Preparation and Characterization of Nano-Hydroxyapatite Particles and Chitosan by Sol–Gel Method (in Vitro Study)

Saja Kareem Esmael[1] , Raghdaa Kareem Jassim[2], Rasha Mahdi[3]

[1]Department of Prosthodontics, College of Dentistry, Uruk University, Baghdad, Iraq, [2]Department of Prosthodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq, [3]Department of dentistry, al – Esraa university college, Baghdad, Iraq

Abstract

Successful osseointegration of dental implants depends on many factors, including implant stability, bone quality, implant surface condition, and implant materials. Implant surface is coated using different techniques to enhance osseointegration. Sol–gel technique is one of the modern and easy techniques in the production of more bioactive hydroxyapatite (HA).

In this study, nano-HA and chitosan were used to coat titanium disc via the sol–gel technique.

Materials and Method: The nano-HA/chitosan composite was prepared using calcium nitride and phosphorus pentoxide solutions. The solutions were drop wisely mixed on a magnetic stirrer. Then, 3 mg of chitosan was added to the mixture, and the mixture was stirred for 15 h. Titanium samples were dip coated in the mixture of chitosan/HA on the stirrer at slow speed for 90 min to precipitate the coating layer. Afterward, the samples were removed and dried in a hot air oven. They were sintered at 400 °C for 1 h. X-ray diffraction (XRD), light microscopy, scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDX), FTIR analysis, and atomic force microscopy (AFM) were used to analyze the surface and thickness.

The results of XRD test showed that HA particles had sharp diffraction peaks, indicating high crystallinity of the structure due to the sol–gel preparation. Results of optical light microscopy showed that the coated layer was fairly distributed on titanium samples. SEM results of the coated samples showed different surface features, which present a roughness with the appearance of a crystal pool with irregular accumulation of small, spherical-like granules. EDX analysis showed the presence of ions that comprised HA. AFM analysis of the coated sample indicated peaks and projections with average surface nano-roughness (10.1 nm) and grain size (2022–2057 nm). FTIR analysis showed the presence of a band associated with HA particles and amide groups associated with chitosan. The average of thickness readings was 60 μm.

Keywords: *dental implant, dip coating, sol–gel, chitosan, hydroxyapatite, nanoparticles.*

Introduction

Osseointegration is the apparent direct attachment or connection of osseous tissue to an inert, alloplastic material without intervening fibrous connective tissue; it is the process and resultant apparent direct connection of an exogenous material's surface and the host bone tissues [1] . Several factors affect the success of osseointegration. One of these factors is implant stability, which is composed of two types: primary stability, which occurs due to mechanical support of implant to the surrounding bone immediately after implant insertion [2] and secondary stability, which occurs due to biological support after bone regeneration and remodeling [3] . Other factors include bone quality, surgical technique, implant surface condition, implant material, and implant surface [4] . The type of material that covers the implant surface is an important factor in enhancing osseointegration for initial stability. Therefore, several attempts have been made to enhance osseointegration at the bone/implant interface by making various coatings to support the main implant [5] . Titanium and its alloy are commonly

used in the fabrication of artificial joints and tooth roots due to their excellent mechanical properties that closely resemble natural bone [6] . Hydroxyapatite (HA) is a commonly used material for coating implant surface because it resembles human bone in its chemical and crystallographic structures, and it has the advantage of acting as a barrier that reduces the release of metallic ions [7] . The use of nanophase particles of HA increases the number of particles and surface areas, thereby increasing the reactivity of HA and enhancing bioactivity [8] . The sol–gel technique is one of the modern techniques for preparing HA to enhance the surface bioactivity of implant and increase bone attachment [9] . Anuar et al. (2013) produced HA nanoparticles using the sol–gel method [10] . Taherian et al. (2014) studied the effects of different sol–gel synthesis processes on HA nanopowders [11] . Chitosan has many medical applications because of its biocompatibility and non-toxicity [12] . It can clot blood to aid in hemostatic action, and it can stimulate macrophages to initiate collagen deposition, which accelerates the healing process [13] .

Materials and Methods

Preparation of sol–gel

Nano-HA was prepared using the sol–gel technique according to a previously reported method with slight modifications [16] .

Preparation of first solution: An electronic balance (accuracy 0.0001 g, Germany) was used to weigh 10.78 g of calcium nitride (Ca(NO3)2). Ca(NO3)2 was then mixed with 125 ml of ethyl alcohol on a magnetic stirrer (SH – 3, England). Stirring was performed for 1 hour until the solution was completely dissolved.

Preparation of second solution: The solution was prepared by mixing 5 g of phosphorus pentoxide (P2O5) with 125 ml of ethyl alcohol on a magnetic stirrer for 1 hour until completely dissolved.

After the two solutions were mixed, Ca(NO3)2 was added drop wise on P2O5 for 2 h under continuous stirring. Next, 5 g of potassium hydroxide (KOH) was added to the mixture to complete the reaction. Finally, 3 g of chitosan was added to the mixture, and the mixture was stirred for 15 h.

Sample preparation

Circular commercially pure titanium (Cp-Ti) discs of 10 mm diameter and 2.5 mm thickness were prepared as follows: Cp-Ti rod grade 2 (Orotig S.r.l., Italy) was cut and mirror polished using 500 micron roughness silicon carbide for 15 min. The discs were then cleaned in an ultrasonic bath with ≥99.8% ethanol to eliminate debris and contamination. After 15 min, the cleaning process was completed; the discs were washed with distilled water for 10 min and left at room temperature to dry [17] .

Dip coating procedure

The titanium samples were dip coated in the mixture of chitosan/HA on the stirrer at slow speed for 90 min to precipitate the coating layer. Then, the samples were removed and dried in a hot air oven (200 °C) (IMS/406, France).

Heat treatment (sintering)

Sintering was performed using a tube furnace (Carbolite Type MTF 12/38A. BAMFORD, England). A pilot study was performed using the three heating methods as follows:

A. Coated at the sol stage and sintered at 400 °C for 1 h

B. Coated at the gel stage and sintered at 125 °C for 1 h

C. coated by gel stage and sintering at 400^0C for one hour.

Examination of specimens after sintering by X-ray diffraction analysis revealed that samples of titanium were coated with nano-hydroxyl apatite and chitosan at the gel stage and sintered at 400^0C for one hour showed the best results.

Analysis of coating layer

The coated surface of the specimens was analyzed as follows:

Thickness measurement:

The thickness of the coating layer was measured by using microprocess coating thickness gauge (TF-C-UVIS-SR, USA). Three readings were obtained, and their average was 60 μm.

XRD analysis:

The Shimadzu XRD-6000 (Japan) diffractometer was used to provide information about the chemical composition and crystallographic structure of materials.

The indexing of peaks was based on the Joint Committee of Powder Diffraction Standards (JCPDS).

Optical microscopy examination (light microscopy):

The features of the coating surface were examined by using a light microscope (Nikon Eclipse ME 600 L/441002, Japan) and compared with those of the control sample. The analysis was performed by using a digital camera (DXM1200F) connected to computer with a software program for analysis.

Structural surface characterization by SEM:

- Surface analysis: The surface morphology and topographical characteristics of the coated sample were determined by SEM (TESCAN Vega 111, Czech Republic).

- Material characterization: Material characterization was performed using EDS, and SEM was used for chemical analysis of the sample, depending on the interaction and excitation of X-ray.

AFM:

AFM was used for analysis of surface roughness. It is an advanced type of profile meter used to produce topographical image where a very sharp and inert tip was scanned over the surface of the coated sample.

Results

Thickness measurement

The thickness of the coated layer was measured using a microprocess thickness gauge. The thickness of the coating film increased with time. The average of three readings was 60 μm.

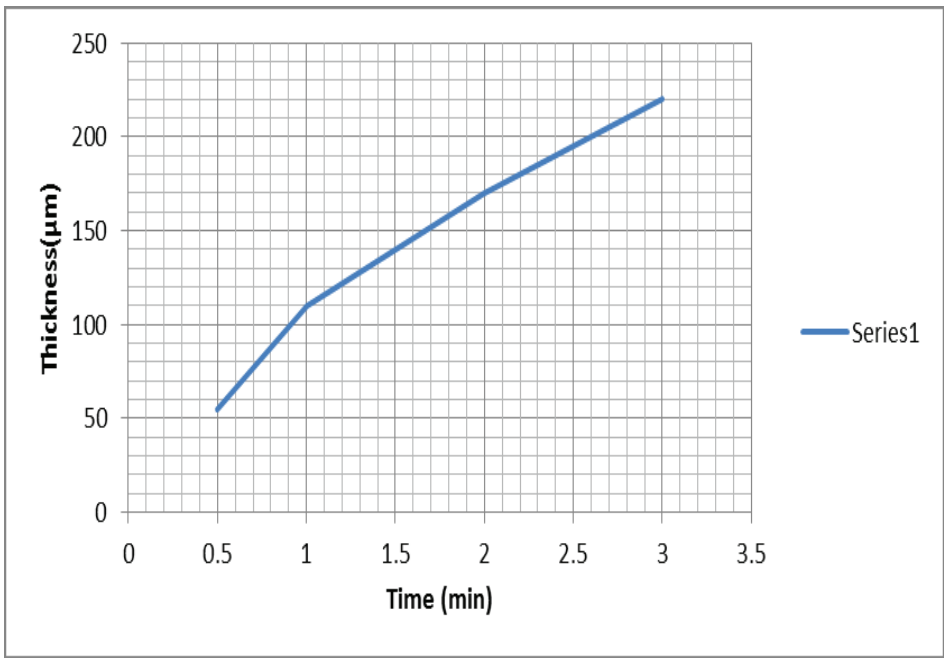
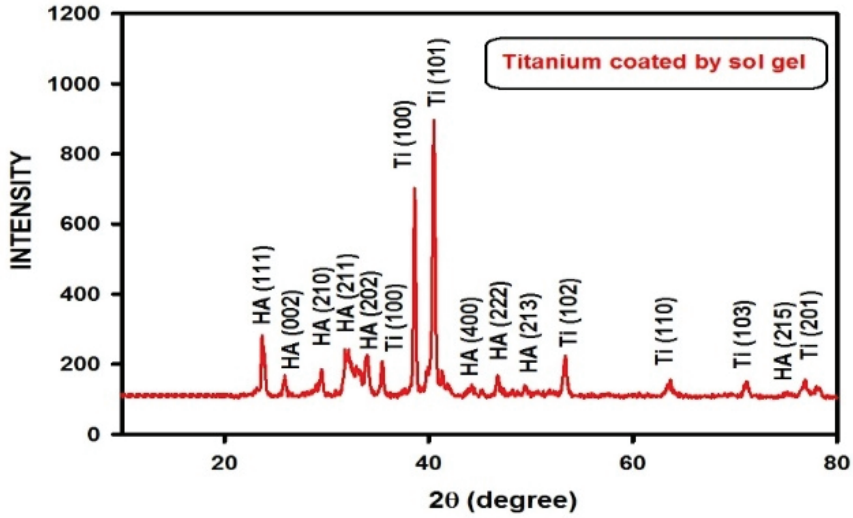


Fig.1 Thickness of nano-HA and chitosan coating layer at 0.5, 1, 2, and 3 min

X-ray diffraction analysis:

According to standard pattern of (JCPDS09 – 0432) the peaks of HA were indexed according to , as shown in Fig. (2) a and b. the Stoichiometric of HA particles displayed peaks with sharp diffraction that representing high crystallinity of the structure. By XRD, There was no identification of impurity phase.



(a) (b)

Fig. 2 XRD for surfaces of all tested groups

FTIR analysis

FTIR analysis results show the coated surface with peaks of HA and chitosan. In Fig. 3.2, FTIR spectra illustrated an OH_ band at 3569 cm−1 and PO3_ 4 bands at 472, 565, 603, and 1032 cm−1 associated with HA. The sharp peak (1637 cm−1) and broad bands for adsorbed water (3000–3500 cm−1) prove water absorption due to the high specific surface that precipitated powders usually have. The CO2_3 groups, which can substitute both PO3_ 4 and OH_ ions in the HA structure, appeared as 1461, 1423, and 875 cm−1 wave numbers. The presence of amide groups I, II, and III at 3500–3700, 1630–1690, and 1735–1750 cm−1, respectively, corresponded with chitosan particles.

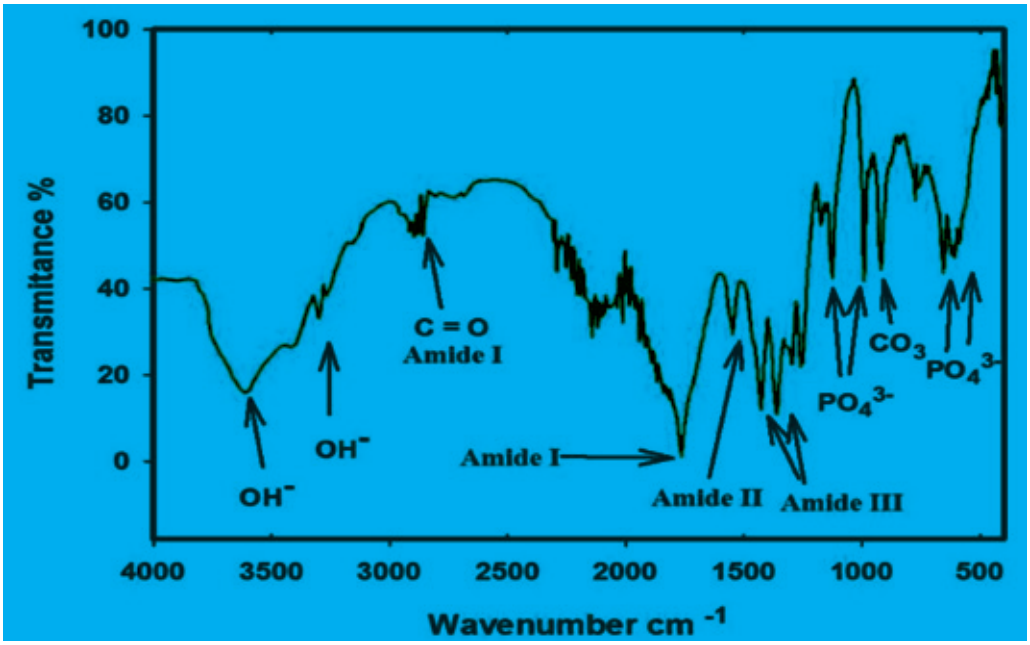


Fig. 3. FTIR spectra of coated surface with chitosan and HA

Light microscopy examination

Light microscopy examinations revealed the surface image of coated and non-coated titanium samples at 250 µm magnification, as shown in Fig. 3.4; lines appeared on the uncoated samples, which resulted from machining, whereas the coated sample showed homogenous coating layer without cracks.

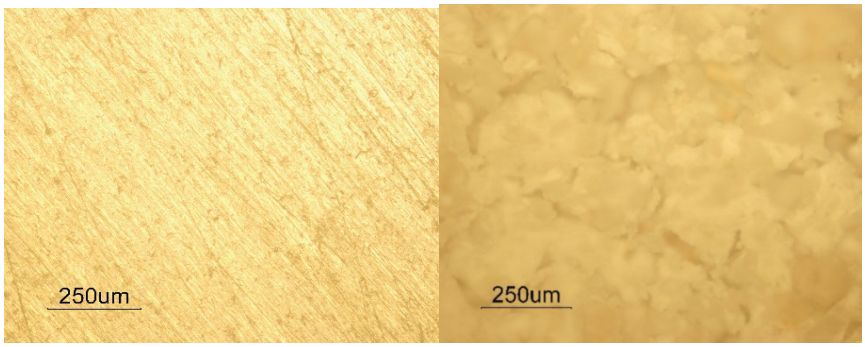


Fig. 4 Light microscopy examination of coated and uncoated samples

AFM analysis

Figure 3.4 shows the surface features of coated surface with mixture of HA and chitosan. Peaks and projections with average surface nano-roughness of 10.1 nm and grain size (2022–2057 nm). Table 1 lists the roughness values obtained from AFM images.

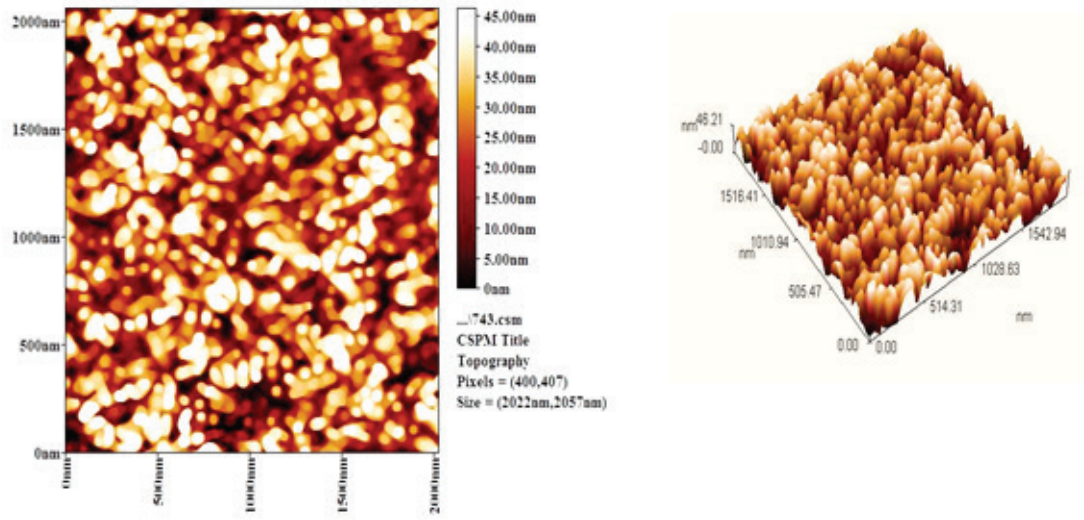


Fig. 5 AFM image for the coated surface with mixture of HA and chitosan

Table 1: Roughness values (nm) obtained from AFM images for all test groups

group	Average diameter of grain	Roughness average
Control (uncoated)	222.15 nm	7.37 nm
Coated sample	70.62 nm	10.1 nm

Discussion

HA has hydroxyl groups that can promote nucleation of phosphate and calcium, which enhances bone formation after implantation [18] . Chitosan has high viscosity in solution [19] , so it was hybridized with sol–gel HA to enhance apatite formation [20] . Therefore, the present study used a HA/ chitosan composite.

HA was successfully coated with titanium via HA sol–gel process. The sol–gel process produces more bioactive HA compared with other techniques [21] .

Dip coating technique was used to successfully implant HA on titanium [22] . Dip coating/sol–gel technique has the advantages of controlling coating thickness [23] .

SEM of the coated film revealed different surface features that appeared as rough surfaces with homogenous and crack-free distribution of the particles, as well as the appearance of crystal pool with accumulation of small, spherical-like granules. This manner of accumulation is one of the properties of HA prepared via sol–gel method [24] . This result is considered as good property, which indicates wide area of contact with living tissue, and is consistent with the results of A. Balamurugan et al., 2002 [25] , who studied the biomedical applications of sol–gel HA. Figure 3.6 shows the existence of O, P, and Ca elements, which are related to HA nanoparticles. The EDX results ensured that the coating layer was evenly distributed with no elements being concentrated in some areas more than others [26] .

FTIR spectra illustrated an OH_ band at 3569 cm−1 and PO3_ 4 bands at 472, 565, 603, and 1032 cm−1 associated with HA. The sharp peak (1637 cm−1) and broad bands for adsorbed water (3000–3500 cm−1) prove water absorption because of the high specific surface area that precipitated powders usually have. The CO2_ 3 groups, which can substitute both PO3_ 4 and OH_ ions in the HA structure, appeared at 1461, 1423, and 875 cm−1 wave numbers. The presence of amide groups I, II, and III at 3500–3700, 1630–1690, and 1735–1750 cm−1, respectively, corresponded with chitosan particles. The result ensured the formation of HA by the appearance of phosphate bands, at 3500–3700, 1630–1690, and 1735–1750 cm−1, respectively, corresponded with chitosan particles. The result ensured the formation of HA by the appearance of phosphate bands [27].

Conclusion

Sol – gel technique was successful method to prepare composite of nano HA/chitosan that showed uniform and homogenous thickness of coatings that improved surface properties of titanium implant when compared with uncoated surface. The use of dip coating procedure was benefit to achieve thin, homogenous and crack free coating film on CP – Ti surface.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, Uruk University, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

Reference

1. Keith J. Ferro, Editor and Chairman, Glossary of Prosthodontic Terms Committee, journal of prosthodontic dentistry. 2017
2. Natali AN, Carniel EL. Investigation of viscoelastoplastic response of bone tissue in oral implants press fit process. J Biomed Mater Res B Appl Biomater.2009; 91:868–875.
3. Greenstein G, Cavallaro J, Romanos G, Tarnow D. Clinical recommendations for avoiding and managing surgical complications associated with implant dentistry: A review. J Periodontol 2008;79:1317–1329
4. Fawad J, Hameeda B, Roberto C, Georgios E. Romanos4: Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation, Interventional Medicine & Applied Science, 2013;5 (4):162–167.
5. Bill GX, Damian E, Gordon G. Bioactive Coatings for Orthopaedic Implants—Recent Trends in Development of Implant Coatings, Int. J. Mol. Sci. 2014;15: 11878-11921.
6. Kensuke K , Masazumi O. Hydroxyapatite Coating of Titanium Implants Using Hydroprocessing and Evaluation of Their Osteoconductivity, Bioinorganic Chemistry and Applications, Volume 2012; Article ID 730693, 7 pages.
7. Roxana family, Mehran Solati-Hashjin, Shahram Namjoy Nik and : Surface modification for titanium implants by hydroxyapatite nanocomposite,

Caspian J Intern Med 2012; 3(3): 460-465.

8. Meng XC, Lv KL, Zhang JX, Qu DL. Caries inhibitory activity of the Nano-HA in Vitro. J Key Eng Mater 2007; 330-332: 251-4.
9. A Balamurugan, S Kannan, S Rajeswari. bioactive so – gel hydroxyapatite surface for biomedical applications, Trends Biomater. Artif. Organs. 2002;16 (1): 18-20.
10. Adilah A. Characterizations of hydroxyapatite (HAp) nanoparticles produced by sol-gel method, Advances in Environmental Biology, October Special Issue for International Conference of Advanced Materials Engineering and Technology, Pages: 2013;3587-3590.
11. Mohamadhassan T, Ramin R. Effect of different sol–gel synthesis processes on microstructural and morphological characteristics of hydroxyapatite-bioactive glass composite nanopowders, Journal of Advanced Ceramics, 2014;3(3): 207–214.
12. Rizzi G, Scrivani A, Fini M. Biomedical coatings to improve the tissue biomaterial interface. Int J Artif Organs. 2004;27:649-657.
13. Magdalena K, Danuta C, Antoni N. potential use of chitosan – based material in medicine, progress on chemistry and application of chitin. 2010;15.
14. Foster L, Thomson K, Marc H, Butt J, Watson S. Chitosan – vancomysin composite biomaterial as a laser activated surgical adhesive with regional antimicrobial activity. Biomacromolecules 2010;11(12): 3563–3570.
15. Pauline R, Be´range` T, Ste´phane B. Functionalization of Titanium with Chitosan via Silanation: Evaluation of Biological and Mechanical Performances, PLoS ONE,2012; 7(7).
16. S Manocha, Parth J, Bhavini P, LM Manocha. Synthesis and Characterization of Hydroxyapatite Nanoparticles using Sol-Gel Method, Eurasian ChemTech Journal , 2011;13 :85-88.
17. Raghdaa K, Zena A, Abdalbasit A. The Effect of Implant Screw Coating with Nano-Hydroxyapatite and Magnesium Chloride Mixture on Osseointegration: Biomechanical and Histological Study, International Journal of Medical Research & Health Sciences, 2017; 6(11): 41-53
18. Gupta R , Kumar A . Bioactive materials for biomedical applications using sol–gel technology. Biomed. Mater. (Bristol, U.K.) 2008;3:034005/1–034005/15.
19. Hua Liu, Hong Li, Wenjun Cheng, Yuan Yang, Minying Zhu and Changren Zhou: Novel injectable calcium phosphate/chitosan composites for bone substitute materials, Acta Biomaterialia , 2006;2 :557–565.
20. Qiaoling Hu, Baoqiang Li, Mang wang, Jiacong Shen, preparation and characterization of biodegradable chitosan / hydroxyapatite nanocomposite rods via in situ hybridization : a potential material as internal fixation of bone fracture. Biomaterials , 2004;25 (5):779 – 785.
21. Catauro M , Bollino F , Papale F , Mozetic P , Rainer A , Trombetta M, Biological response of human mesenchymal stromal cells to titanium grade 4 implants coated with PCL/ZrO2 hybrid materials synthesized by sol–gel route: In vitro evaluation. Mater. Sci. Eng., C 2014e ; 45:395–401.
22. Bora Mavis , A Cuneyt Tas: Dip Coating of Calcium Hydroxyapatite on Ti-6Al-4V Substrates, J. Am. Ceram. Soc., 2000;83 (4): 989–91.
23. Mehdi M, Rabi A , Jamaliah I. sol – gel bioactive glass coating for improvement of biocompatible human body implant, MJoM , 2010 ; 16 (3) : 149-163.
24. Yurisa M, Midhat N, Adila A. Preparation of hydroxyapatite nanoparticles by sol – gel method with optimum processing parameters, AIP conference proceedings 2014; 1660: 1.
25. A Balamurugan, S Kannan, S Rajeswari : BIOACTIVE SOL-GEL HYDROXYAPATITE SURFACE FOR BIOMEDICAL APPLICATIONS – IN VITRO STUDY, Trends Biomater. Artif. Organs. 2002;16 (1) :18-20.
26. Andersson J, Areva S, Spliethoff B. Sol–gel synthesis of a multifunctional, hierarchically porous silica/apatite composite. Biomaterials 2005; 26:6827–6835.
27. Fathi M, A Hanifi , V Mortazavi. Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder. Journal of Materials Processing Technology, 2008;202: 536-542.