JOURNAL OF ENGINEERING SCIENCES
ЖУРНАЛ ІНЖЕНЕРНИХ НАУК
ЖУРНАЛ ИНЖЕНЕРНЫХ НАУК

Web site: http://jes.sumdu.edu.ua

DOI: 10.21272/jes.2017.4(2).f1 Volume 4, Issue 2 (2017)

UDC 544.032.4

Formation of hydroxyapatite coatings with addition of chitosan from aqueous solutions by thermal substrate method

Yanovska G. O.[1], Bolshanina S. B.[1*], Kuznetsov V. M.[2]

[1] Sumy State University, 2 Rymskogo-Korsakova St., 40007, Sumy, Ukraine;
[2] Institute of Applied Chemistry of the National Academy of Sciences of Ukraine, 3 Rymskogo-Korsakova St., 40007, Sumy, Ukraine

Article info:
Paper received: September 27, 2017
The final version of the paper received: December 1, 2017
Paper accepted online: December 3, 2017

[*]**Corresponding Author's Address:**
svet.bolshanina@gmail.com

Abstract. The aim of this investigation was obtaining of biocompatible coatings for medical implants based on biopolymer chitosan and hydroxyapatite, which is the main mineral component of bone tissue. Coatings were obtained by thermal substrate method, because it allows low temperature deposition and provides possibility to incorporate into coating structure biomolecules, unstable at high temperatures. As a way of chitosan incorporation into coating composition co-precipitation method was proposed. It allows obtaining uniform coatings with required composition and morphology. The obtained coatings were investigated by using of XRD, SEM with EDS, adhesion was tested by test-tape method. It was assigned that chitosan addition decreased hydroxyapatite crystallinity, so the range of concentrations from 0.01 to 0.1 g/L was chosen at pH = 6.5. It was determined that variation of chitosan concentrations in the initial solution influenced on morphology and structure of hydroxyapatite coatings as well as on the antibacterial properties and the use in orthopedics and dentistry. The best characteristics were obtained for hydroxyapatite-chitosan coatings deposited from solution with chitosan concentration 0.025 g/L.

Keywords: hydroxyapatite, chitosan, coating, deposition, thermal substrate method.

F

1 Introduction

Metal ion release of medical implants lead to inflammatory effects in the physiological environment. That's why the application of bioactive coatings for implant materials is a very promising way to solve this problem. Chitosan (CS) and hydroxyapatite (HA) are among the best bioactive biomaterials in bone tissue engineering due to their excellent biocompatibility in the physiological environment [1]. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is widely used in dentistry and orthopedics. It is one of the most thermodynamically stable forms of calcium phosphate which occurs in the bone as a major component (from 60 to 65 %) [2]. Chitosan is an alternative polymer for use in orthopedic applications due to its good biocompatibility, biodegradability, porous structure, suitability for cell growth, osteoconduction and intrinsic antibacterial nature [3–5]. Chitosan is an N-deacetylation product of chitin. It is a copolymer consisting of β-(1→4)-2-acetamido-d-glucose and β-(1→4)-2-amino-D-glucose unit linkages [6–7]. It has good solubility in various organic acid solutions and sufficient resistance in alkali environments. In addition, chitosan is flexible and has a high resistance upon heating due to the intermolecular hydrogen bonds formed between hydroxyl and amino groups [8–10]. When chitosan is dissolved in a diluted organic acid solution, its free amino groups are protonated, although it is insoluble in an aqueous solution at pH > 7 [11].

Several methods are available for the application of HA coatings onto metal substrates [12]. But only few of them are acceptable for obtaining CS-HA coatings, because of high temperatures of processes and possibility of chitosan degradation. Among methods for HA-CS coating deposition are simple mixing and heating method [13], biomimetic method [14-16], low temperature wet chemical method [17], electrochemical deposition [18], electrochemistry assisted deposition [19, 20], electrophoretic deposition [21, 22]. In our work we proposed thermal substrate method [23, 24] (TSM) for deposition of

HA-CS coatings onto Ti_6Al_4V substrates from aqueous solutions. The main advantage of this method is a thermal activation near substrate surface which immersed in aqueous solutions containing Ca^{2+} and PO_4^{3-} for hydroxyapatite synthesis with promoted crystallization and formation of film-like deposits [24]. Several factors influence the HA nucleation and crystallization: chitosan concentration in the initial solution, pH value of the solution, Ca^{2+} and PO_4^{3-} ion concentrations, the heating time and temperature, substrate surface modification, etc.

At the present work an attempt has been made for the first time HA-CS coatings deposition onto Ti6Al4V substrates by TSM. The aim of our study was to compare various concentrations of chitosan inserted into hydroxyapatite coatings. The main characteristics of obtained coatings were investigated and compared. The influence of chitosan onto calcium-phosphate formation during deposition was also studied.

2 Experimental

2.1 Materials

Ti_6Al_4V specimens, 36×1.9×0.36 mm in size, were used as substrates for HA-CS coatings deposition. They were polished with sandpaper, washed in acetone (15 min), 96 % ethanol (15 min) and three times rinsed with distilled water under ultrasound.

Biomedical grade chitosan (200 kDa molecular weight) was supplied by the Haidebei Marine Bio Ltd. (Jinan, China) with 91 % degree of the deacetylation. Solutions with various chitosan concentrations were prepared by dissolving the 1 g of chitosan fibers in 1 liter of 1 % CH_3COOH solution with vigorous stirring. Chitosan solution with concentration 1 g/l was diluted to required CS concentrations: 0.01, 0.025, 0.05, and 0.1 g/l by mixing with the initial solution for HA synthesis which contains $CaCl_2$ (10 mmol/dm^3) and NaH_2PO_4 (6 mmol/dm^3).

2.2 Obtaining of HA-CS coating by thermal substrate method

Chitosan was inserted into hydroxyapatite coatings by co-deposition of hydroxyapatite and chitosan. The initial solution for coating deposition was prepared by mixing solution which contains $CaCl_2$ (10 mmol/dm^3) and NaH_2PO_4 (6 mmol/dm^3) with chitosan solution (1 g/l) in various proportions (Table 1).

The thermal substrate method for obtaining of HA coatings based on the main principle that the solubility of HA in aqueous solutions decreases with increasing substrate temperature [2, 24]. Alternating current passed through the system to heat the substrate. By this method hydroxyapatite directly coated the substrate without precipitation in the initial solution. The experimental arrangement for coating deposition is described in the work [24].

Co-deposition of hydroxyapatite coatings was carried by TSM method under following conditions: substrate temperature 100–105 ºC, pH of the initial solution 6.5–6.85, time of deposition – 180 min.

Table 1 – The composition of the initial solution for co-deposition of HA-CS coatings, ml

Volume of reagents	Chitosan concentration (g/l)				Without chitosan
	0.001	0.025	0.05	0.1	
Chitosan solution in 1 % CH_3COOH (1 g/l)	2	5	10	20	–
$CaCl_2$ (10 mmol/dm^3)/ NaH_2PO_4 (6 mmol/dm^3)	198	195	190	180	200

2.3 Analysis techniques

The crystallinity and structure of the coatings were examined using an X-ray diffractometer DRON 4-07 ("Burevestnik", Russia) connected to a computer-aided system for the experiment control and data processing. The Ni-filtered CuK_α radiation (wavelength 0.154 nm) with a conventional Bragg–Brentano ϑ-2ϑ geometry was used. The current and the voltage of the X-ray tube were 20 mA and 40 kV, respectively. The samples were measured in the continuous mode at a rate of 1.0 deg/min, with 2θ-angles ranging from 15° to 55°. All experimental data were processed by means of the program package DIFWIN-1 ("Etalon PTC" Ltd, Russia). Identification of crystal phases was done using a JCPDS card catalog (Joint Committee on Powder Diffraction Standards.

The surface morphology of HA-CS coatings was examined by Scanning electron microscopy (SEM). These investigations were performed in combination with X-ray emission spectroscopy using the REMMA-102 device (SELMI, Sumy, Ukraine). The surface chemical composition was determined with an energy dispersive X-ray (EDX) detector. The analytical signal of the characteristic X-ray emission was integrated by scanning the 50×50 μm^2 area of the sample surface.

Adhesion of obtained coatings was measured by tape-test method as described elsewhere [25].

3 Results and discussion

Chitosan dissolution in acetic acid described as follows:

$$CS–NH_2 + HOOC–CH_3 \rightarrow CS–NH_3^+ + CH_3COO^- \quad (1)$$

Viala et al. (26) established that the presence of calcium and phosphate ions in the solution allows the soluble form of CS to exist when below pH 6.7. At pH 6.65-7.0 calcium and phosphate ions existed in the initial solution and deposited on the substrate. Simultaneously, the following reaction of hydroxyapatite formation takes place on the substrate surface:

$$10Ca^{2+} + H_2PO_4^- + 14OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12H_2O \quad (2)$$

Morphology of the CS-HA coatings obtained by TSM method from solutions with chitosan concentrations 0.001–0.1 g/l is shown in Fig. 1.

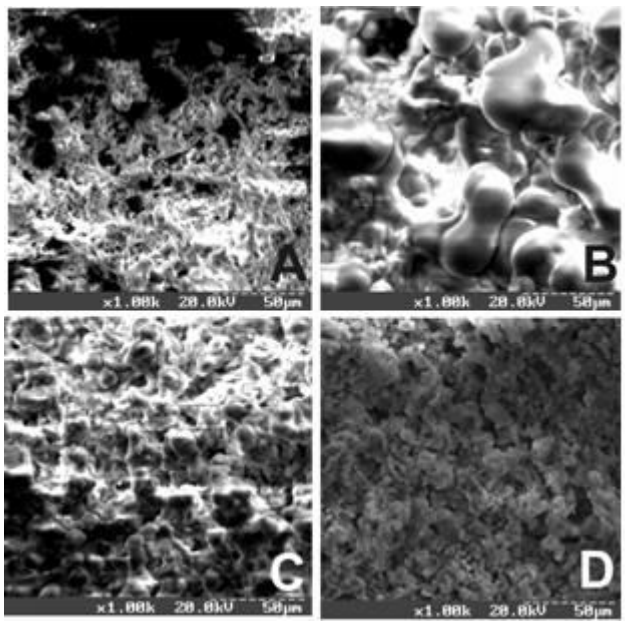

Figure 1 – Morphology of the HA-CS coatings co-deposited by TSM method from aqueous solutions with chitosan concentrations 0.1 g/l (A), 0.05 g/l (B), 0.025 g/l (C), 0.001 g/l (D)

As can be seen in Fig.1 the most uniform coatings with rough surface were obtained from solutions with chitosan concentrations 0.001−0.025 g/l. It could be due to the possible interaction of chitosan macromolecules with components of initial solution. From the XRD-spectra (Fig. 2) it could be seen that with increasing of chitosan concentration in the initial solution for HA synthesis the relation of intensities of the main HA peak (2θ = 31,65°) to diffusive peak of chitosan (2θ = 20º) are decreased.

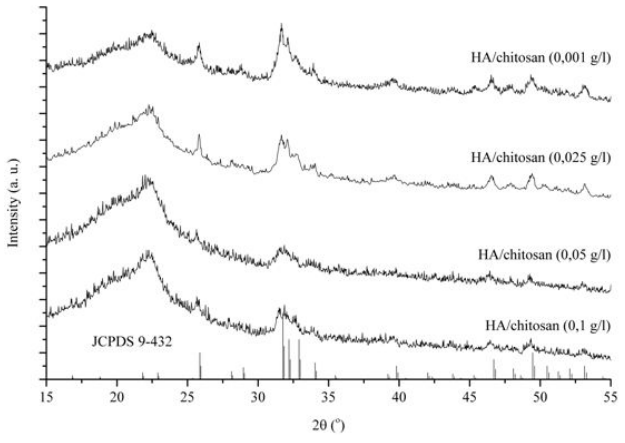

Figure 2 – X-ray diffraction patterns of HA-CS coatings obtained by TSM from aqueous solutions with chitosan concentrations 0.1 g/l (A), 0.05 g/l (B), 0.025 g/l (C), 0.001 g/l (D)

When chitosan is dissolved in acetic acid its amino groups are protonated ($CS{-}NH_3^+$) and bonds with carboxyl groups (CH_3COO^-) presented in solution. If other ions Ca^{2+}, HPO_4^- are also presented in the initial solution some positively charged complexes for example $CH_3COO^-{-}Ca^{2+}$ and $HPO_4^-{-}Ca^{2+}$ can be formed in the solution. Such complexes adsorbed negatively charged PO_4^{3-} with following crystal growth of HA. The presence of Ca^{2+} ions in the initial solution leads to chemical interaction between the calcium ions on HA surface and the amino groups in a chitosan molecule. In general, chitosan forms a chitosan–metal complex in which the metal ion coordinates the amino group in chitosan molecules [26]. Activity of the Ca^{2+} ions is somewhat weaker than that of the transition metal ions [11]. We suggest that small HA crystallites are able to align along the chitosan molecule upon aggregation through the interaction between the Ca^{2+} ions on the HA surface and the amino groups of the chitosan molecule. In other words, the c-axes of HA nano-crystals are parallel to the chitosan molecules due to formation of complexes of Ca^{2+} and amino groups of chitosan, which are the nucleation centers for HA crystals [11]. Measured adhesion strength of obtained HA-CS coatings is presented in the Table 2.

Table 2 – Adhesion strength of HA-CS coatings obtained from solutions with various CS concentrations

Adhesion strength/ composition	HA/CS coatings obtained from solutions with CS concentration (g/L)			HA
	0.025	0.05	0.1	
N/m^2	$2\cdot10^5$	66670	54540	$8\cdot10^4$
MPa	0.2	0.07	0.05	0.08

So the most adhesive strength was observed for HA-CS coatings with CS concentration 0.025 g/L. The obtained coatings due to the existence of CS could reveal antibacterial properties and find its application in dentistry or orthopedics.

4 Conclusions

Novel HA-CS composite coatings were obtained by co-deposition from aqueous solutions having chitosan concentrations (0.001–0.1 g/l) using thermal substrate method. This method is very perspective in comparison with other methods of coatings deposition, because it gives possibility to obtain coatings under temperatures from 40 to 120ºC. Due to the low temperatures of deposition polymer addition and composite coating formation is possible. The obtained HA-CS coatings have developed surface that could improve osteointegration. Concentration of CS 0.025 g/L allows obtaining HA-CS coating with adhesion strength 0.2 MPa.

References

1. Wang, M. (2003). Developing bioactive composite materials for tissue replacement. *Biomaterials*, Vol. 24, 2133–2151.
2. Elliott, J. C. (1994). Structure and chemistry of the apatites and other calcium orthophosphates. *Elsevier*. London, pp. 111–127.
3. Vande V. P. J., Matthew, H. W. T., et al. (2002). Evalution of the biocompatibility of chitosan scaffold. *J. Biomed. Mater. Res.*, Vol. 59, 585–90.
4. Eugene, K., & Lee, Y. L. (2003). Implantable application of chitin and chitosan. *Biomaterials*, Vol. 24, 2339–2349.
5. Di Martino, A., Sittinger, M., & Risbud, M. V. (2005). Chitosan: A versatile biopolymer for orthopedic tissue-engineering. *Biomaterials*, Vol. 26, 5983–5990.
6. Je, J., & Kim, S. (2005). Water soluble chitosan derivatives as a BACE1 inhibitor. *Bioorg. Med. Chem.*, Vol. 13, 6551–6555.
7. Jeon, Y., Shahidi, F., & Kim, S. (2000). Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. *Food Rev. Int.*, Vol. 16, 159–176.
8. Okuyama, K., Noguchi, K, et al. (1999). Structural study of anhydrous tendon chitosan obtained via chitosan/acetic acid complex. *Int. J. Biol. Macromol.*, Vol. 26, 285–293.
9. Ogawa, K., Hirano, S., et al. (1984). A new polymorph of chitosan. *Macromolecules*, Vol. 17, 973–975.
10. Lee, Y. L., Khor, E., & Ling, C. E. (1999). Effects of dry heat and saturated steam on the physical properties of chitosan. *J. Biomed. Mater. Res., Appl. Biomater.*, Vol. 48, 111–116.
11. Yamaguchi, K., Tokuchi, H., et al. (2001). Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites. *J. Biomed. Mater. Res.*, Vol. 55, 20–27.
12. Venkatesan, J., & Kim, S.-K. (2010). Review. Chitosan composites for bone tissue engineering – An overview. *Mar. Drugs*, Vol. 8, 2252–2266.
13. Ding, S. (2007). Biodegradation behavior of chitosan/calcium phosphate composites. *J. Non-Cryst. Solids*, Vol. 353, 2367–2373.
14. Li, Q., Chen, Z., et al. (2006). Biomimetic synthesis of the composites of hydroxyapatite and chitosan–phosphorylated chitosan polyelectrolyte complex. *Mater. Lett.*, Vol. 60, 3533–3536.
15. Verma, D., Katti, K., & Katti, D. (2008). Effect of biopolymers on structure of hydroxyapatite and interfacial interactions in biomimetically synthesized hydroxyapatite/biopolymer nanocomposites. *Ann. Biomed. Eng.* Vol. 36, 1024–1032.
16. Davidenko, N., Carrodeguas, R., et al. (2010). Chitosan/apatite composite beads prepared by in situ generation of apatite or Si-apatite nanocrystals. *Acta Biomater.*, Vol. 6, 466–476.
17. Murugan, R., & Ramakrishna, S. (2004). Bioresorbable composite bone paste using polysaccharide based nano hydroxyapatite. *Biomaterials*, Vol. 25, 3829–3835.
18. Redepenning, J., Venkataraman, G., Chen, J., & Stafford, N. (2003). Electrochemical preparation of chitosan/hydroxyapatite composite coatings on titanium substrates. *J. Biomed. Mater. Res.*, Vol. 66, 411–416.
19. Pang, X., & Zhitomirsky, I. (2005). Electrodeposition of composite hydroxyapatite–chitosan films. *Mater. Chem. Phys.*, Vol. 94, 245–251.
20. Huang, Z., Dong, Y., Chu, C., & Lin P. (2008). Electrochemistry assisted reacting deposition of hydroxyapatite in porous chitosan scaffolds. *Mater. Lett.*, Vol. 62, 3376–3378.
21. Pang, X., Casagrande, T., & Zhitomirsky, I. (2009). Electrophoretic deposition of hydroxyapatite-$CaSiO_3$-chitosan composite coatings. *J. Colloid Interface Sci.*, Vol. 330, 323–329.
22. Pang, X., & Zhitomirsky, I. (2007). Electrophoretic deposition of composite hydroxyapatite-chitosan coatings. *Mater. Charact.*, Vol. 58, 339–348.
23. Sukhodub, L. B., Moseke, C., et al. (2003). Improved thermal substrate method with cooling system for hydroxyapatite coatings on titanium substrates. Annual Report. *Institut für Kernphysik*. Westfalishe Wilhelmsuniversität Münster, pp. 86–88.
24. Yanovska, A., Kuznetsov, V., et al. (2011). Synthesis and characterization of hydroxyapatite-based coatings for medical implants obtained on chemically modified Ti_6Al_4V substrates. *J. Surf. Coat. Technol.*, Vol. 205, 5324-5329.
25. Horkavcová, D., Plešingerová, B., et al. (2008). Adhesion of the bioactive layer on titanium alloy substrate by Tape-test. *Ceramics*. Silikáty, Vol. 52, No. 3, 130–138.
26. Viala, S., Freche, M., & Lacout J. L. (1998). Preparation of a new organic-mineral composite: chitosan-hydroxyapatite. *Ann. Chim. Sci. Mater.*, Vol. 23, Issues 1–2, 69–72.