

Cu AND Zn SUBSTITUTED HYDROXYAPATITE COATINGS ON TITANIUM OXIDE NANOTUBES FORMED BY ELECTROCHEMICAL METHODS

SUBHASHMITA SWAIN, TAPASH RANJAN RAUTRAY*

Biomaterials and Tissue Regeneration Laboratory, Centre of Excellence in Theoretical and Mathematical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar - 751 030, Odisha, India. Email: tapash.rautray@gmail.com

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ABSTRACT

Objective: Hydroxyapatite (HA) coatings on titanium oxide nanotubes (TNTs) provide osteoconduction and hence promote bone healing and apposition, leading to the rapid biological fixation of implants. In the current study, TNT surfaces were formed by electrochemical anodization technique and Cu and Zn were simultaneously substituted in HA coating so as to form a coating with antibacterial properties with good osteoconductive surface.

Methods: Ion substituted HA coatings such as Cu-HA and Zn-HA were deposited cathodically on TNT surfaces. This work elucidated the antibacterial effect and cytocompatibility of Cu and Zn substituted HA coating on TNT. To improve the antibacterial property of pure HA, Cu and Zn were substituted into its structure.

Results: X-ray diffractometer of the coatings showed the formation of Cu-HA and Zn-HA while particle-induced X-ray emission confirmed the coatings to be calcium deficient. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was employed to assess the cell survival rate of Cu-HA and Zn-HA coatings with osteoblast-like cells.

Conclusions: While both the coating showed good bioactivity, better cell activities were observed in the case of Zn-HA coating. Better cell activities on Zn-HA may be attributed to the cytocompatibility nature of Zn-HA and because of its higher roughness.

Keywords: Hydroxyapatite, Antibacterial, Roughness, Bioactivity.

INTRODUCTION

The current gold standard biomaterial for orthopedic and dental applications is titanium. Because of their superior biocompatibility and better mechanical properties, titanium-based materials are preferred over other metals for implant applications. However, due to bioinert properties of titanium, its surface needs to be modified so as to be acceptable by human bone tissues thereby enhancing the clinical success rate [1-3]. It is also understood that this osseointegration can be improved at the nanometer scale [4,5] by suitable techniques. Titanium oxide (TiO₂) nanotubes can improve cell attachment and growth due to its large specific area [3]. TiO₂ nanotubes (TNTs) can be prepared by various techniques, but anodization is a frequently used method because of its easy handling and cheap commercially. This technique can be used to produce ordered nanostructures and strongly adherent TiO₂ layers [6]. Living tissues well accept the bone-like apatite on the implant surface on which cell adhesion and differentiation can occur. The apatite can also promote a strong bond between tissues and implant surface [7,8].

Hydroxyapatite (HA) has been extensively used as a coating material on titanium because of its similarity with a bone composition having better bioactive properties, and it has the potential to directly bond at the interface with host bone. However, due to its limitations in using as an implant material for its relatively slow rate of biological interaction, fragility, and less mechanical strength, it has to be coated on titanium surface [9,10]. Implant-associated infections lead to implant failure as a result of which the infection rate for implants in the orthopedic industry is 4.3% per annum in the USA. Bacteria aggregation occurs in a hydrated polymeric matrix forming a film on the implant surface as soon as infections take place, and it cannot be destroyed by the antibacterial agents coming from outside sources. As a result of which, these infections lead to implant failure, revision surgery, and even member amputation asking for high medical costs. Therefore, it becomes an essential practice to use an antibacterial implant to

avoid initial adhesion of bacteria [11]. To enhance the bioactivity and biocompatibility of HA and retard the risk of bacterial infection, the implants are surface modified to control the properties such as chemical and biological behavior. The doping of some ions such as Zn²⁺, Sr²⁺, Mg²⁺, Cu²⁺, Na⁺, and F⁻ constituting human bone can be substituted in the HA crystal structure for better biological activities [10,12].

Several studies reported that Cu²⁺ and Zn²⁺ in the HA coatings have beneficial effects in preventing bacterial adhesion. Cu²⁺ and Zn²⁺ in trace quantities are necessary for various metabolic processes in living organisms, whereas higher quantities are potentially toxic [12]. These doped metal ions exhibit antimicrobial activity on implanted materials, and they are not cytotoxic at low concentrations. The effects of Cu²⁺ and Zn²⁺ on microorganisms have been proved to be safe in the literature. Cu is an essential trace element necessary for human metabolism, and it plays a major role in the cross-linking of collagen and elastin of bone [13]. Moreover, Zn has beneficial effects on bone metabolism where it stimulates osteoblast proliferation and mineralization and promotes osteoblast marker gene expressions as well as calcium deposition on mesenchymal stem cells inhibiting bone resorption by reducing osteoclast formation and adsorption capability having anti-inflammatory response [14,15]. In this work, TNTs were fabricated by anodization technique and subsequently Cu-Ha and Zn-HA were deposited on its surface.

METHODS

Sample preparation

Rectangular samples of Ti with working area 1 cm×2 cm were used as the substrate material. The Ti samples were mechanically polished using 600 and 1200 grit SiC papers. After polishing, the specimens were thoroughly cleaned with distilled water and subjected to ultrasonication for about 10 minutes in acetone. Then, the samples were dried and etched in an acidic solution containing 1 part 50% hydrofluoric, 4 parts 1 N HCl, and 5 parts deionized water for 30 seconds. Then, the obtained

samples were again washed in deionized water and dried at room temperature [16].

TNT formation

TNTs were synthesized on Ti surfaces using the electrochemical anodization technique. The electrochemical anodization setup consists of two-electrode system. The cleaned Ti plate was used as the anode and connected to the positive terminal of the direct current power supply while platinum plate was used as the cathode that was connected to the negative terminal to complete the electrolytic cell. The cathode and anode were maintained at a distance of 3 cm, and the electrolyte used in this work contained 1 M Na_2SO_4 + 0.5 wt% NaF. TNT coatings were obtained at potentiostatic 20 V for 1 hrs at room temperature. Subsequent to anodization, the samples were washed in deionized water and then dried at room temperature. To achieve the desired structure of the sample's crystallinity, the anodized specimens were subjected to heat treatment at 500°C for 2 hrs [17].

HA, Cu-HA, and Zn-HA formation on nanoporous titanium

The heat treated TNTs were taken for deposition of HA, Cu-HA, and Zn-HA onto it by cathodic deposition. Cathodic deposition of HA was conducted at room temperature in an electrolyte containing $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ to obtain control HA. Cu-HA was prepared using the electrolyte containing $\text{Cu}(\text{NO}_3)_2$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and $(\text{NH}_4)_2\text{HPO}_4$, whereas Zn-HA was prepared in electrolyte containing $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and $(\text{NH}_4)_2\text{HPO}_4$. All these solutions were of a molar ratio of (Ca+ respective ion) to P as 1.67 and starting pH of solutions were 4.5 having (Ca/respective ion) ratio as 9:1. The deposition was carried out by applying the constant potential of 15 V for 20 minutes for each sample. During cathodic deposition, the solution in the beaker was continuously stirred to obtain a uniform electrolyte concentration. Once the coatings were obtained, the specimens were immersed in 0.1 M NaOH solution for 2 hrs. Further, the coated specimens were rinsed with distilled water and subsequently dried at room temperature in air [18,19].

X-ray diffractometer (XRD; X'pert-APD, Philips, Netherlands) was carried out to determine the structure of coating using Cu K α radiation ($\lambda=0.154$ nm, 40 kV, 20 mA) and scanned in the 2θ range of 15-85° [20]. Field emission scanning electron microscopy (FE-SEM; JSM-6700, JEOL, Tokyo, Japan) was employed to observe the surface morphology and thickness of coating layer and energy dispersive X-ray (EDX) was used for the elemental analysis of the coatings. Surface measuring instrument (SURFTEST, Mitutoyo, Japan) was used to measure the Roughness (Ra) of the coatings. Coating wettability or hydrophilicity was measured using a contact angle detection system (OCA15 Plus, Dataphysics, Germany), and it was measured using drops of ultrapure distilled water (0.6 μl) and by capturing the image with delivery span of 10 seconds. Using a universal testing machine (Instron; 4202) under tension mode in accordance with ASTM C633-79 tensile pull-off adhesion strength was estimated [21]. Titanium (diameter 3 mm) was used as a stub and was pasted to the coating side. The stub was secured to a tension adaptor with a ball joint to ensure a pure axial alignment during loading of the interface. A tensile load at a crosshead speed of 1.5 mm/minutes was applied to the coating/substrate interface until failure occurred. The adhesion strength was calculated as the load at failure divided by the coated area bonded to the stub [22].

RESULTS

Peaks of Cu and Zn are seen in the Cu-HA and Zn-HA samples, respectively, as evident from XRD pattern. SEM micrograph of the as formed TNT surface is depicted in Fig. 1, and the cross-section of the nanotube surface is shown in Fig. 2. The SEM of Cu-HA and Zn-HA are shown in Figs. 3 and 4, respectively. SEM shows that the nanotubes are present uniformly throughout the Ti substrate. It is evident from Figs. 3 and 4 that dense ion substituted HA were coated on TNT surfaces. Cu-HA and Zn-HA coatings with bead-like particles conglomerated with hexagonal HA crystal structures with 10% Cu and Zn concentration. Morphology of both the coatings of HA showed hexagonal structure.

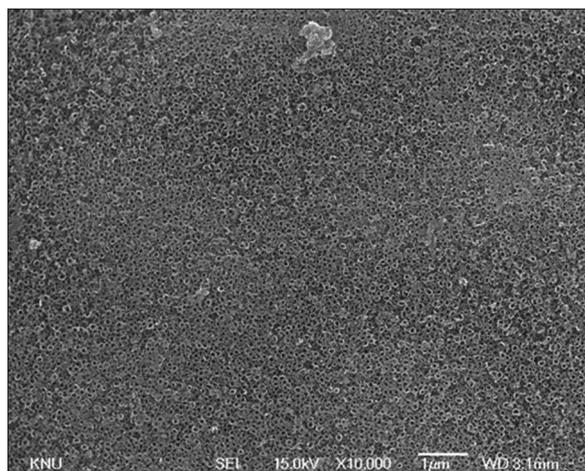


Fig. 1: Scanning electron microscopy of titanium oxide nanotube surface

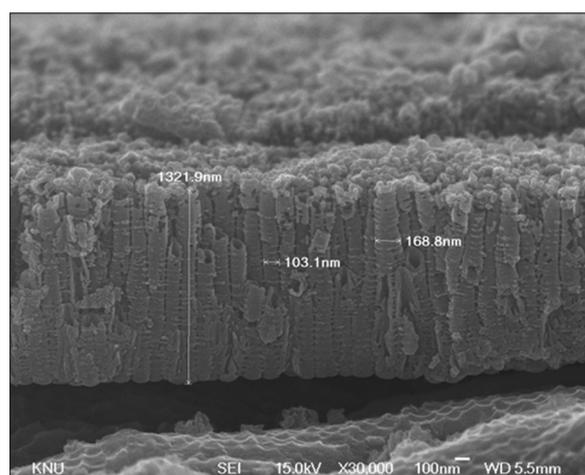


Fig. 2: Scanning electron microscopy of the cross-section of titanium oxide nanotube surface

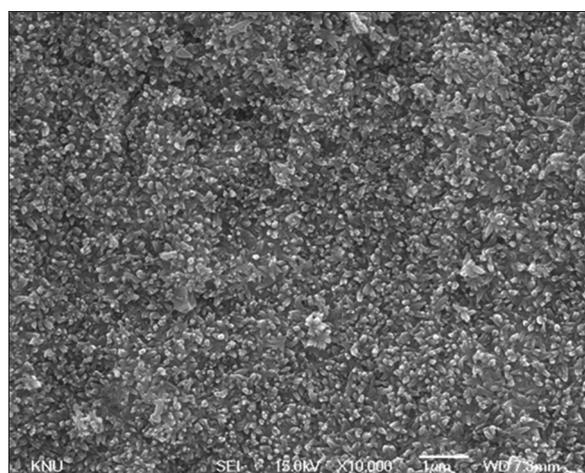


Fig. 3: Scanning electron microscopy of Cu-HA surface

Uniform distribution of HA was observed on both the TNT surfaces. The pores diameter of the nanotubes was observed in the range of 70-170 nm. Porosity was in the range of 60-80%, and the tube lengths were measured to be up to 1.32 μm . The synthesized nanotubes are self-organized, and the walls of the tubes had ripples with less Ra. Particle-induced X-ray emission (PIXE) spectroscopic technique was

used for the elemental analysis of the coatings. PIXE analyses showed the presence of Cu and Zn in the coatings. High surface to volume ratio having different morphologies was observed in the Cu-HA and Zn-HA coatings.

It is evident that implant surfaces having higher Ra have the better bond strength to the coating produced on it. So, in the current coating, the HA coatings are supposed to have better bonding strength with the TNT surface and subsequently the coated implant has better bond strength with the body tissues. The (Ca+Zn)/P and (Ca+Cu)/P ratio of the coatings were found to be calcium deficient, and they are 1.51 and 1.6, respectively, i.e., lower than the standard stoichiometry HA value of 1.67.

DISCUSSION

Roughened surfaces are more suitable for bone anchoring and biomechanical stability [23]. The surface Ra of both the coatings are higher than the control HA surface. The Ra of Zn-HA coatings was found to be higher than the Cu-HA coating. Since higher surface Ra favors better osteoblast activities, the Zn-HA surface has an advantage as far as biological properties are concerned. On the other hand, the contact angle of Cu-HA and Zn-HA coatings were lower than the control HA surface. The contact angle of Zn-HA coating was found to be 51°, i.e., lower than the Cu-HA coating with a value of 59°. It suggests that the Zn-HA coating is more hydrophilic than Cu-HA surface. Hydrophilic surfaces enhance the formation of proteins and osseointegration. So, Zn-HA is supposed to show better cell activities on its surface as compared to Cu-HA surface. Zn-HA coating also showed another advantageous property in the form of adhesion strength. Adhesion strength is a very important characteristic since coatings will exhibit a weight loss when abraded against bone. Zn-HA with 8.8 MPa showed better adhesion strength than Cu-HA coating with a 7.8 MPa value. Hence, Zn-HA is predicted to have higher cell attachment than the Cu-HA coating. It has been specified that the adhesion strengths of the HA coatings used as implants should have at least 18 MPa [24]. However, coatings obtained by cathodic deposition can give less values of adhesion strength that is up to 10 MPa. On the other hand, as low as 10 MPa of adhesion strength of HA coatings were also obtained using plasma spray technique [25]. The low adhesion strength values of our coatings can be attributed to the two layers of coatings on Ti substrate. Fig. 5 shows the results of cell proliferation of osteoblast-like cells evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on the control, Cu-HA, and Zn-HA surfaces. Zn-HA-coated surfaces showed significantly higher cell viability than the control.

The favorable effects of Zn on bone growth have motivated researchers to incorporate this trace element in HA for better biological coating applications [10-12]. The addition of nanoparticles in HA crystal structure effectively improves the corrosion resistance of the substrate [8,9]. Therefore, the simultaneous incorporation of Zn and Cu ions into HA coating is encouraging for the enhancement of the bioactivity, corrosion resistance, and antibacterial property of the biomedical coating. Cathodic electrodeposition (CED) is attracting increasing attention as a superior method for the preparation of bioceramic coatings, inorganic composite coatings, and organic protective coatings [10]. Therefore, the application of CED would be suitable for the preparation of Cu-HA and Zn-HA coating.

Development of bone-implant interfaces depends on the direct interactions of bone matrix and osteoblasts with the biomaterials. Osteoblast adhesion is, therefore, essential for bone-biomaterial interactions. Since Zn-HA coating has higher Ra, hydrophilicity, and mechanical strength, it is envisaged that this coating will show better protein accumulation and osseointegration in the implanted condition.

CONCLUSIONS

Thin coatings of Cu-HA and Zn-HA were deposited on TNT surfaces. Zn-HA showed higher adhesion strength as compared to other coatings.

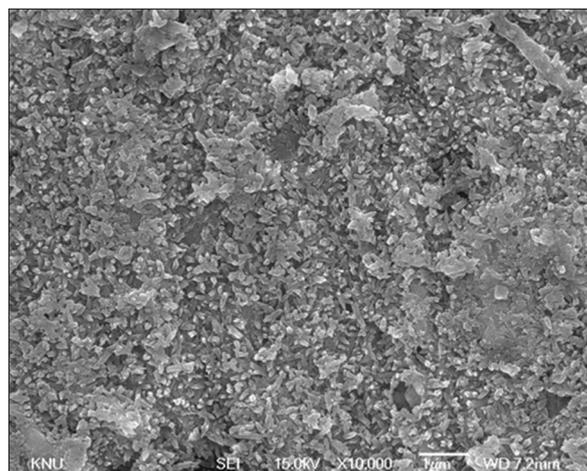


Fig. 4: Scanning electron microscopy of Zn-HA surface

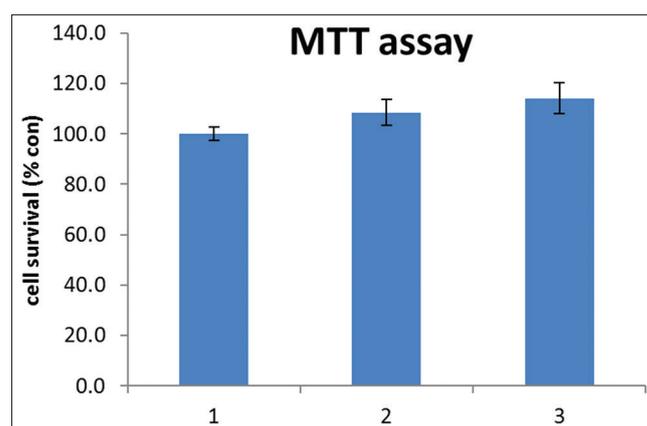


Fig. 5: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay of (1) HA, (2) Cu-HA, and (3) Zn-HA surface

Table 1: Properties of coating

Parameters	HA (ctrl)	Cu-HA	Zn-HA
Surface roughness (Ra) (μm)	0.54	0.58	0.68
Contact angle (degrees)	64	59	51
Adhesion strength (MPa)	7.1	7.8	8.8

HA: Hydroxyapatite

Moreover, hydrophilicity and surface Ra of Zn-HA was higher than Cu-HA coating. Having better mechanical, surface Ra, hydrophilic and biological properties, Zn-HA is most suitable as a bioimplant surface as compared to HA or Cu-HA coating that can have better cell activities.

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REFERENCES

- Rautray TR, Narayanan R, Kwon TY, Kim KH. Surface modification of titanium and titanium alloys by ion implantation. *J Biomed Mater Res B Appl Biomater* 2010;93(2):581-91.
- Rautray TR, Narayanan R, Kim KH. Ion implantation of titanium based biomaterials. *Prog Mater Sci* 2011;56:1137-77.
- Wang Y, Wen C, Hodgson P, Li Y. Biocompatibility of TiO₂ nanotubes with different topographies. *J Biomed Mater Res A* 2014;102(3):743-51.
- Brammer KS, Kim H, Noh K, Loya M, Frandsen CJ, Chen L, *et al.* Highly bioactive 8 nm hydrothermal TiO₂ nanotubes elicit enhanced

- bone cell response. *Adv Eng Mater* 2011;13(3):B88-94.
5. Chang CH, Lee HC, Chen CC, Wu YH, Hsu YM, Chang YP, *et al.* A novel rotating electrochemically anodizing process to fabricate titanium oxide surface nanostructures enhancing the bioactivity of osteoblastic cells. *J Biomed Mater Res A* 2012;100(7):1687-95.
 6. Oh SH, Finões RR, Daraio C, Chen LH, Jin S. Growth of nano-scale hydroxyapatite using chemically treated titanium oxide nanotubes. *Biomaterials* 2005;26(24):4938-43.
 7. Sobieszcyk S, Klotzke R. Nanotubular titanium oxide layers for enhancement of bone-implant bonding and bioactivity. *Adv Mater Sci* 2011;11(1):17-26.
 8. Rautray TR, Narayanan R, Kwon TY, Kim KH. Accelerator based synthesis of hydroxyapatite by MeV ion implantation. *Thin Solid Films* 2010;518:3160-3.
 9. Thian ES, Huang J, Vickers ME, Best SM, Barber ZH, Bonfield W. Silicon-substituted hydroxyapatite (SiHA): A novel calcium phosphate coating for biomedical applications. *J Mater Sci* 2006;41(3):709.
 10. Huang Y, Zenga H, Wang X, Wang D. *???* *Appl Surf Sci* 2014;290:353-8.
 11. Hu H, Zhang W, Qiao Y, Jiang X, Liu X, Ding C. Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO₂ coatings on titanium. *Acta Biomater* 2012;8(2):904-15.
 12. Bir F, Khireddinea H, Touatib A, Sidanea D, Yalaa S, Oudadesse H. *???* *Appl Surf Sci* 2012;258:21-7030.
 13. Radovanovic Z, Jokic B, Veljovic D, Dimitrijevic S, Kojic V, Petrovic R, *et al.* Antimicrobial activity and biocompatibility of Ag⁺ and Cu²⁺ -doped biphasic hydroxyapatite/alpha-tricalcium phosphate obtained from hydrothermally synthesized Ag⁺ - And Cu²⁺ - Doped hydroxyapatite. *Appl Surf Sci* 2014;307:513-9.
 14. Huo K, Zhang X, Wang H, Zhao L, Liu X, Chu PK. Osteogenic activity and antibacterial effects on titanium surfaces modified with Zn-incorporated nanotube arrays. *Biomaterials* 2013;34(13):3467-78.
 15. Zhao S, Dong W, Jiang Q, He F, Wang X, Yang G. *???* *Biomed Biotechnol* 2013;14:518-25.
 16. Rautray TR, Kim KH. *Key Engineering Materials*. Vol. 493-494. Switzerland: Trans Tech Publications; 2012. p. 181-5.
 17. Rautray TR, Swain S, Kim KH. *???* *Adv Sci Lett* 2014;20:801-3.
 18. Huang Y, Han SG, Ding QQ, YanYJ, Pang XF. *???* *Spectrosc Spectral Anal* 2013;33:2379-82.
 19. Rautray TR, Kim KH. Nanoelectrochemical coatings on titanium for bioimplant applications. *Mater Technol* 2010;25:143-8.
 20. Lee KW, Bae CM, Jung JY, Sim GB, Rautray TR, Lee HJ, *et al.* Surface characteristics and biological studies of hydroxyapatite coating by a new method. *J Biomed Mater Res B Appl Biomater* 2011;98(2):395-407.
 21. **ASTM C633-79, 1980.**
 22. Narayanan R, Kwon TY, Kim KH. TiO₂ nanotubes from stirred glycerol/NH₄F electrolyte: Roughness, wetting behavior and adhesion for implant applications. *Mater Chem Phys* 2009;117:460-4.
 23. Rautray TR, Vijayan V, Panigrahi S. Analysis of Indian cholesterol gallstones by particle-induced X-ray emission and thermogravimetry-derivative thermogravimetry. *Eur J Gastroenterol Hepatol* 2006;18(9):999-1003.
 24. Kar A, Raja KS, Misra M. Electrodeposition of hydroxyapatite onto nanotubular TiO₂ for implant applications. *Surf Coat Technol* 2006;201(6):3723-31.
 25. Mohammadi Z, Ziaei-moayyed AA, Sheikh-mehdi AM. *???* *Appl Surf Sci* 2007;253:4960-65.