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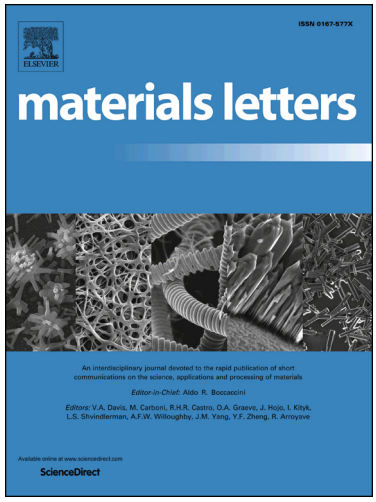

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Simultaneous anodization/anaphoretic electrodeposition synthesis of nano calcium phosphate/titanium oxide composite coatings assisted with chitosan oligosaccharide lactate

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Abstract

In this paper novel *in situ* one-step simultaneous anaphoretic deposition process of amorphous calcium phosphate (ACP) and titanium oxide (TiOx) with and without chitosan oligosaccharide lactate (ChOL) on titanium substrate was performed. The coatings were investigated by SEM, XRD and FTIR techniques, whereas roughness and adhesion were measured. It was shown that novel process occurs, with improved coatings adhesion and excellent coverage of the surface. At 90V the surface is smoother, and there is possibility for crystallization of the components at prolonged deposition times.

Keywords: amorphous calcium phosphate; chitosan oligosaccharide lactate; *in situ* anaphoretic deposition; titanium oxide.

1. Introduction

Calcium phosphates (CPs), mainly hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), along with Ti and TiO_2 layers, have found vast applications in preventive and regenerative medicine due to their excellent biocompatibility, nontoxic properties and participate in the normal metabolism of organisms [1,2]. Particularly significant application of CPs is reflected in their coating form. The properties of metal implants suitable for use in orthopedics have been enhanced by coating with CPs due to their good osteoconductive properties [3]. Electrophoretic deposition method (EPD) is effective method for coating bioceramics on the implants [4,5]. The factors which affect the EPD coating quality are deposition period, applied voltage, and suspension concentration.

Due to the poor mechanical properties of CP-based biomaterials, combining CPs with biopolymers is gaining increasing interest. Chitosan (CH) based CPs have found applications in bone tissue engineering,

where chitosan enabled room temperature processing of composite materials with corrosion protection of implant [5,6]; CH and heparin coatings showed increased deposition rate and improved blood compatibility [7]. Cataphoretically synthetized CH/HAp coatings on Ti substrate in two-step process are obtained [8], some chitosan multilayered coatings were used as multifunctional implants [9] and amorphous calcium phosphate (ACP) was shown to be good candidate for protein protection [10]. New research on chitosan oligosaccharide lactate (ChOL) coated HAp for drug delivery have indicated the advanced properties of this derivative compared to CH [2]. However, there are neither published studies about the ACP/ChOL composite coating, nor about anaphoretical deposition of ACP/ChOL on titanium for the biomedical application. Moreover, there is no record about formation of such composite coating in single step process.

Thus, the aim of this work was investigation of possible simultaneous anodization/anaphoretic electrodeposition synthesis of nano CP/TiOx composite coatings assisted with ChOL.

2. Materials and Methods

An aqueous $Ca(NO_3)_2$ (150ml; 26.6 wt%) was added to the $(NH_4)_3PO_4$ (7ml H_3PO_4+165ml NH_4OH+228ml H_2O) at 50°C for 60 minutes, while stirring at the rate of 100 rpm. The obtained gel was washed with distilled water and centrifuged at 4000 rpm and 5°C for 1h. The resulting precipitate was freeze-dried at -30°C and 0.37 bar for 2h.

Absolute ethanol suspensions containing: 1wt% of nanosized ACP for ACP/TiOx deposition and 1wt% of ACP and 0.05wt% of ChOL (Sigma, average Mn 5,000) for ACP/TiOx/ChOL deposition; all with 10wt% NaOH and at pH value of 10 were prepared. The suspensions were ultrasonicated for 15 min. Titanium plates (20mm×10mm×0.89mm, Aldrich, 99.7% purity) were used as substrates. Before deposition, Ti plates were abraded with silicon carbide paper and mechanically polished with alumina pastes.

Ti plate as working electrode and 1Cr18Ni9Ti stainless steel plates (10mm from the anode) as counter electrode were used. The applied voltages were 60V and 90V. Hewlett Packard HP6024A potentiostat/galvanostat was used as power supply. The suspensions were constantly stirred during electrodeposition. The coatings were obtained for 3min at 25°C and constant voltage regime.

The surface morphology was analyzed by field-emission scanning electron microscopy (Tescan Mira 3 XMU FEG-SEM). Fourier transform infrared spectroscopy (FTIR) was carried out using Michelson MB Series Bomen FTIR spectroscope (Hartmann Braun). Structural and phase analysis of the composites

were examined by X-ray diffraction (XRD) measurements on Philips PW 1050 powder diffractometer 25°C. Linear roughness of composites was measured using roughness tester TR-200 (Innovatest). Adhesion of the coatings was measured according to ASTM D 3359-02 standard, and recorded by Olympus CX41 optical microscope.

3. Results and discussion

Fig. 1a and c show SEM images of ACP/TiOx composite coatings synthetized at 60V and 90V, while Fig. 1b and d show SEM images of ACP/TiOx/ChOL composite coatings synthetized at 60V and 90V, respectively. Images have shown that synthesized coatings cover the substrate surface completely, and that the coating consists of agglomerated nanosized particles of size less than 100 nm. Two morphologically different coatings can be observed: the ACP/TiOx agglomerates are larger, and the surface is rougher than in the case of ACP/TiOx/ChOL. Also noticeable is smoother surface at higher voltage in both cases and there is no cracking of ACP/TiOx/ChOL at 90V.

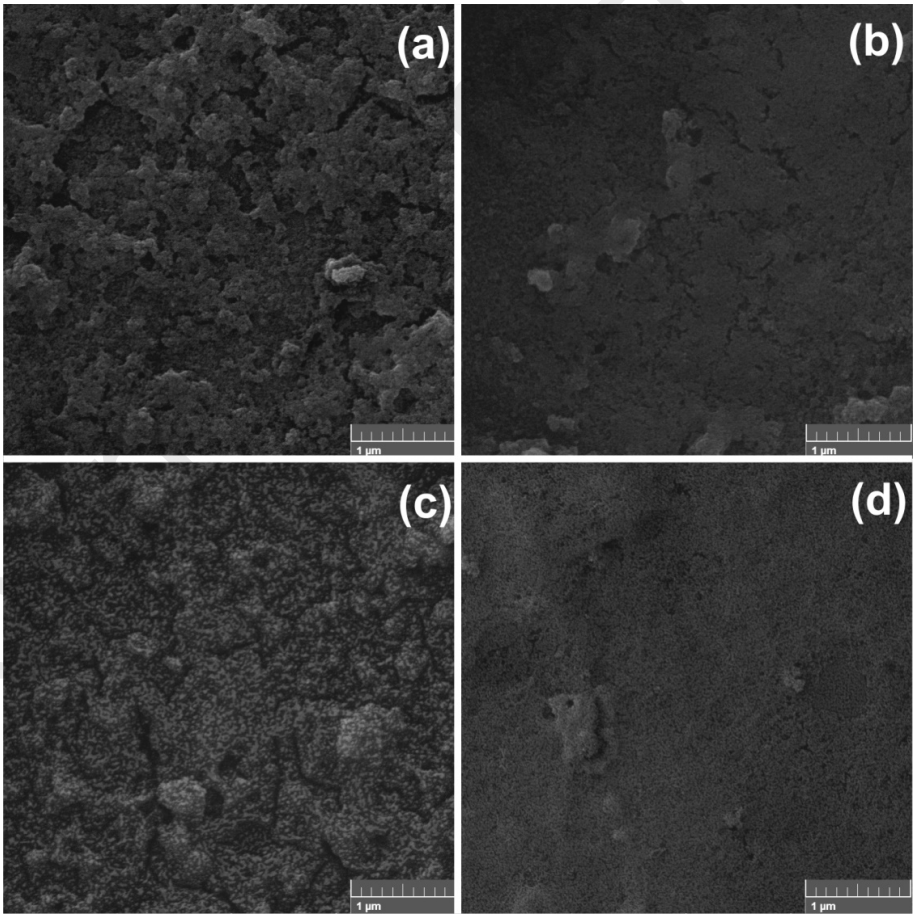

Fig. 1. SEM images of synthetized ACP/TiOx and ACP/TiOx/ChOL coatings. (a) ACP/TiOx 60 V, (b) ACP/TiOx 90 V, (c) ACP/TiOx/ChOL 60 V, (d) ACP/TiOx/ChOL 90 V

In accordance with mass composition of the suspension (Section 2) and EDS analyses of the anodic TiOx growth [11], the average TiOx/ChOL mass ratio (out of 15 EDS measurements) appears to be 1:2.

As demonstrated in Fig. 2a, the diffraction pattern of coatings XRD measurements show typical ACP diffusion maximum at about 2θ =30° [12]. However, diffraction peaks at 2θ of 25.8°, 31.7° and 32.2° for 90V samples can be assigned to (0 0 2), (2 1 1) and (1 1 2) reflections of HAp crystal lattice.

As there is local temperature increase during *in situ* process, it is possible that ACP transforms to low-crystalline HAp. Other authors have also confirmed the possibility of transformation of ACP into low-crystalline HAp under the influence of temperature [13]. Also noticeable is ACP/TiOx/ChOL samples diffraction peak at 2θ=29.4° that can be assigned to chitosan [14]. The obtained results suggest that increase in temperature causes thermal degradation of ChOL to products similar to chitin and chitosan [15]. The diffraction peak at 2θ=37.3° belongs to TiO_2 phase [16]. Findings prove that *in situ* one-step ACP/TiOx/ChOL deposition occurs.

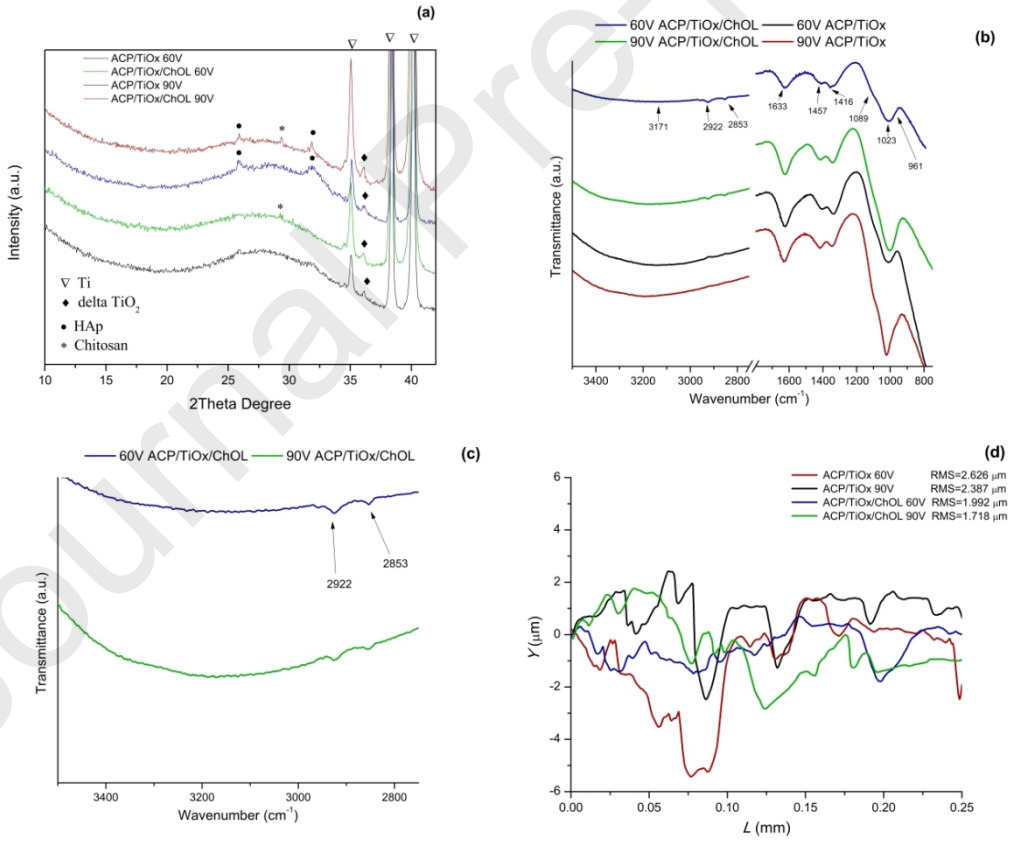

Fig. 2. (a) XRD and (b) FTIR spectra of ACP/TiOx and ACP/TiOx/ChOL. (c) enlarged FTIR spectra, (d) linear roughness profiles

Fig. 2b shows FTIR spectra of synthesized coatings. All the spectra display typical PO_4^{3-} characteristic absorption bands of ACP that are observed in the 950–1100 cm^{-1}. The most distinguished adsorption peak is at 1023 cm^{-1} with two noticeable shoulders at 1089 and 961 cm^{-1} (υ1 and υ3 phosphate modes) [17]. Peaks at 1416 and 1457 cm^{-1} are attributed to CO bending vibrations from CO_3^{2-} in ACP [11]. The wide band at 3171 cm^{-1} and peak at 1633 cm^{-1} are attributed to the OH− of absorbed water [11,17]. Two peaks at 2922 and 2853 cm^{-1} in ACP/TiOx/ChOL samples (Fig. 2b and c) are attributed to CH vibrations of ChOL [18].

The root mean square roughness (RMS) results are shown in Fig. 2d. It can be seen that the surface is rougher for ACP/TiOx compared to ACP/TiOx/ChOL samples, where more pronounced smaller profile shapes (less agglomerates) can be observed. Also, the samples prepared at 60V are rougher compared to the ones prepared at 90V in both cases.

Adhesion of composite coatings is one of the most important features for possible future biomedical use of these composite materials, and it was performed as described in Section 2. The optical images of the coatings after performing adhesion testing are shown in Fig. 3a-d.

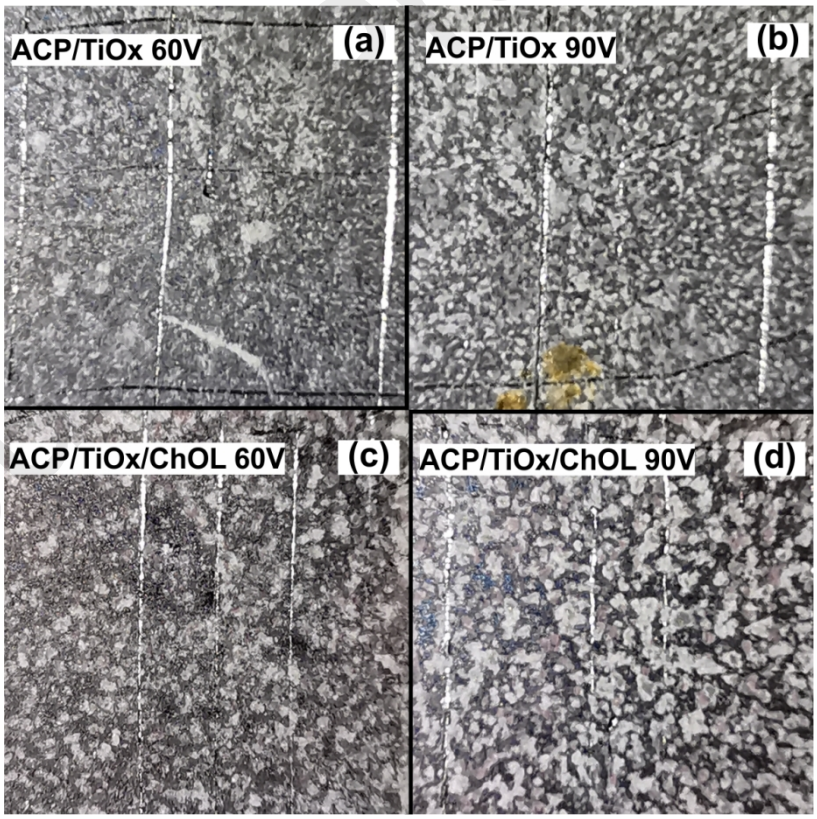

Fig. 3. Optical images of composites after performing adhesion testing. Size 5mm×5mm.

It can be concluded that, after testing, adhesion has the highest level 5 (no delamination and no flaking). Comparing to similar coatings prepared by EPD methods [19], this novel method is huge improvement, having in mind that the sintering of the coatings is omitted.

4. Conclusions

Nano ACP/TiOx and ACP/TiOx/ChOL composite coatings have been successfully synthesized for the first time by novel simultaneous *in situ* method of anaphoretic deposition on titanium substrate. The formation of these composite coatings was confirmed by SEM, XRD and FTIR. Excellent coverage of the surface with firm deposit and good adhesion is obtained. Also noticeable is transformation of ACP to low-crystalline HAp at higher voltages due to local temperature increase during *in situ* process. Well adhered ACP and ACP/ChOL film on TiOx could improve Ti implants` surface osteoconductive properties for use in orthopedics, which represents our next research.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] C. Qi, J. Lin, L.H. Fu, P. Huang, Calcium-based biomaterials for diagnosis, treatment, and theranostics, Chem. Soc. Rev. 47 (2018) 357–403. doi:10.1039/c6cs00746e.

[2] N.L. Ignjatović, M. Sakač, I. Kuzminac, V. Kojić, S. Marković, D. Vasiljević-Radović, V.M. Wu, V. Uskoković, D.P. Uskoković, Chitosan oligosaccharide lactate coated hydroxyapatite nanoparticles as a vehicle for the delivery of steroid drugs and the targeting of breast cancer cells, J. Mater. Chem. B. 6 (2018) 6957–6968. doi:10.1039/c8tb01995a.

[3] W. Habraken, P. Habibovic, M. Epple, M. Bohner, Calcium phosphates in biomedical applications: Materials for the future?, Mater. Today. 19 (2016) 69–87. doi:10.1016/j.mattod.2015.10.008.

[4] İ. Aydın, A.İ. Bahçepınar, M. Kırman, M.A. Çipiloğlu, HA Coating on Ti6Al7Nb Alloy Using an Electrophoretic Deposition Method and Surface Properties Examination of the Resulting

Coatings, Coatings. 9 (2019) 402. doi:10.3390/coatings9060402.

[5] X. Pang, T. Casagrande, I. Zhitomirsky, Electrophoretic deposition of hydroxyapatite-CaSiO3-chitosan composite coatings, J. Colloid Interface Sci. 330 (2009) 323–329. doi:10.1016/j.jcis.2008.10.070.

[6] Q.H. Zhang, B.S. Hou, Y.Y. Li, G.Y. Zhu, H.F. Liu, G.A. Zhang, Two novel chitosan derivatives as high efficient eco-friendly inhibitors for the corrosion of mild steel in acidic solution, Corros. Sci. (2019) 108346. doi:10.1016/j.corsci.2019.108346.

[7] F. Sun, X. Pang, I. Zhitomirsky, Electrophoretic deposition of composite hydroxyapatite-chitosan-heparin coatings, J. Mater. Process. Technol. 209 (2009) 1597–1606. doi:10.1016/j.jmatprotec.2008.04.007.

[8] A. Pawlik, M.A.U. Rehman, Q. Nawaz, F.E. Bastan, G.D. Sulka, A.R. Boccaccini, Fabrication and characterization of electrophoretically deposited chitosan-hydroxyapatite composite coatings on anodic titanium dioxide layers, Electrochim. Acta. 307 (2019) 465–473. doi:10.1016/j.electacta.2019.03.195.

[9] J.P. Govindharajulu, X. Chen, Y. Li, J.C. Rodriguez-cabello, Chitosan-Recombinamer Layer-by-Layer Coatings for Multifunctional Implants, Int. J. Mlecular Sci. 18 (2017) 1–16. doi:10.3390/ijms18020369.

[10] Y. Yang, G. Wang, G. Zhu, X. Xu, H. Pan, R. Tang, The effect of amorphous calcium phosphate on protein protection against thermal denaturation, Chem. Commun. 51 (2015) 8705–8707. doi:10.1039/c5cc01420d.

[11] M.R. Pantović Pavlović, S.G. Eraković, M.M. Pavlović, J.S. Stevanović, V. V. Panić, N.L. Ignjatović, Anaphoretical/oxidative approach to the in-situ synthesis of adherent hydroxyapatite/titanium oxide composite coatings on titanium, Surf. Coatings Technol. 358 (2019) 688–694. doi:10.1016/j.surfcoat.2018.12.003.

[12] K. Pakravanan, M. Rezaee Roknabadi, F. Farzanegan, A. Hashemzadeh, M. Darroudi, Amorphous calcium phosphate nanoparticles-based mouthwash: preparation, characterization, and anti-bacterial effects, Green Chem. Lett. Rev. 12 (2019) 278–285. doi:10.1080/17518253.2019.1643412.

[13] V. Uskoković, S. Marković, L. Veselinović, S. Škapin, N. Ignjatović, D.P. Uskoković, Insights

into the kinetics of thermally induced crystallization of amorphous calcium phosphate, Phys. Chem. Chem. Phys. 20 (2018) 29221–29235. doi:10.1039/c8cp06460a.

[14] C. Lai, Y. Chen, S. Zhang, Study on Chitosan-Lactate Sponges with Oriented Pores as Potential Wound Dressing, Mater. Sci. Appl. 04 (2013) 458–470. doi:10.4236/msa.2013.48056.

[15] A.K. Pal, V. Katiyar, Thermal degradation behaviour of nanoamphiphilic chitosan dispersed poly (lactic acid) bionanocomposite films, Int. J. Biol. Macromol. 95 (2017) 1267–1279. doi:10.1016/j.ijbiomac.2016.11.024.

[16] W. Li, R. Liang, A. Hu, Z. Huang, Y.N. Zhou, Generation of oxygen vacancies in visible light activated one-dimensional iodine TiO2 photocatalysts, RSC Adv. 4 (2014) 36959–36966. doi:10.1039/c4ra04768k.

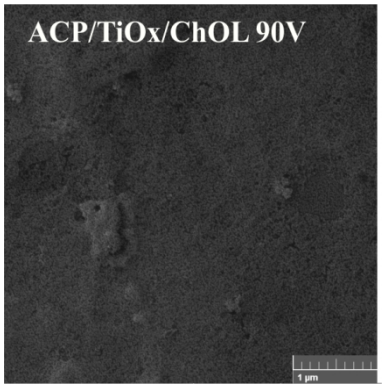
[17] W. Kong, K. Zhao, C. Gao, P. Zhu, Synthesis and characterization of carbonated hydroxyapatite with layered structure, Mater. Lett. 255 (2019) 126552. doi:10.1016/j.matlet.2019.126552.

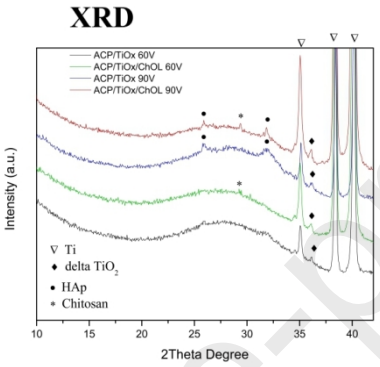
[18] M.F. Cervera, J. Heinämäki, N. de la Paz, O. López, S.L. Maunu, T. Virtanen, T. Hatanpää, O. Antikainen, A. Nogueira, J. Fundora, J. Yliruusi, Effects of spray drying on physicochemical properties of chitosan acid salts., AAPS PharmSciTech. 12 (2011) 637–649. doi:10.1208/s12249-011-9620-3.

[19] M. Pantovic-Pavlovic, M. Pavlovic, S. Erakovic, T. Barudzija, J. Stevanovic, N. Ignjatovic, V. Panic, Relationship between the properties of an interlayer formed by in situ Ti anodization and anaphoretically deposited hydroxyapatite, J. Serbian Chem. Soc. 84 (2019) 105–105. doi:10.2298/JSC190730105P.

CRediT author statement

Marijana R. Pantović Pavlović: Investigation, Methodology, Writing – Original Draft, **Miroslav M. Pavlović:** Formal Analysis, Validation, Visualization, Writing – Review and Editing, **Sanja Eraković:** Data Curation, **Jasmina S. Stevanović:** Funding acquisition, **Vladimir V. Panić:** Supervision **and Nenad Ignjatović:** Conceptualization, Methodology, Supervision, Writing – Review and Editing





Highlights

- Nano calcium phosphate/titanium oxide/chitosan oligosaccharide lactate composites
- Novel *in situ* one-step anaphoretic electrodeposition with improved adhesion
- Transformation of amorphous calcium phosphate to hydroxyapatite

Conflict of interest

The authors confirm that the manuscript submitted for review is original, has been written by the stated authors who are all aware of its content and approve its submission and has not been published previously. The article is currently not being considered for publication by any other journal elsewhere and will not be submitted for such a review while under review by *Materials Letters***.** There is **no existence of conflict of interest, and if accepted, the article will not be published elsewhere in the same form, in any language, without the written consent of the publisher. Manuscript has been approved by the responsible authorities – institutions where the work has been carried out.**

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: