Journal of Mechanical Engineering Research and Developments
ISSN: 1024-1752
CODEN: JERDFO
Vol. 44, No. 6, pp. 162-168
Published Year 2021

Fabrication of Zinc calcium phosphate for antibacterial applications

Roaa Saad, Shaker J. Edrees, Aseel Hadi

Department of Ceramics Engineering and Building Materials, Faculty of Materials Engineering, University of Babylon, Babylon, Iraq.

*Corresponding Author Email: stud.roaa.saad@uobabylon.edu.iq, mat.shaker.jahil@uobabylon.edu.iq, mat.Aseel.hadi.@uobabylon.edu.iq, [3]aseel16484@yahoo.com.

ABSTRACT

Zinc calcium phosphate is one of the important biological and antibacterial materials. The major objective of this research is estimating the possibility of forming zinc calcium phosphate [$Ca_{19}Zn_2(PO_4)_{14}$] by substituted Ca^{2+} by Zn^{2+} in the hydroxyapatite structure. In this study was prepared pure hydroxyapatite and zinc calcium phosphate at percent of zinc (0, 8) mol% ZnO by Sol-Gel technique. The resultant powders was sintering at 1100º C. The precursor materials are calcium nitride, di-ammonium hydrogen phosphate, zinc nitride, and ammonium hydroxide solution. At 8 mol% of ZnO, zinc calcium phosphate was successfully obtained. Testing the morphological properties was done by using Transmission electron microscopy (TEM), Field Emission- Scanning Electron Microscopy (FE-SEM), and Energy Dispersive X-Ray Spectroscopy (EDS). TEM results found the particles like rode with nano size. FE-SEM results tested after sintering at 1100ºC found that the particles agglomerated more when adding 8mol% ZnO. For testing the bioactivity was done by immersion the compacted the samples in simulated body fluid (SBF). XRD and FE-SEM tests shown that appetite layers formed on the samples surface, giving an evidence of their bioactivity. The antibacterial activity tested against *Escherichia coli*. Generally, adding zinc to the hydroxyapatite resulted the smaller particle size obtained, that mean high surface area of bioceramic powder, and subsequently improve the bone bonding ability and improve the bioactivity compared with pure hydroxyapatite.

KEYWORDS

Zinc calcium phosphate, TEM, SEM, SBF, HA.

INTRODUCTION

Currently, one of the most critical issues for the biomedical engineering field is development of next-generation materials [1]. The greatest potency for bone replacement is illustrated by materials composed of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, a calcium phosphate mineral that forms a strong bond with bone tissue, exhibits osteoconductive properties, resistance of bioresorption, and no adverse effects on the human organism [2-3]. Calcium phosphate material synthesis similar to that found in bone and suitable for orthopedic and dental applications [4]. The osseointegration property is required to minimize damage to surrounding tissues and to maximize implant efficiency. However, by substituting with ions found in natural bone apatites, the biological and physicochemical properties of HA can be enhanced. Due to the presence of minor constituents such as cations (Mn^{2+}, Zn^{2+}, Mg^{2+}, Na^{+}, Sr^{2+}) or anions (CO_3^{2-} or HPO_4^{2-}) , the majority of natural apatites are non-stoichiometric [5-6]. The lattice parameters, dissolution kinetics, and other physical properties can be affected by trace ions that are incorporated into apatites [7]. Research interest is the synthesis of Zn-substituted hydroxyapatite is important because bone and teeth are made of hydroxyapatite. In small amounts, zinc is found in the dentin of human teeth, and in large amounts it is present in the bone [8]. The formation of microbes at the positions of implantation is one of the major failures in the implantation process [9].Another problem of implantation is the contamination by bacteria that adhere on the surface of the biomaterials as an impediment of orthopedics[10].

The antibacterial behaviour of Zn-HA has been reported [11] while its nano-rods are reported to have improved act against oral cavity bacteria [12]. But the structural implication of the dopant has not been well reported. The authors in reference [13] reported that the complete inhibitory concentration against *E. coli* was 280 $\mu g \cdot mL^{-1}$ when the size of ZnO-NPs was 13 nm, while the complete inhibitory concentration against *S. aureus* was 80 $\mu g \cdot mL^{-1}$.Our previous study has institute the zinc location into the hydroxyapatite structure [14]. The sol-gel technique has found an advantage in easy control of chemical composition and synthesis temperatures. Stoichiometric, Homogeneity, and purity are characteristics of the sol-gel technique; also, using small particles allows for a higher firing temperature and ability to generate homogeneous and fine-grained structures are its advantages [15]. As well as easily using the sol–gel technique, trace elements like zinc can be doped into HA. This research has investigated the relationship between substituted zinc and microstructure and properties of HA that synthesized by sol-gel technique and how zinc affects the formation of an anti-bacterial biomaterial.

MATERIALS AND METHODS

Hydroxyapatite was obtained by sol-gel approach. In this method an appropriate amounts was made of calcium nitrate tetrahydrate (CN) and di-ammonium hydrogen phosphate (DAP) dissolved in distilled water in separate beakers using magnetic stirrer for about 30 minutes. Then, the DAP solution added drop by drop to the CN solution at 75 ºC under stirring. NH_4OH used to adjust the PH of the obtained solution and maintained above 10 .The solution kept under magnetic stirrer and continuously stirred for 12 hours. Then allowed to cool for 24 hours, washed with distilled water and ethanol at least three times and filtered. Finally, dried in hot oven at 85° C for overnight and hydroxyapatite obtained according to the equation (1).

$$10Ca(NO_3)_2.4H_2O+6(NH_4)_2HPO_4+8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 +20NH_4NO_3+46H_2O \quad (1)$$

To prepare zinc calcium phosphate, the same procedure above was done except that substituted of 8mol % of calcium nitride by zinc nitride.

Figure 1 shows the steps of the work research.

Figure 1. The steps of preparation of HA and Zinc calcium phosphate.

To confirm the particle morphology and calculate the particle size of obtained powders, a TEM device must be used. EDS was used to confirm the purity of the resultant powders.

RESULTS AND DISCUSSION

Transmission electron microscopy (TEM)

Figure 2 shows TEM micrographs of the prepared powders particles at percents (0and 8) mol% of ZnO at magnification 100 nm and these images are the most specified technique used to estimate the particle size. In figure (2, A) show the agglomerations particles at size 50nm . It was observed that ZnHA exhibited a rod-like morphology mimicking that of the natural bone apatite.

In figure (2, B) showed that the particles smaller size (33 nm) and highly agglomerated than that 0 mol% of ZnO that explained when the percent increased the agglomerations increased that attributed to the increasing in zinc content.

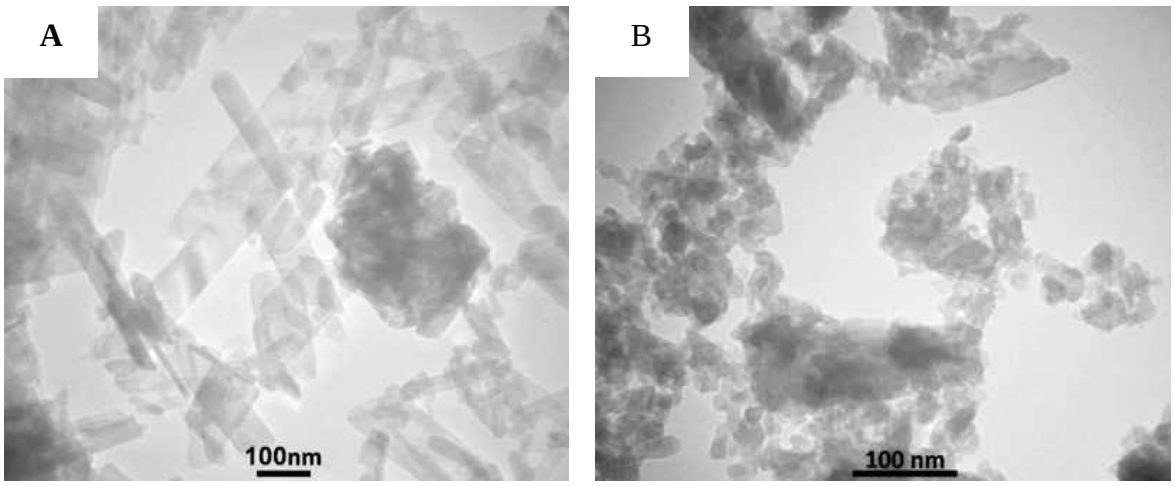

Figure 2: TEM images (A) 0 mol % ZnO and (B) 8 mol % ZnO of dried gel.

FE-SEM results

In figure 3 shows the compacted samples of pure HA (0mol% ZnO) at 1100 ºC. As noticed in figure (3, a) that there was different distributed particles and their shape nearly rounded .In figure (3, b) the size of particles in nano size ranging from (33.97 to 45.14) nm. In figure 4 the compacted bioceramic sample at 8mol% ZnO sintering at 1100 ºC . As noticed observed the phase of zinc calcium phosphate [$Ca_{19}Zn_2(PO_4)_{14}$]. The particles are spherical and the size in nano ranging from (38.38 to 55.58) nm.

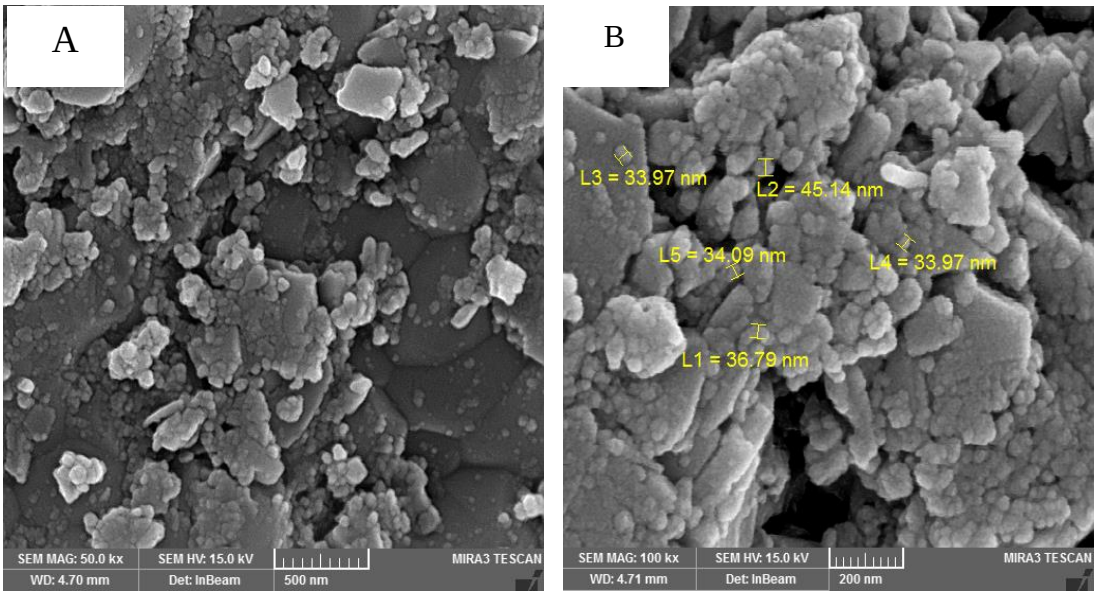

Figure 3. FE-SEM images of 0 mol% ZnO at 1100 ºC of compacted bioceramic samples at different magnifications.

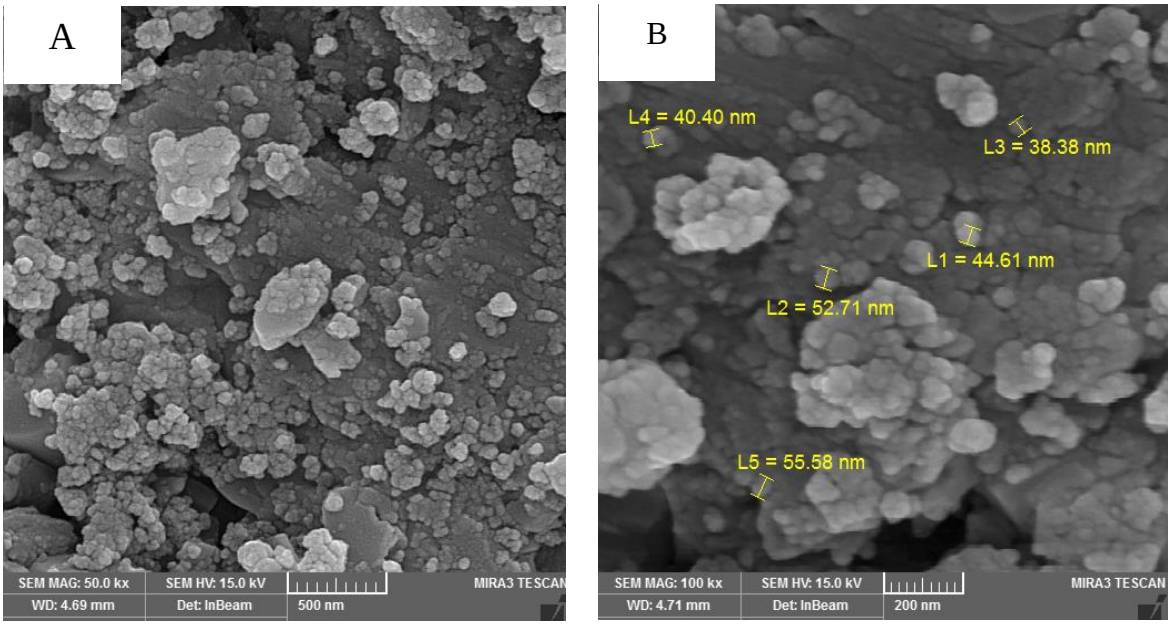

Figure 4. FE-SEM of 8 mol% ZnO at 1100 ºC of compacted samples at different magnifications.

EDS results

As noticed in figure 5 for 0 mol% of ZnO revealed the main elements of hydroxyapatite without any impurities, while in figure 6 the 8 mol % of ZnO sintered at 1100 ºC that the percent of Ca decreasing with increasing zinc oxide and the intensity of peaks decreased. This result indicated that the calcium oxide substituted by zinc oxide which incorporated into the structure of hydroxyapatite forming new phase (zinc calcium phosphate).

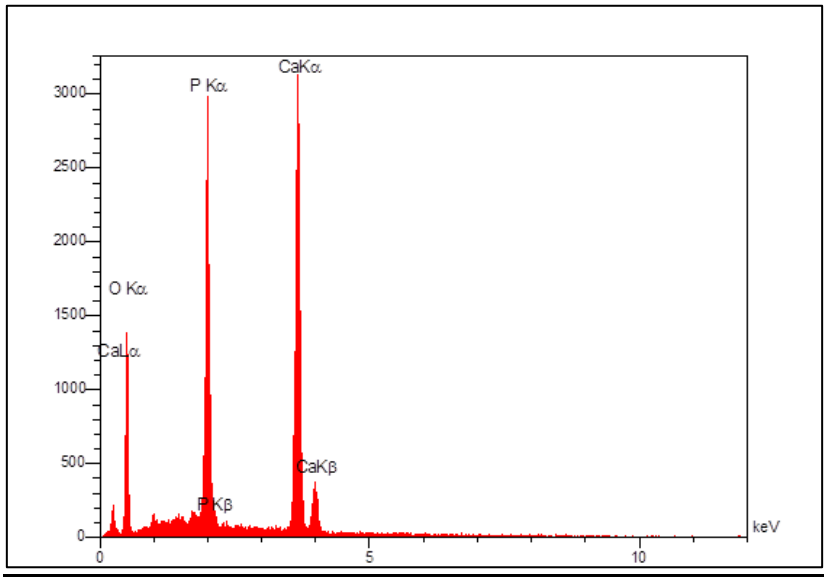

Figure 5. EDS results at 0 mol % ZnO of compacted bioceramics samples at 1100 ºC.

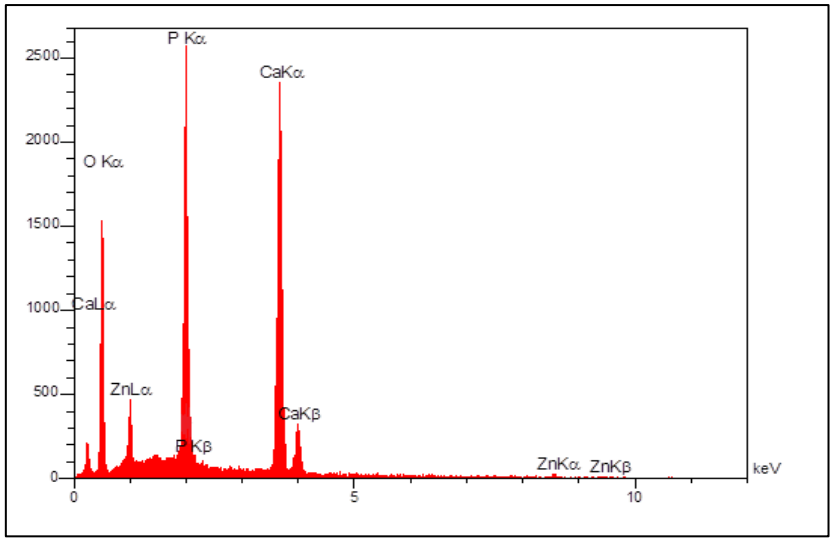

Figure 6. EDS results at 8 mol% ZnO of compacted bioceramics samples at 1100 ºC.

FE-SEM and EDS after immersion in simulated body fluid (SBF)

The compacted bioceramic sample immersed in SBF for 14 days to examine the bioactivity of the zinc calcium phosphate. As clear in figure 7 the layer of HA formed on the surface of the samples that confirms the bioactivity of zinc calcium phosphate.

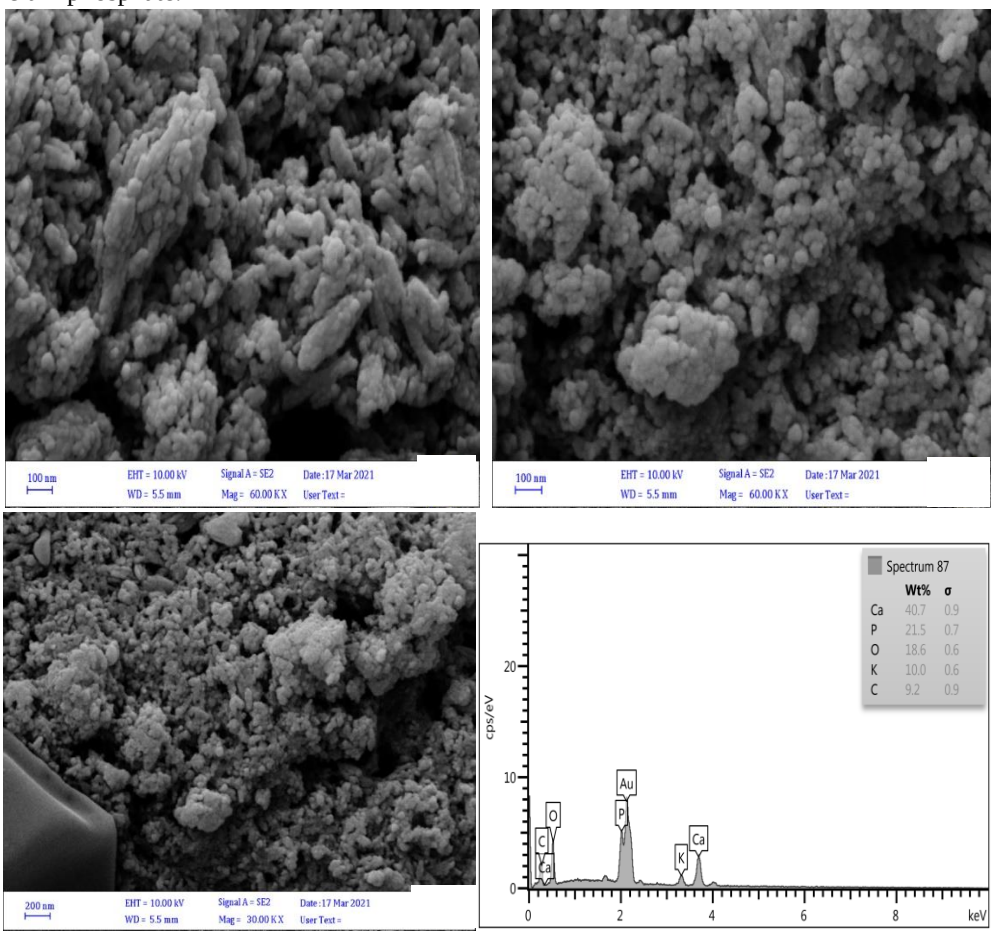

Figure 7. FE-SEM and EDS results of 8 mol% ZnO compacted sample at different magnifications after immersion in SBF for 14 days.

Antibacterial test

The samples were implanted in (*Escherichia coli*) bacteria which are (0 and 8) mol% ZnO. Antimicrobial activity of bioceramic samples measured by inhibition zone (mm) around the sample disc as shown in Figure 8. The inhibition zones for 0 mol%, and 8 mol% samples are (0), (12mm), respectively. As clear in the figure no antibacterial action was observed in the HA sample like the previous research [16] while the percent of 8mol% of ZnO shows antibacterial activity against (E. coli).

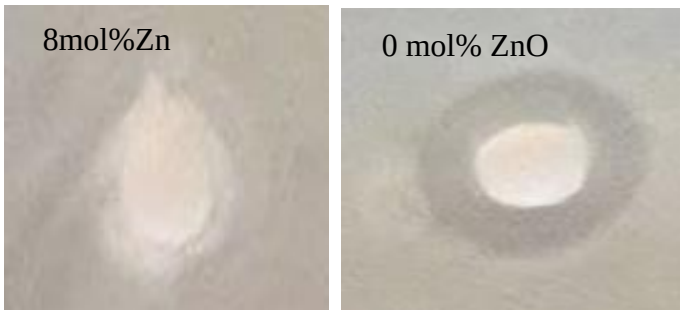

Figure 8. Antibacterial activity of (0, and 8) mol% of ZnO co-doped specimens against *E. Coli* bacteria.

The proposed mechanism is based on Zn ions' ability to form strong bonds with functional groups (carboxylates, imidazoles, thiols, and amines) found in bacterial cell membrane proteins.

CONCLUSIONS

Hydroxyapatite and zinc calcium phosphate was successfully synthesis by sol-gel method. TEM results revealed that the particles in nano rang with low porosity while 8mol% the particles became smaller. FE-SEM before immersion in SBF solution results found that the particles almost rounded at 0 mol% and the particles at 8mol% are spherical. The bioactivity of the compacted samples confirmed when forming of HA layer on the samples as conformed by FE-SEM test after immersion. EDS test confirmed that the zinc successfully incorporated into hydroxyapatite structure forming zinc calcium phosphate. The percent of 8mol% ZnO revealed antibacterial activity against *Escherichia coli*.

REFERENCES

[1] P.D. Sarkisov, N. Michailenko, Yu., and E.E. Stroganova, "Glass-Based Bioactive Calcium Phosphate Materials", Proc. XIX Int. Congress on Glass, Edinburg, Pp. 23, 2001.

[2] Tret'yakov, D. Yu, and O.A. Brylev, "New Generation of Inorganic Functional Materials", Ross. Khim. Zh., Vol. 7, No. 4, Pp. 10–16, 2000.

[3] W. Suchanek, and M. Yoshimura, "Processing and Properties of HA-Based Biomaterials for Use as Hard Tissue Replacement Implants", J. Mater. Res. *Soc.* Vol. 13, No. 1, Pp. 94–103, 1998.

[4] J.M. Sautier, J.R. Nefussi, N. Forest, Cells Mater., Vol. 1, Pp. 209, 1991.

[5] I. Mayer, J.D.B. Featherstone, J. Cryst. Growth, 219, Pp. 98, 2000.

[6] S.B. Abdelkader, I. Khattech, C. Rey, and M. Jemal, Thermochim. Acta, Vol. 376, No.25, 2001.

[7] I.R. Gibson, K.A. Hing, J.D. Revell, J.D. Santos, S.M. Best, W. Bonfield, Key Eng. Mater, Pp. 203- 218.

[8] A. Bandyopadhyay, S. Bernard, W. Xue, and S. Bose, "Calcium Phosphate-Based ResorbableCeramics: Influence of MgO, ZnO, and SiO_2 Dopants", Journal of the American Ceramic Society, Vol. 89, No. 9, Pp. 2675–2688, 2006.

[9] S. Samani, S.M. Hossainalipour, M. Tamizifar, and H.R. Rezaie, "In vitro antibacterial evaluation of sol-gel-derived Zn-, Ag-, and (Zn+Ag)-doped hydroxyapatite coatings against methicillin-resistant Staphylococcus aureus", J. Biomed. Mater. Res. A., 2013.

[10]K. Kaviyarasu, A. Mariappan, K. Neyvasagam, A. Ayeshamariam, P. Pandi, P.R. Palanichamy, C.G. Gopin athan, T. Mola, M. Maaza, "Photocatalytic performance and antimicrobial activities of HAp-TiO_2 nanocomposite thin films by sol-gel method Surf", Interfaces, Vol. 6 , Pp. 247-255, 2017.

[11] X. Wang, A. Ito, Y. Sogo, X. Li, and A. Oyane, "Zinc-containing apatite layers on external fixation rods promoting cell activity", Acta Biomater., Pp. 962-968, 2010.

[12] V. Stanić, S. Dimitrijević, J. AntićStanković, M. Mitrić, B. Jokić, I.B. Plećaš, and S. Raičević, "Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders", Appl. Surf. Sci., 256 (20), Pp. 6083-608, 2010.

[13] K.M. Reddy, K. Feris, J. Bell, D.G. Wingett, and C. Hanley, "Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems", Appl. Phys. Lett., 2007. 90213902, https://doi.org/10.1063/1.2742324

[14] S. Gomes, J.M. Nedelec, E. Jallot, D. Sheptyakov, and G. Renaudin, "Unexpected mechanism of Zn^{+2} insertion in calcium phosphate bioceramics", Chem Mat., 23, Pp. 3072-85, 2011.

[15] H. Podbielska, and A. Ulatowska-Jarza, "Sol-gel technology for biomedical engineering", Bulletin of the Polish Academy of Sciences Technical Science, Vol. 53, No.3, Pp. 261-271, 2005.

[16] N. Iqbal, M.R.A. Kadir, N.H. Mahmood, N. Salim, G.R. Froemming, H.R. Balaji, and T. Kamarul, "Characterization, antibacterial and in vitro compatibility of zinc–silver doped hydroxyapatite nanoparticles prepared through microwave synthesis", Ceramics International, Vol. 40, No. 3, Pp. 4507-4513, 2014.