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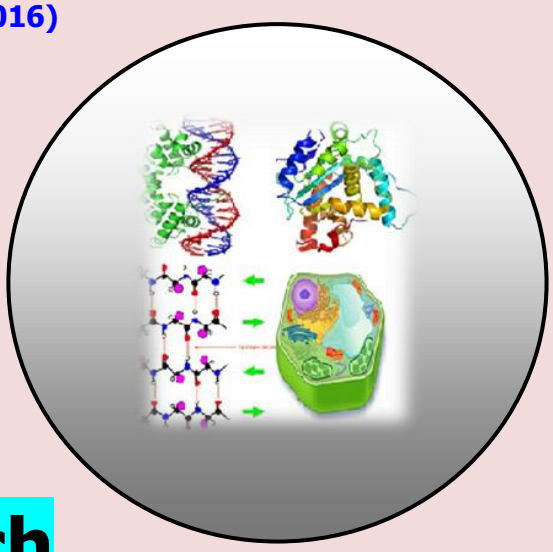

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RESEARCH PAPER

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Synthesis and Characterization of Polymer Doped Composite for Orthopedic Application

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ABSTRACT

Research on Hydroxyapatite for bone replacement is one of the most active areas of ceramic biomaterials. It is a desirable implant material due to its biocompatibility and osteoconductive properties. Alteration of elements like Mg, Sr, Zn ions into the structure of calcium phosphates is the focus of extensive investigation. Among the bio composite materials, mineral/polymer composite material possesses major advantages due to their high mechanical consistency and outstanding biocompatibility for applications in load bearing areas. Our studies deals with the synthesis of copper/zinc/PEG (polyethylene glycol) composite co-substituted with hydroxyapatite nanoparticles (Ca/Cu/Zn/PEG–HA) by chemical precipitation method. The synthesized polymer composite material was characterized by XRD, FTIR, SEM with EDAX, antimicrobial and cell viability studies. The as-synthesised polymer composite material functions effectively in orthopedic applications.

Keywords: HAP, Biomaterials, PEG and Bio activity.

INTRODUCTION

Calcium phosphates are primarily used as bone substitutes due to their biocompatibility, low density, chemical stability in biomedical industry [Nayak et al., 2010]. The molecular composition of hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. Calcium phosphate is one of the most widely investigated biocomposite materials and used as bioceramics for tooth and bone substitution due to their structural similarity to the mineral part of calcified tissues [Sakthivel et al., 2015]. During the past few years, considerable research works have been directed towards the synthesis of different biomaterial for orthopedic applications [Sakthivel et al.,2014, Hu et al.,2016, Bhowmick et al., 2016]. HA alone is not exhibit the biological activity. Moreover HA can be directly stick to the bone. Unexpectedly, due to low reliability, the HA cannot presently be used for heavy load bearing applications, like artificial bones or teeth. HA can be prepared by using different methods such as hydrothermal method, chemical precipitation technique, hydrothermal-micro emulsion method, ultra sonication method sol–gel method and biomimetic preparation [Anitha et al., 2014, Sanosh et al., 2009]. The most widely used technique for the preparation of HA is Chemical precipitation technique [Shakir et al., 2015]. The raw materials used in this technique at a reasonable cost controlling the process parameters such as particle size and shape, particle distribution and agglomeration are feasible to improve the properties of HA. The HA exhibit greater surface area. Moreover, nanophase ceramics clearly represent a unique and promising

class of dental/orthopedic implant formulations with enhanced osteointegrative properties. However, the brittleness and poor mechanical property of pure HA limit its use for the regeneration of non-load-bearing bone defects and bone tissue engineering applications. Composite biomaterials like metal and polymer matrix are utilized to improve the mechanical property of nano HA. Generally, composite biomaterials are prepared by using biocompatible/biodegradable and synthetic/natural polymers. It has been widely used in orthopedic field such as bone defect filler and as a coating for metallic prostheses to enhance their biological properties. Many in vitro studies analyzed that the copper, silver and zinc ions in the implant coatings act as main role in prevention or minimization of initial bacterial adhesion [Wang et al., 2015]. Copper and zinc ions in the larger amounts are potentially toxic so the uses of those ions in the small quantities are essential for various metabolic processes in most of the living organisms [Stani et al., 2010]. Bone formation in vitro and in vivo, zinc has also a stimulatory effect in this process [Rezakhani et al., 2012]. There are three major mechanisms for the antimicrobial activity of copper and zinc ions. First, metal ions bind to proteins and deactivate them. Second, metal ions can interact with microbial membrane, which causes structural change and permeability. Finally, metal ions interact with microbial nucleic acids, inhibiting microbial replication. Several new analysis of research, antimicrobial activity of copper and zinc-co substituted hydroxyapatite were reported [Ahmadzadeh et al., 2016].

In addition to this, the right choice of the composition of both filler and polymer matrix are essential to obtain appropriate polymer composites [Dhanalakshmi et al., 2012]. Recently, attempts have been made to develop nanocomposites, wherein mineralized nano HA particles are embedded in polyethylene glycol (PEG) polymeric matrices. Extensive studies have been made on both natural and synthetic (polyethylene, polyamide, chitosan, polystyrene, poly [vinyl alcohol], poly [ethylene glycol/PEG] polymers to overcome the mechanical problems associated with bioceramics in bone tissue engineering applications [Yasmin et al., 2015, Bach et al., 2013, Pandharipande et al., 2016, Shao et al., 2010, Saranya et al., 2017]. Among these different raw materials, PEG remains one of the broadly used polymer group of biomaterials applied for biomedical implants. In accordance to the related synergistic effect resulting from the combination of HA and PEG, the present work describes the synthesis of a nano structured HA/Cu/Zn/PEG composite under controlled environment, evaluate their physical-chemical characteristics and discuss their biological properties which act as a active materials for orthopedic applications.

RESEARCH METHODOLOGY

Materials

The analytical grade calcium nitrate tetrahydrate (Ca $(NO_3)_2.4H_2O$, Merck), copper nitrate ($Cu(NO_3)_2.6H_2O$, 97%, Merck), zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$, Merck) and dipotassium hydrogen phosphate K_2HPO_4, (99%, Sigma aldrich) were used as the sources for calcium (Ca), copper (Cu), zinc (Zn) and phosphorous (P) respectively. All reagents used were of analytical reagent grade without further purification and deionized water was employed as the solvent throughout the experiments.

Pure HA, Cu/Zn/PEG-HA were synthesized by chemical precipitation method using $Ca(NO_3)_2.4H_2O$, K_2HPO_4, $Cu(NO_3)_2.6H_2O$ and $Zn(NO_3)_2.6H_2O$, respectively. Three sets of powders were synthesized with same phosphorus sources, for series 1 Pure (HA): using $Ca(NO_3)_2.4H_2O$ and K_2HPO_4, series 2 HA/Cu/Zn/PEG: using $Ca(NO_3)_2.4H_2O$, $Cu(NO_3)_2.6H_2O$, $Zn(NO_3)_2.6H_2O$ and K_2HPO_4.

Series 1

For the synthesis of HA, solution 1 is 0.5M $Ca(NO_3)_2.4H_2O$ whose pH is adjusted to 10 by adding ammonia solution. About 0.3M solution of K_2HPO_4 was considered as solution 2. Solution 2 was added drop wise to solution 1 and stirred for 2 hrs and the pH was constantly adjusted and maintained at 10 using ammonia solution during the reaction. After mixing, the obtained precipitate was kept in stirring for 4 hrs to ensure the homogeneous mixture and then dried in a hot air oven at 120 C. The dried powder was washed three times with ethanol and deionized water. It was then calcined for 8hrs and sintered at 600 C for 4hrs.

Series 2

HA/Cu/Zn/PEG is synthesized by dissolving $Ca(NO_3)_2.4H_2O$, $Cu(NO_3)_2.6H_2O$, $Zn(NO_3)_2.6H_2O$ and PEG to 0.5 M in double distilled water, mixed well and the pH was adjusted to 10 using ammonia solution and 0.3 M K_2HPO_4 was dissolved in double distilled water. The method of synthesis and other processes like stirring, drying process were similar to the previous synthesis method of HA.

Characterization

The FT-IR spectral analysis was performed for functional group analysis for as-synthesized HA and Cu/Zn/PEG-HA samples by using Nicolet 380 FT-IR spectrophotometer over the range from 4000 to 400 cm-1 with a number of scans 32 and 4 cm resolution using KBr(JASCO 6100 FTIR spectrometer) and then pressed into discs for the analysis. The phase composition, purity and the crystallinity of the as-developed HA and HA/Cu/Zn/PEG nanocomposite were analysed by the XRD (Seifert, X-ray diffractometer Siemens D500 Spectrometer) in the range between 20◦ ≤ 2 ≤ 60◦ with Cu K radiation generated at 35 kV and 25 mA. The crystallite size of the samples was calculated from the XRD pattern. The surface morphology and the elemental composition of the as-developed samples of HA, HA/Cu/Zn/PEG were determined using FESEM (Philips XL-305 FEG) operated at an accelerating voltage of 15 kV equipped with EDAX. 3.1 Antibacterial activity the *in vitro* antibacterial activity of the as-synthesized HA, Cu/Zn/PEG–HA nanoparticles has been investigated against clinically isolated bacterial pathogen i.e., Staphylococcus epidermidis. The given samples were dissolved in 1 ml of DMSO separately and mixed well which was used to perform antimicrobial screening tests. The inoculums of all microorganisms were prepared from fresh overnight broth cultures (Tripton soy broth with 0.6% yeast extract – Torlak, Belgrade) that were incubated at 37 °C. The resulting broth cultures were used for both tests. The agar diffusion test was performed at Muller-Hinton agar (Institute for Immunology and Virology, Torlak, Belgrade). The culture turbidity was adjusted to 0.5 McFarland equivalents (1.5 x 108 CFU). The antibacterial activity of leaf extracts of selected plants were determined using agar well diffusion method. A suspension of test culture (50 µl) was swabbed on the Muller Hinton Agar (MHA) using sterile cotton swab. In each seeded plate, four wells were made using sterile cork borer (5 mm diameter). Then, different concentrations / volumes (25, 50, 75 µl/µg) of each sample was separately loaded into wells and allowed to diffuse at room temperature. 25 µl of ciprofloxacin (1 µg/µl) for bacteria was served as positive controls. The bacterial plates were incubated at 37° C for 24 hours. After appropriate incubation period, the diameter of zone of growth inhibition was measured (in mm).

In vitro cytotoxicity assay

Human cervical cancer cells (HeLa cells) were purchased from American Type of Culture collection (Manassas, VA). An in vitro cytotoxicity test method was performed for the given test sample as per ISO 10993:5.Test sample in duplicates were added to the cells. And the cells were incubated in the growth medium containing different concentrations of HA/Cu/Zn/PEG. Afterward, cells were washed and treated with MTT solution (1 mg/ mL, final concentration) for 4 h. Finally, the supernatant was removed, and DMSO (150 lL) was added to solubilize the formed formazan salt and read at 570 nm using a spectrovisible photometer. Cytotoxicity and cell viability were calculated by below formula.

Cytotoxicity = [(AbsControl – AbsTreated)/ AbsControl] * 100

Cell viability= (AbsTreated / AbsControl) * 100

The cell cytotoxic level was quantified as a percentage compared to blank.

RESULTS AND DISCUSSION

XRD: X-ray diffraction pattern for the synthesised HA and HA/Cu/Zn/PEG composites, are shown in Fig (1a-b). The more intense peaks are observed at 2 theta values are 30.88^{o}, 37.8^{o}, 30.1^{o}, 31.7^{o}, 35.1^{o}, 29.6^{o}, 31.3^{o}. From these values sharp intense peak of 31.77^{o}, 29.6^{o} corresponds to exhibit pure HA. The polymer composite peaks are observed at 37.80˚. The broad peaks and very wide baseline are obtained, which indicates the presence of inorganic component. The patterns are in good agreement with JCPDS (09-0432) and confirmed the pure phase of HA polymer composite. The particle size of HA crystalline size is 20 - 100 nm in length and width of 2 - 4 nm.

FTIR: FTIR spectra of pure HA and Cu/Zn/PEG-HA nanocomposite are shown in Fig.2(a-b). The spectral data for HA clearly reveals the characteristic absorption corresponding to phosphate and hydroxyl groups. The FTIR spectra of pure nano HA and HA/Cu/Zn/PEG composites are as shown in Fig. 1a and 1b. The peak at 471-472 cm^{-1} corresponds to PO_4^{3-} group in HA. The bands at 1036 to 1048 cm^{-1} and 566 to 570 cm^{-1} are obtained, respectively to P-O stretching vibration of regular tetrahedral PO_4^{3-} groups. The band observed at 633 cm^{-1} corresponds to O-P-O bending and symmetric P-O stretching modes. The symmetric stretching mode of phosphate group was observed at 963.26cm^{-1}. Similarly the bands obtained at 1413 and 857 to 874 cm^{-1} are assigned to carbonate ions.

The bands observed in the region between 2067 and 2069 cm^{-1} are related to their harmonic overtones or combination bands. The lattice H_2O exists in the range of 1603 to 1608 cm^{-1}, while the bands observed at 3393 to 3572.45 cm^{-1} overlap the –OH group. The band at 2855 and 2925 cm^{-1} corresponds to C-H stretching of PEG. A new peak of stretching is observed at 2926 cm^{-1}, which indicates the addition of PEG and this confirms the chemical bond interactions between HA and PEG.

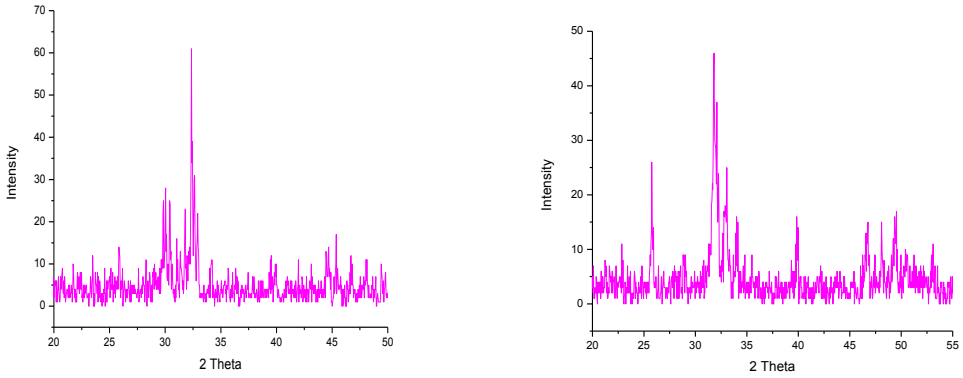

Fig. 1 .XRD pattern of (a) pure HA, (b) HA/Cu/Zn/PEG.

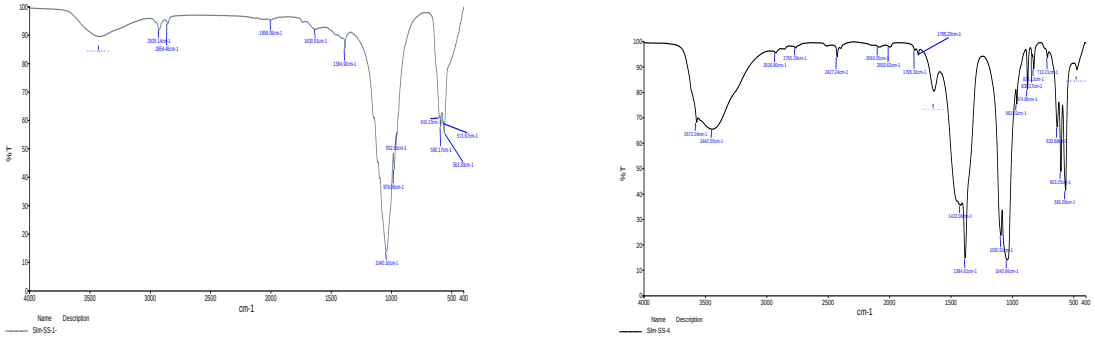

Fig. 2. FTIR spectra of (a) pure HA, (b) HA/Cu/Zn/PEG.

SEM

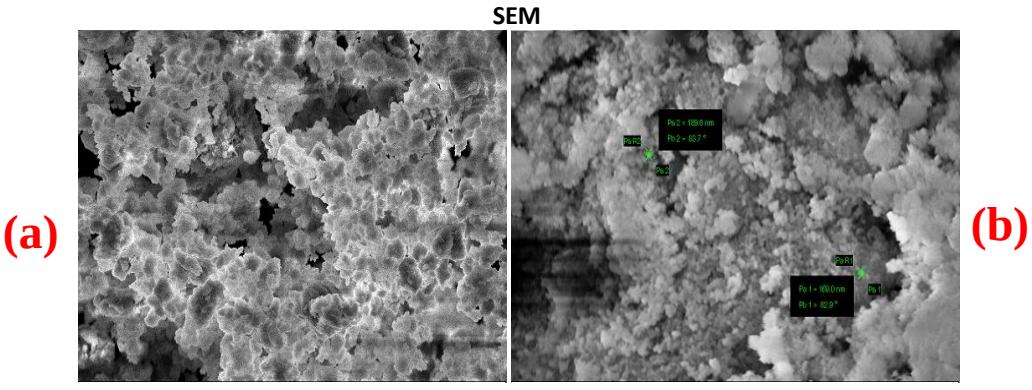

Fig. 3. FESEM image for (a) pure HA, (b) HA/Cu/Zn/PEG.

The surface morphology of the synthesized composite materials were analysed by scanning electron microscope (SEM) operating at an accelerating voltage of 20 KV. The SEM image of pure HA(a), HA/Cu/Zn/PEG(b) nanocomposite materials were exhibit in Fig.3(a-b). SEM image shows plate like structure with nano rod size ranging between 2-20 µm. It can be seen that all the samples consist of similar agglomerates that are composed of fine crystallites.

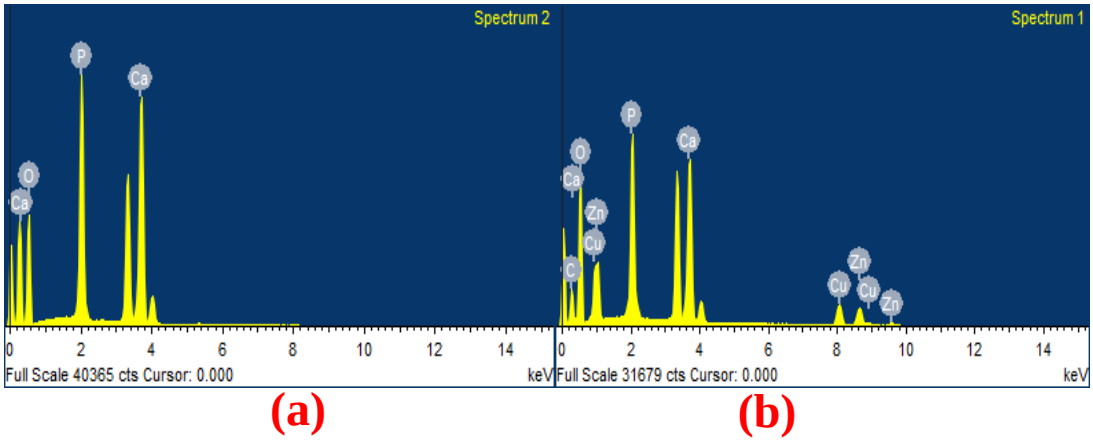

Fig.4. EDAX images for pure (a) HA, (b) HA/Cu/Zn/PEG.

EDAX

The elemental composition of pure HA (a), HA/Cu/Zn/PEG(b) composites were analysed using EDAX study with optimal concentration (0.1 M),and their spectra are shown in Fig.4(a-b). The EDAX study which confirms the presence of calcium (Ca), copper (Cu), zinc (Zn), phosphorus (P) and oxygen (O) and carbon(C) in the structure of the as-synthesized composite materials.

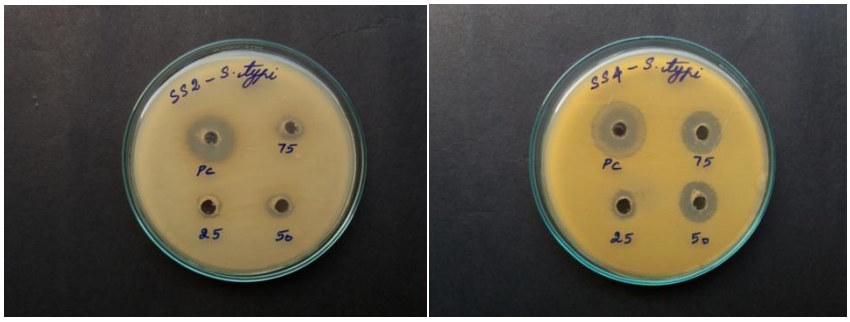

Fig.5.Antimicrobial images of (a) pure HA, (b) HA/Cu/Zn/PEG.

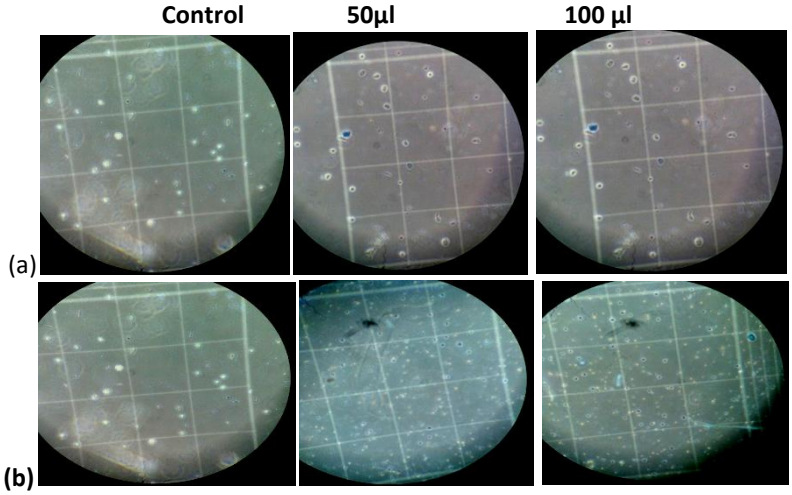

Figure 6. The images of cell viability against HeLa cells for (a) Pure HA, (b) HA/Cu/Zn/PEG nanohybrids at 24 h.

Antimicrobial study

Dissolution studies of antimicrobial materials are a necessary step in assessing the appropriateness of their utility. Antimicrobial materials with low solubility have good antibacterial properties for a longer period. The results of antimicrobial disk diffusion tests showed that affects S. epidermidis. The average inhibition zones were 2 and 3 mm for *S. epidermidis*.

The results of the quantitative antimicrobial tests carried out in liquid medium for all samples are shown in Fig. 5 (a-b). The HA/Cu/Zn/PEG shows more antimicrobial activity than pure HA which shows lower inhibition. We suppose that the reason is that the larger quantity of incorporated Zn(II) ions are in the surface of ZnHA samples particles. Several authors established that zinc ions and copper ions are mostly incorporate in the surface of hydroxyapatite and hinder the crystal growth.

Cell viability

The cell viability of pure HA and Cu/Zn/PEG/HA nanocomposites were tested against HeLa cell lines. Minimum essential medium is supplemented with foetal bovine serum. For this technique, Trypan blue dye was utilized. The cell viabilities of pure HA and Cu/Zn/PEG/HA nanocomposites against HeLa cells for 24h are shown in Fig.6 (a-b). At present, pure HA did not show any harmful effect towards HeLa cells in a concentration range 50-100 µl and even at such higher concentration as 100µl, HA still no suppression on cells. The overall look on the data exposed that Cu/Zn/PEG/HA nanocomposite had greater suppression efficiency on Hela cells. It was revealed that Cu/Zn/PEG/HA nano composite with good controlled release property and also shows moderate anticancer activity in vitro bioassay test.

CONCLUSION

The nano size and homogenous in composition of all samples were analysed by XRD, FTIR and SEM. The incorporation of copper, zinc ions and PEG doped HA samples are showed by using the chemical analysis. The antibacterial analysis of as-synthesized HA/Cu/Zn/PEG powder showed strong antibacterial activity against S. epidermidis. Finally it can be concluded, according to the results of antimicrobial tests, the antimicrobial efficiency of the materials is a function of the types and quantities of metal ions as well as strain of microorganism. In vitro studies of the prepared HA/Cu/Zn/PEG composite suggested their promising activity against human cervical cancer cells (HeLa cells). This facile strategy for the synthesis of nanocomposite materials would find widespread applications in diverse areas.

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REFERENCES

Nayak K. (2010). "Hydroxyapatite Synthesis Methodologies: An Overview," International Journal of ChemTech Research., vol. 2, June 2010, pp. 903-907.

Sakthivel P. and Ragu A. (2015). "Synthesis and Characterization of Nano Hydroxyapatite with Polymer Matrix Nano Composite for Biomedical Applications," International Journal of Chemical, Environmental & Biological Sciences (IJCEBS)., vol. 3, 2015, pp. 2320–4087.

Sakthivel P, Ragu A, and Senthilarasan K. (2014). "Synthesis and Characterization of Nano Hydroxyapatite with Agar-Agar Bio-Polymer Int. Journal of Engineering Research and Applications," vol. 4, July 2014, pp. 55- 59.

Hu J, Tong H, Zhu Y, Shen X, Chen L, and Ran J. (2016). "A detailed study of homogeneous agarose/hydroxyapatite nanocomposites for load-bearing bone tissue," International Journal of Biological Macromolecules, vol. 82, 2016, pp. 134–143.

Bhowmick A, Mitra T, Gnanamani A, Das M, and Kundu P. P. (2016). "Development of biomimetic nanocomposites as bone extracellular matrix for human osteoblastic cells," Carbohydrate Polymers., vol.141, 2016, pp. 82–91.

Anitha P and Pandya H. M. (2014). "Synthesis, Characterization and Antimicrobial Activity of Nano Hydroxyapatite Via a Novel Sol Gel Method," Nanotechnology Research and Practice, vol. 3, 2014, pp.120-126..

Sanosh K P, Balakrishnan A, chu M, Kim T N, and Cho S. (2009). "Preparation and characterization of nano-hydroxyapatite powder using sol–gel technique," Bull. Mater. Sci.,vol. 32, 2009, pp. 465–470.

Shakir M, Jolly R, Khan M. S, Iram N, and Khan H. M. (2015). "Nano-hydroxyapatite/chitosan–starch nanocomposite as a novel boneconstruct: Synthesis and in vitro studies," International Journal of Biological Macromolecules, vol, 80, 2015, pp. 282–292.

Wang Y, Li Y, Hao H, and Zhang S. (2015). "Selenium-substituted hydroxyapatite nanoparticles and their in vivo antitumor effect on hepatocellular carcinoma," Colloids and Surfaces B: Biointerfaces., vol.15, 2015, pp. 30404-5.

Stani V, Anti-Stankovi J, Dimitrijevi S, Mitric M, and Jokic B. (2010). "Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders," Applied Surface Science, vol. 256 , 2010, pp. 6083–6089.

Rezakhani A, Kashani Motlagh M. M. (2012). "Synthesis and characterization of hydroxyapatite nanocrystal and gelatin doped with Zn2+ and cross linked by glutaraldehyde," International Journal of Physical Sciences, vol. 7, 2012 ,pp. 2768 – 2774.

Ahmadzadeh, E, Tabatabaei M and Talebnia F. (2016). "Osteoconductive composite graft based on bacterial synthesized hydroxyapatite nanoparticles doped with different ions: From synthesis to in vivo studies," Nanomedicine: Nanotechnology, Biology and Medicine, vol.16, 2016.

Dhanalakshmi C. P, Narayanan V and Vijayalakshmi L. (2012). "Synthesis and preliminary characterization of polyethylene glycol (PEG)/hydroxyapatite (HAp) nanocomposite for biomedical applications," International Journal of Physical Sciences., vol. 7, 2012, pp. 2093 – 2101.

Yasmin A. R. and Kalyani D. (2015). "Naturally Derived Porous Hydroxyapatite/ Polymer Biocomposite of Cuttlebone and Eggshell for Dental and Orthopedic Applications," IJRASET., vol. 3, June 2015.

Bach L. G, Vo T. S, Islam M. R, Kim S. K, and Lim K.T. (2013). "Poly (allyl methacrylate) functionalized hydroxyapatite nanocrystals via the combination of surface-initiated RAFT polymerization and thiol–ene protocol: A potential anticancer drug nanocarrier," Journal of Colloid and Interface Science., vol. 394, 2013, pp. 132–140.

Pandharipande S. L. and Sondawale S. S. (2016). "Review on the characterization methods of Hydroxyapatite and its Bio-composites," International Journal of Science, Engineering and Technology Research (IJSETR), vol.5, no. 7, July 2016.

Shao Z. F, Gang G, Wang X. H, Liang H, Wei Y. Q and Qian Z. Y. (2010). "Structure and Morphology Changes during in Vitro Degradation of Electrospun Poly(glycolide-co-lactide) Nanofiber Membrane," J. Phys. Chem. C, vol. 43, no. 114, 2010 pp. 18372–18378.

Saranya S, Abinaya R, Padma R and Prema Rani M. (2017). "Structural, Optical and Antimicrobial Activity of Cu Doped HAp: Synthesized by Solgel Method for Biomedical Applications," Research & Reviews: Journal of Material Sciences, Vol. 5, Issue 3, 2017.

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