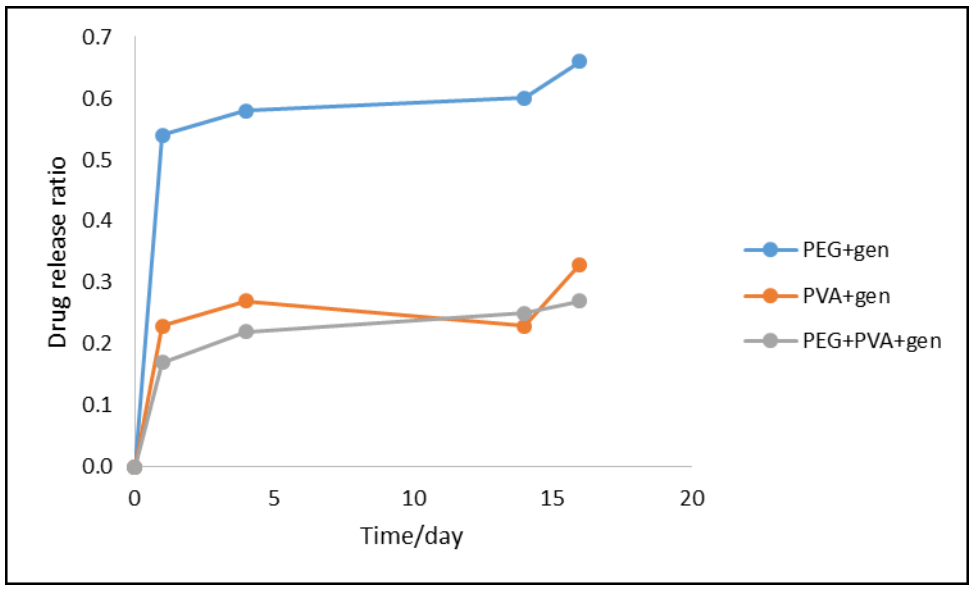


Fig. 10. The amount of drug released from coatings of TNT + PEG, TNT + PVA, TNT + PEG + PVA at 37°C in the PBS solution for 16 days

of the coating.

Percentage viability studies

Fig. 9 shows the cell viability percentage in samples of TNT, PEG + gen, PVA + gen and PEG + PVA + gen. Due to its non-interactive nature, TNT has a lower cell viability percentage than all other samples. Cell viability percentage in the PVA + gen sample (97.39%) is higher than TNT (95.6%) due to the presence of gentamicin because gentamicin is an antibiotic. The percentage of cell viability in the PEG + PVA + gen sample (99.1%) is higher than

that of the PVA + gen due to the presence of PEG because PEG has few antimicrobial properties that protect the cell by eliminating them. In the PEG + gen sample, cell viability percentage (99.5%) is higher than PEG + PVA + gen sample because in PEG + PVA + gen sample, due to the simultaneous presence of PVA, PEG has caused the gen to show less antimicrobial properties.

Drug release kinetics

Fig. 10 shows the percentage of the released drug for titanium dioxide nanotube samples coated with PEG, PVA and PEG +PVA in PBS buffer solution over 16 days. In the sample of titanium dioxide nanotube coated with polyethylene glycol and polyvinyl alcohol, the release of the gentamicin drug is more controlled than in other samples. (The release percentage of TNT sample coated with polyethylene glycol and polyvinyl alcohol equal to 0.27%). It is due to the simultaneous presence of polyethylene glycol and polyvinyl alcohol that both control drug releases.

CONCLUSION

biocompatibility and corrosion properties of titanium dioxide nanotubes coated with PEG + gen, PVA + gen and PEG + PVA + gen using different methods XRD, EDX, Fe-SEM, Dynamic polarization and electrochemical impedance were performed. The results showed that the sample of titanium dioxide nanotubes coated with PEG and PVA has higher corrosion resistance (corrosion current is 1.6843×10^{-6}) than other samples. The simultaneous presence of PEG and PVA has increased the number of coated layers, reducing the possibility of corrosive ions reaching the surface of titanium dioxide nanotubes. This paper also investigated the release of gentamicin from coated titanium dioxide nanotubes using a spectrophotometer. Drug release in PEG and PVA coated titanium dioxide nanotube samples were more controlled due to the simultaneous presence of PEG and PVA, which are the drug release controllers (The drug release ratio is 0.27%). In this article, the antibacterial properties of the samples were also investigated. Among the samples, the sample of titanium dioxide nanotubes coated with gentamicin has more antibacterial properties (optical density is 0.5) because gentamicin is an antibiotic. In samples coated with a polymer (PVA, PEG), the antibacterial property is reduced because the polymer prevents the drug from showing antibacterial properties. The results of cytotoxicity showed that the sample of titanium dioxide nanotubes coated with PEG and gentamicin had a higher cell viability percentage (cell viability percentage is 99.5%), but in the sample of titanium dioxide nanotubes coated with PEG and PVA due to the presence of PVA the viability percentage of the cell is smaller.

Acknowledgment

Authors are grateful to council of Kashan University for supporting this work by grant no 463561-19. The authors wish to thank Yazd University Research Council for supporting of this research.

CONFLICT OF INTEREST

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

REFERENCES

1. Vallittu PK, Närhi TO, Hupa L. Fiber glass–bioactive glass composite for bone replacing and bone anchoring implants. Dent Mater. 2015;31(4):371-381.
2. Liu X, Chu P, Ding C. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Materials Science and Engineering: R: Reports. 2004;47(3-4):49-121.
3. Wang H, Lin C, Hu R. Effects of structure and composition of the CaP composite coatings on apatite formation and bioactivity in simulated body fluid. Appl Surf Sci. 2009;255(7):4074-4081.
4. Albrektsson TO, Johansson CB, Sennerby L. Biological aspects of implant dentistry: osseointegration. Periodontol 2000. 1994;4(1):58-73.
5. Kumeria T, Mon H, Aw MS, Gulati K, Santos A, Griesser HJ, et al. Advanced biopolymer-coated drug-releasing titania nanotubes (TNTs) implants with simultaneously enhanced osteoblast adhesion and antibacterial properties. Colloids Surf B Biointerfaces. 2015;130:255-263.
6. Shafei A, Sheibani S. Visible light photocatalytic activity of Cu doped TiO2-CNT nanocomposite powder prepared by sol–gel method. Mater Res Bull. 2019;110:198-206.
7. Behpour M, Ghoreishi SM, Mohammadi N, Soltani N, Salavati-Niasari M. Investigation of some Schiff base compounds containing disulfide bond as HCl corrosion inhibitors for mild steel. Corros Sci. 2010;52(12):4046-4057.
8. Nakhaei Pour M, Rangkooy HA, Jahani F, Golbaghi A, Shojaee-Farah Abady H, Nematpour L. Removal of styrene by the synthesized ZnO nanoparticles coated on the activated carbon adsorbent in the presence of UV irradiation. Environmental Health Engineering and Management. 2019;6(4):225-232.
9. Kunrath MF, Leal BF, Hubler R, de Oliveira SD, Teixeira ER. Antibacterial potential associated with drug-delivery built TiO2 nanotubes in biomedical implants. AMB Express. 2019;9(1).

10. Aw MS, Gulati K, Losic D. Controlling Drug Release from Titania Nanotube Arrays Using Polymer Nanocarriers and Biopolymer Coating. J Biomater Nanobiotechnol. 2011;02(05):477-484.
11. Sawhney AS, Pathak CP, Hubbell JA. Interfacial photopolymerization of poly(ethylene glycol)-based hydrogels upon alginate-poly(l-lysine) microcapsules for enhanced biocompatibility. Biomaterials. 1993;14(13):1008-1016.
12. Luo F, Xu G, Liu Y, Xie C, Xiao X. PEG-Coated Titania Nanatubes for Controlled Drug Release. Proceedings of the International Conference on Chemical, Material and Food Engineering: Atlantis Press; 2015.
13. Deng C, Wang B, Dongqin X, Zhou S, Duan K, Weng J. Preparation and Shape Memory Property of Hydroxyapatite/Poly (Vinyl Alcohol) Composite. Polymer-Plastics Technology and Engineering. 2012;51(13):1315-1318.
14. Qiu K, Netravali AN. A Composting Study of Membrane-Like Polyvinyl Alcohol Based Resins and Nanocomposites. Journal of Polymers and the Environment. 2013;21(3):658-674.
15. Evaluation of Gentamicin's Antimicrobial Resistance Pattern Against Uropathogens in a Tertiary Health Care Center in Dhaka City, Bangladesh. Journal of Health, Medicine and Nursing. 2019.
16. Tuomi JT, Björkstrand RV, Pernu ML, Salmi MVJ, Huotilainen EI, Wolff JEH, et al. In vitro cytotoxicity and surface topography evaluation of additive manufacturing titanium implant materials. J Mater Sci Mater Med. 2017;28(3).
17. Abdelaziz M, Abdelrazek EM. Effect of dopant mixture on structural, optical and electron spin resonance properties of polyvinyl alcohol. Physica B: Condensed Matter. 2007;390(1-2):1-9.