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# Naturally Derived Porous Hydroxyapatite/ Polymer Biocomposite of Cuttlebone and Eggshell for Dental and Orthopedic Applications

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**Abstract--**Hydroxyapatite [HAp,  $Ca_{10}(PO_4)_6(OH)_2$ ], a well-known mineral component and chemically similar to inorganic constituent of bone and teeth, possess good osteoconduction and osseointegration properties. Bio inspired lightweight materials such as cuttlebone and eggshell are rich in  $CaCO_3$ . Since they possess good permeability, porosity and compressive strength much interest are shown in orthodontics and orthopedic applications. Several attempts had been made to synthesize HAp from cuttlebone and eggshell. The aim of the study is to synthesize a biocomposite materials with a blend of cuttlebone and eggshell HAp by a simple wet chemical method. The newly formed composite showed a good crystalline nature. The presence of calcium phosphate in HAp is confirmed by X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analyses. Mold prepared by solvent/solution casting method showed appreciable compressive strength and porosity (80-85%). Further, invitro biocompatibility studies were done with MG-63 osteoblast cells.

**Keywords:** Hydroxyapatite; cuttlebone; eggshell; orthodontics; orthopedic.

## I. INTRODUCTION

Synthesis of hydroxyapatite (HAp) with porous morphology is a defied work in the field of bone tissue engineering [1, 2]. In recent decade HAp with osteoconduction and osseointegration properties has shown tremendous interest in oral/orthopedic surgery for bone repair or replacement [3]. The chemical composition of hydroxyapatite (HAp) is similar to that of natural bone tissue exhibits non toxicity, non-inflammatory, non-pyrogenic response, no fibrous tissue formation between implant and bone, in addition to that they possess the ability to bond directly to the host bone and hence deliberated as an elite biocompatible material in bone graft substitute and used in bulk form, as a coating and/or cements [4, 5]. Currently, HAp is the material used in various biomedical applications, e.g. as a replacement for bony and periodontal defects, alveolar ridge, middle ear implants, tissue engineering systems, drug delivery agent, dental materials [6-14]. Although synthetic HAp has been widely used in biomedical field, natural materials are most preferred in medical applications. Over last decade, several attempts to synthesize hydroxyapatite from natural sources such as coral [15], bovine bone [16] and sea shells [17] are seen. Utilization of the same resource will lead to extinction of species. So it's crucial to discover a new alternative material with comparable properties, should be easily accessible, renewable and cost effective. Eggshell and cuttlefish bone being an inexpensive, abundance and rich in calcium source can be converted into HAp granules. An eggshell is made up by a three layered structure, viz. the cuticle, the spongy layer and the lamellar layer [17]. The cuticle layer represents the outermost surface and consist of a number of proteins. Spongy and lamellar layer form a matrix constituted by protein fibers bonded to calcite (Calcium Carbonate) crystals [18]. The eggshells constitute 11% of the total weight of the egg and are composed of calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%) [19]. According to the Food and Agricultural Organization of the United Nations, the world's egg production was approximately 64 million metric tons in 2010, and India occupies the third position with 3.4 million metric tons. A huge amount of egg shells being produced daily are of no use causing environmental pollution [20]. HAp being derived from egg shell is a low cost material and at the same time reduces environmental pollution related problems. Cuttlebone (CB) is a cheap, easily available natural biomaterial with similar chemistry and crystallography as coral [21, 22]. CB is a rigid structural component consists of a dorsal shield which act as an external wall and an internal lamellae matrix. The lamellar matrix is formed by a parallel sheet structure and is interconnected, which results in highly porous properties and consists primarily of Aragonite, a crystallized form of  $CaCO_3$  [23]. Due to this unique property, several studies have explored the transformation of CB into hap. Moreover, cuttlebone Hap as a filler in acrylic bone cements showed enhanced osseointegration and no evidence for secondary infection during *in vivo* testing. Further it shows appreciable mechanical properties when compared to those of commercial bone cement [24].

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Several methods have been reported for preparing hydroxyapatite such as chemical precipitation [25], sol-gel process [26], hydrothermal method [27] etc. However, among these methods wet chemical method is more promising owing to its ease of production, low working temperature, inexpensive equipment and also maximum purity [28]. Aragonite and Calcite can be easily sintered to hydroxyapatite by wet precipitation at a temperature of  $>900^{\circ}\text{C}$  showed highest crystallinity [29]. Brittleness and poor mechanical stability of pure HAp limits its usage in the regeneration of non-load-bearing bone defects and tissue engineering applications [30]. Composite biomaterials like metal and polymer matrix are used to improve the mechanical compatibility of Nano HAp. Further, polymer coating enhances mechanical properties such as porosity and compressive strength of HAp fillers [31]. PEG is one of the widely used polymer and its unique structural feature plays a crucial role in tailoring the applications such as tissue scaffolding and biodegradable scaffolds [32]. The aim of the study is to synthesize a biocomposite materials with a blend of cuttlebone and eggshell HA by a simple wet chemical method. The newly formed composite showed a good crystalline nature. The presence of calcium phosphate in HAp are confirmed by X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analyses. Mold prepared by solvent/solution casting method showed an appreciable compressive strength and porosity. (80-85%). Further, invitro biocompatibility studies were done with MG-63 osteoblast cells.

### II. MATERIALS AND METHODS

#### A. Synthesis Of Hydroxyapatite From Eggshell

Eggshells boiled for 15 minutes and the protein membrane was removed manually. Eggshells were washed with ethanol to remove the organic residues and dried at  $60^{\circ}\text{C}$ . Dried eggshells were grinded in the agate mortar into a fine powder. By weighing it was found that 41.8 grams of powder obtained from 15 eggshells. Hydroxyapatite is synthesized as follows: Eggshell powder was dissolved in conc. nitric acid. The reaction is given below



Then 0.6 M ammonium di-hydrogen phosphate was added drop wise to achieve Ca/P ratio and stirred for overnight to form white colored HAp precipitate. The pH of the reaction was adjusted to 9 by adding dil. ammonium hydroxide solution. The precipitate was thoroughly washed and filtered. The residue was dried in an oven at  $90^{\circ}\text{C}$  for 15 hours. Then the final powder was sintered at  $1000^{\circ}\text{C}$  for 3 hours [17].

#### B. Synthesis Of Hydroxyapatite From Cuttlefish Bone

Cuttlebones as a whole (4 no's) were collected from local fish market at Madhavaram in North Chennai. Small pieces of sample were cut from whole cuttlebone using a sharp knife. Aragonite ( $\text{CaCO}_3$ ) was obtained from the lamellae of cuttlefish bone powder by using mortar and pestle and heated in a furnace at  $350^{\circ}\text{C}$  for 3 hours [2, 29]. Wet precipitation method was carried out by mixing 1M  $\text{CaCO}_3$  and 0.6 M of  $\text{H}_3\text{PO}_4$  solution with a magnetic stirrer for 30 minutes to achieve molar ratio of Ca/P=1.67. The prepared solution was heated at a temperature of  $150^{\circ}\text{C}$  for 15 hrs. The resultant powder was cooled at room temperature and then washed with distilled water using a magnetic stirrer. Washing was performed repeatedly in distilled water till it remains at the neutral pH value. This is done to eliminate acidic byproducts. The last wash was performed with methanol to limit agglomeration of HAp during drying. Next, the sample was filtered and dried in oven at  $70^{\circ}\text{C}$ . Hydroxyapatite formed was further sintered at a temperature of  $900^{\circ}\text{C}$  for 1 hour and the final product was white in color [33, 34].

#### C. Preparation Of Hap Scaffold With Peg

To analyze the compressive strength and porosity HAp powder were molded into different shape by solvent/solution casting method [35, 36]. Polymer solution was prepared by dissolving Polyethylene glycol-600 in the distilled water and stirred for 30 minutes. Then HAp powder from different sources were dissolved in polymer solution and stirred for 1 hour. The solution was dried in a Hot air oven at  $70^{\circ}\text{C}$  and dried for 24 hours. Then the mold was cut into a desired shape.

#### D. Characterization Of Hap And Hap/Peg

Synthesized HAp powder were mixed in different proportion (50CB HAp: 50ES HAp; 70CB HAp: 30ES HAp) and characterized by scanning electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy. Finally MTT assay has been performed on MG 63 Osteoblast cells.

- 1) *X-Ray Diffraction:* XRD analysis was performed using a Rigaku Miniflex ii C (Japan) diffractometer with Cu-K $\alpha$  incident radiation (30 kV, 10 Ma) ( $\lambda=1.5405 \text{ \AA}$ ). The diffraction patterns were collected at room temperature over the  $2\theta$  range of  $10-80^{\circ}$  and at step size angle of  $0.8^{\circ}$ .
- 2) *Fourier Transform Infrared Spectroscopy:* Perkin-Elmer Spectrum RS I spectrophotometer was used for the identification of functional groups in the HAp. The samples were first ground in a mortar, and then mixed with pure moisture-free KBr

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powder in a ratio of 1:100, in a form of thin pellet. Infrared spectra were recorded in the region 4000–400  $\text{cm}^{-1}$ , with 3600 scans performed at a resolution of 1  $\text{cm}^{-1}$ .

- 3) **Bulk Density And Porosity Values:** The bulk density and porosity values were determined using a liquid displacement method. Scaffold initial dry weight (W) was recorded and immersed in a known volume of ethanol (V1). Test tube is tightly capped to drive ethanol into the porous structure of scaffold. After 30 minutes, the total volume of ethanol and ethanol infiltrated scaffold were noted as (V2). Scaffold was removed and the residual volume is noted (V3). The bulk density of the scaffold can be calculated by  $W/(V2-V3)$  and the porosity of the scaffold by  $(V1-V3)/(V2-V3) \times 100\%$  [3].
- 4) **Mechanical Properties:** Porous scaffold (3mm breadth×12mm width) was subjected to a compression test using an Instron test machine by applying a load via 1000N load cell at a cross head speed of 0.001 mm/min under ambient conditions. Load was applied on the top of the scaffold until it cracks. The results were interrupted from the stress vs. strain curve.
- 5) **Scanning Electron Microscopy:** The surface topography and microstructure of the HAp samples were observed using scanning electron microscopy (VEGA 3 TESCAN) by coating gold on the surface to reduce charging of the samples.
- 6) **Cell Viability/Cytotoxicity:** Human osteoblastic cells (MG 63) cell line was procured from the National Center for Cell Sciences (NCCS) Pune, India. The cells were grown in culture flasks containing DMEM supplemented with 10% FBS in a 95% air and 5%  $\text{CO}_2$  in a humidified atmosphere at 37°C. Indirect MTT assay was carried out to test the cytotoxicity. Cell viability was assessed by MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyl-tetra-zolium bromide) method. Human osteoblastic cells (MG63) at a concentration of 10,000 cells were seeded to the 96 well cell culture plate. Briefly, 50 mg of hydroxyapatite (HAp) scaffold was weighed and soaked in 500  $\mu\text{l}$  of the DMEM medium for 24 h. The supernatant termed as conditioned medium was taken at different volumes (10 $\mu\text{l}$ , 20 $\mu\text{l}$ , 50 $\mu\text{l}$  and 100 $\mu\text{l}$ ) and made up to 1 ml with medium and added to the wells containing cells and incubated for 24 h. The media were removed from the wells and 100 $\mu\text{l}$  of 0.5% MTT solution was added to each well and incubated for another 4 h at 37°C. DMSO was used to dissolve the formed formazan crystals, and the optical densities (OD) were determined using the spectrophotometer (BioTEk micro plate reader, USA) at 570 nm. The intensity of the colors obtained (red and blue respectively) is directly proportional to the viability and metabolic activity of the cell population and inversely proportional to the toxicity of the material.

### III. RESULTS AND DISCUSSION

#### A. XRD Analysis

Fig 1 shows the X-ray diffraction patterns of the synthesized HAp and of the two different sources. As shown in this figure, HA samples synthesized had similar XRD patterns and no other crystalline phase was observed other than HA. Identification of the phases was realized by comparing the experimental XRD pattern to standards compiled by the International Centre for Diffraction Pattern (ICDD) using the JCPDS Card No 09-432 for hexagonal HAp structure. Well-resolved characteristic peak of highest intensity was obtained at 2theta value of 31.77° corresponding to 211 plane of HAp [37]. Complete crystallization of the powders has been confirmed due to sharp peak intensity and well-resolved peaks in XRD patterns of the powders [17, 33].

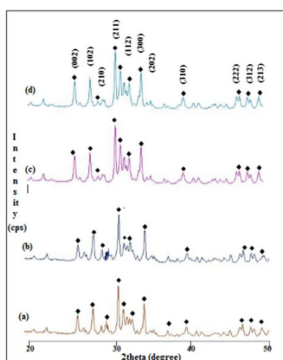


Fig 1 XRD of a) Eggshell HAp b) Cuttlebone HAp c) 50:50 HAp of CB and ES d) 70:30 HAp of CB and ES.

The crystallite sizes of HAp particles from both sources were calculated using Debye Scherer's equation  $D = 0.9 k/B \cos\theta$ , where D represents mean grain size, B stands for full width at half maximum of the peak, k is the diffraction wavelength (0.154059 nm) and  $\theta$  is the diffraction angle. The Bragg reflection at 002 planes of HAp was considered to calculate the crystallite size [28]. This indicates that the various proportions of HAp from CB and ES (50:50 HAp of CB and ES; 70:30 HAp of CB and ES) has not hindered the formation of HA phases resembles the same peaks of eggshell and cuttlebone synthesized HAp. These characteristic feature resemble those of naturally occurring bone apatite. The crystallite sizes are found

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to be 68nm, 67nm, 69nm and 67nm for eggshell, cuttle bone, 50:50 Hap and 70:30 Hap respectively. Due to their crystallite dimension in nanometer the HAp particle can be reinforced with fibers in a better way to improve the mechanical strength and hence favours the bone cell attachment and growth [38].

### B. FTIR Analysis

FT-IR spectrum of HAp produced by wet precipitation method of the two different composition from cuttlebone and eggshell are shown in Fig 2 which shows all the characteristic bands for hydroxyapatite. In the HAp derived from cuttlebone, the major peaks are identified at approximately  $560 - 630 \text{ cm}^{-1}$ ,  $1030 - 1090 \text{ cm}^{-1}$  due to the asymmetric stretching mode of  $\text{PO}_4$  group indicates the presence of  $(\text{PO}_4)^{3-}$  and  $\text{OH}^{-1}$  group at  $3568 \text{ cm}^{-1}$  indicates the hydroxyl group and also contain broad peaks at approximately  $1410 - 1570 \text{ cm}^{-1}$  in the spectra suggesting the presence of  $\text{CO}_3^{2-}$  substituted in the HAp. HAp from Eggshell shows the characteristic bands where  $(\text{PO}_4)^{3-}$  modes were detected at  $1079 \text{ cm}^{-1}$ ,  $1033 \text{ cm}^{-1}$ ,  $560 \text{ cm}^{-1}$  in the spectra and  $\text{OH}^{-1}$  mode was at  $3447 \text{ cm}^{-1}$ . The bands at  $3751.7 \text{ cm}^{-1}$  and  $1637.2 \text{ cm}^{-1}$  corresponds to absorbed  $\text{H}_2\text{O}$  [39-41]. Both the composition showed the same functional groups in cuttlebone and eggshell HAp.

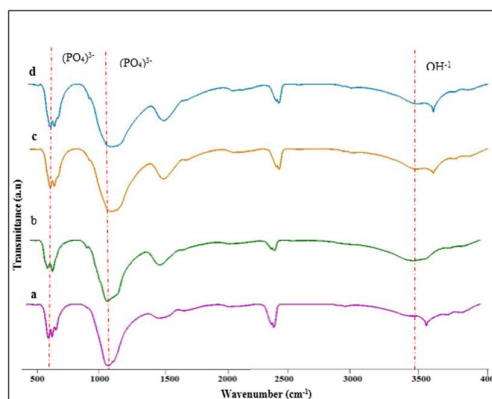


Fig 2 FTIR of a) Eggshell HAp b) Cuttlebone HAp c) 50:50 HAp of CB and ES d) 70:30 HAp of CB and ES.

### C. Bulk Density, Porosity And Compressive Strength Analysis

Hap scaffold prepared by solution/solvent casting method is analyzed for porosity and bulk density by liquid displacement method. Fig 3 shows the stress vs. strain curve obtained for the HAp/PEG scaffold from compressive strength analysis. From the curve, the values are interpreted and listed in the Table 1. The compressive strength obtained at the maximum force of 101.1 N is 2.84 MPa. The porosity and compressive strength value obtained is appreciable for the applications of cortical bone [42].

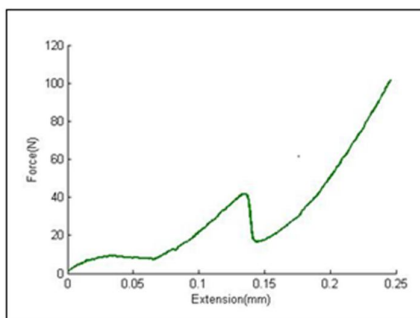


Fig 3 Stress Vs. strain Curve

TABLE I

VALUES FROM STRESS VS STRAIN CURVE

|                            |        |
|----------------------------|--------|
| Compressive strength (MPa) | 2.824  |
| Max Force (N)              | 101.1  |
| Yield strength (Mpa)       | 0.2598 |
| Yield Force (N)            | 9.35   |

### D. SEM Analysis

Microstructures of the Hap derived from cuttlebone and eggshell were studied by Scanning Electron Microscopy (SEM). Images were shown in Fig 4 (a,b,c & d) which clearly shows that the synthesized HAp consisted of agglomerates. Individual fine particles with spherical and semi-spherical shapes were observed in Fig 4a & 4b. Images showed that biocompatible composites consisted of a highly porous network structure in the range between  $1 \mu\text{m}$  and  $5 \mu\text{m}$  [43]. The agglomerates (Fig 4c and 4d) were of irregular shape like oval shape and spherical shape [44]. Agglomerates as big as  $5 \mu\text{m}$  could also be seen, however, the predominant sizes ranges between 1 and  $3 \mu\text{m}$ . Crystallites of nano-sized particle with a tendency to create porous formation

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which are more beneficial and permits the circulation of body fluid throughout the coating when it is used as biomaterial [40].

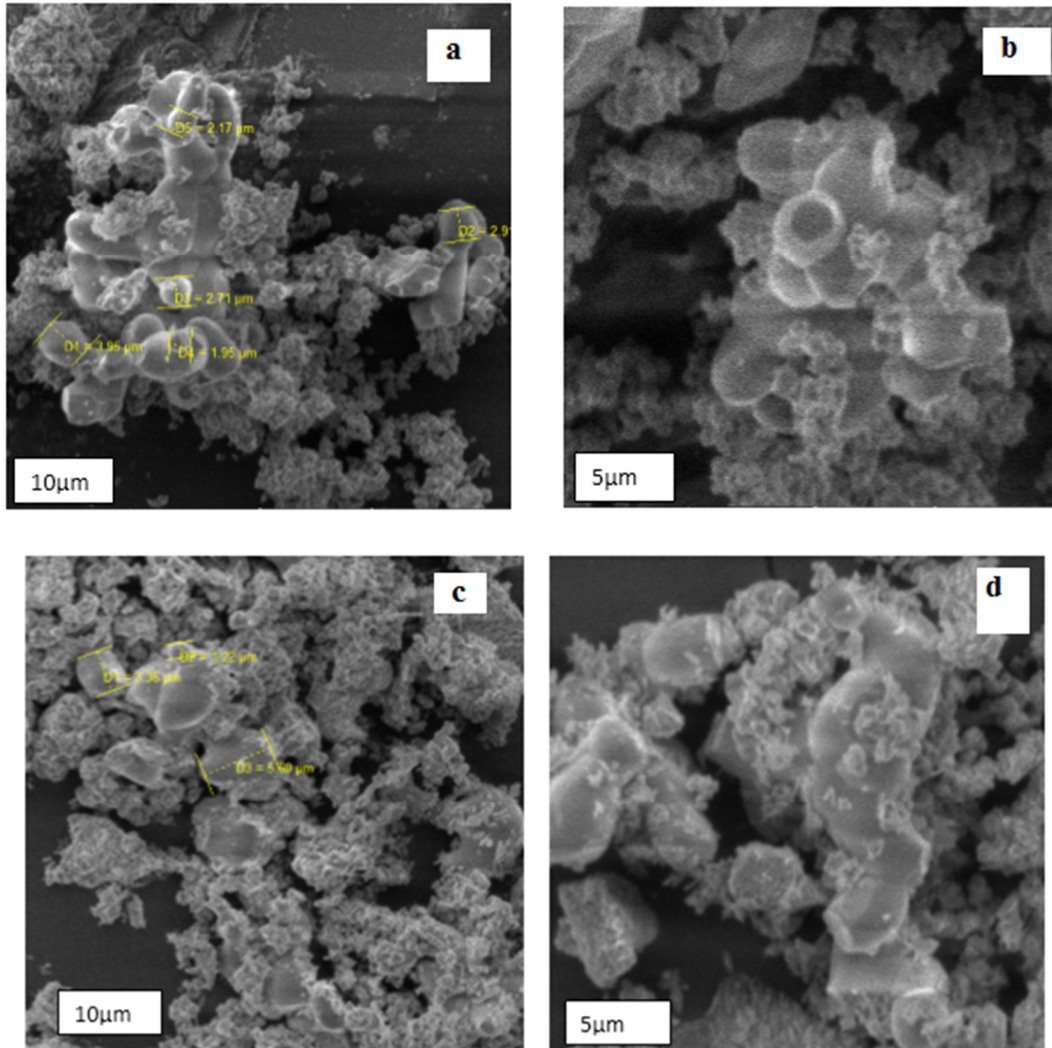


Fig 4 SEM images of a & b 50:50 HAp of CB and ES; c & d 70:30 HAp of CB and ES.

### E. MTT Assay

All data were analyzed using GraphPad Prism 5.0 Software (GraphPad Software, La Jolla, USA) and expressed as mean  $\pm$  standard error of mean (SEM). From the graph it was observed that for 24 hours the cell viability test shows a constant OD value which is similar to the positive control value [1,23].

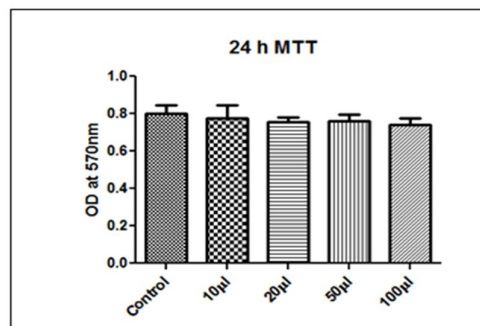


Fig 5. Mean  $\pm$  SEM of observations denotes at  $p < 0.05$  compared with control

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## IV. CONCLUSIONS

The present study reveals that the phase pure HAp were prepared in two different proportions from the cuttlebone and eggshell by wet precipitation method in a cost effective manner. Two composite material revealed their crystalline nature of nano size by XRD analysis. FTIR analysis showed the presence of functional groups such as calcium and phosphate in HAp. Porous scaffold showed 85% of porosity with compressive strength 2.84 MPa which is beneficial to be used as filler and load bearing in biomedical applications. SEM images showed that the scaffold had pore diameters in the range 2~5  $\mu\text{m}$  and pores were interconnected which promotes cellular vascularization. This was supported by invitro assay. The experimental results revealed that the HAp granules had fine biocompatibility. Altogether, our findings clearly suggest that the HAp granules obtained from two different sources may be used as a bone substitute. However, further invivo research studies has to be carried out to explore the benefits of HAp granules in tissue engineering.

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## REFERENCES

- [1] Xin lee et al (2013) "Biotemplated synthesis of macroporous materilas for bone tissue engineering scaffolds and experiments in vitro and vivo" ACS Appl. Mater. Interfaces, 5:5557–5562.
- [2] Ivankovic H et al (2008) "Preparation of highly porous hydroxyapatite from cuttlefish bone", J Mater Sci: Mater Med 20:1039-1046
- [3] Beom-su kim et al (2013) "Improvement of the compressive strength of the cuttlefish bone-derived porous hydroxyapatite scaffold via polycaprolactone coating", J Biomed Mater Res Part B 101:1302-1309
- [4] Jaebeom Lee et al (2011) "Nanoscale hydroxyapatite particles for bone tissue engineering" Acta Biomaterialia 7:2769–2781
- [5] Shih-Ching Wu et al (2013) "A hydrothermal synthesis of eggshell and fruit waste extract to produce nanosized hydroxyapatite", Ceramics International 39:8183–8188
- [6] Furukawa T et al (2000) "Histomorphometric study on high-strength hydroxyapatite/ poly(Llactide) composite rods for internal fixation of bone fractures", J Biomed Mater Res 50:410–9.
- [7] Trombelli L et al (2010) "Single flap approach with and without guided tissue regeneration and a hydroxyapatite biomaterial in the management of intraosseous periodontal defects", J Periodontal 81:1256–63.
- [8] Strietzel FP et al (2007) "Lateral alveolar ridge augmentation using a synthetic nano-crystalline hydroxyapatite bone substitution material", Clin Oral Implants Res 18:743–51.
- [9] Ye Q, Ohsaki K et al (2001) "Histological reaction to hydroxyapatite in the middle ear of rats", Auris Nasus Larynx 28:131–6.
- [10] Seol YJ et al (2009) "Fabrication of a hydroxyapatite scaffold for bone tissue regeneration using microstereolithography and molding technology", Microelectron Eng 86:1443–6.
- [11] Lv Q et al (2009) "Fabrication, characterization, and in vitro evaluation of poly(lactic acid glycolic acid)/nano-hydroxyapatite composite microsphere-based scaffolds for bone tissue engineering in rotating bioreactors", J Biomed Mater Res 91:679–91.
- [12] Itokazu M et al (1998) "Synthesis of antibiotic-loaded interporous hydroxyapatite blocks by vacuum method and in vitro drug release testing", Biomaterials; 19:817–9.
- [13] Jabr S. Al-Sanabani et al (2013) "Application of Calcium Phosphate Materials in Dentistry" International Journal of Biomaterials", Volume 2013, Article ID 876132, 12 pages.
- [14] Blackwood D et al (2009) "Electrochemical cathodic deposition of hydroxyapatite: improvements in adhesion and crystallinity", Mater Sci Eng C 29:1233–8.
- [15] I. Manjubala et al (2000) J. Mater. Sci. Mater. Med. 11:705-709.
- [16] M. E. Bahrololoom et al (2009) "Characterisation of natural hydroxyapatite extracted from bovine cortical bone ash", Journal of Ceramic Processing Research. Vol. 10, No. 2, pp. 129-138.
- [17] Sudip mondal et al, (2011) "synthesis, characterization and in vitro cytotoxicity assessment of hydroxyapatite from different bioresources for tissue engineering application", Bull. Mater. Sci., vol. 35, no. 4, pp. 683–691.
- [18] Eric M. Rivera et al (1999) "Synthesis of hydroxyapatite from eggshells", Materials Letters 41:128-134.
- [19] S.Sasikumar and R.Vijayaraghavan (2006) "Low Temperature Synthesis of Nanocrystalline Hydroxyapatite from Egg Shells by Combustion Method", Trends Biometra. Artif. Organs, Vol (19)2: pp 70-73
- [20] Dennymol P. V et al (2014) "Morphological Diversity in Nanohydroxyapatite Synthesized from Waste Egg Shell: Verification and Optimization of Various Synthesis Parameters", The International Journal Of Science & Technolege vol 2 (ISSN 2321 – 919X)
- [21] Manoli F, Dalas E (2000) "Calcium carbonate crystallization on xiphoid of the cuttlefish. J Cryst Growth", 217:422–8.
- [22] Lowenstam HA, Weiner S (1989) On bio mineralization. Oxford: Oxford University Press.
- [23] Pankaj Sarin et al (2011) "Porous Biphasic Calcium Phosphate Scaffolds from Cuttlefish Bone", Journal of the American Ceramic Society, 94 [8] 2362–2370
- [24] Joseph Cadman et al (2012) "Cuttlebone: Characterisation, Application and Development of Biomimetic Materials", Journal of Bionic Engineering 9:367–376
- [25] M. H.Santose et al (2004) "Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process", Mater. Res 7: 625-30
- [26] Khelendra Agrawal et al (2011) "Synthesis and Characterization of Hydroxyapatite Powder by Sol-Gel Method for Biomedical Application", Journal of Minerals & Materials Characterization & Engineering, Vol. 10, No.8, pp.727-734.

## International Journal for Research in Applied Science & Engineering Technology (IJRASET)

- [27] J S Earl et al (2006) "Hydrothermal synthesis of hydroxyapatite", Journal of Physics: Conference Series 26:268–27
- [28] Prabhakaran K et al (2005) "Development of calcium phosphate based apatite from hen's eggshell" Bull Mater Sci 28:115–119
- [29] Rocha J.H.G et al (2005) "Scaffolds for bone restoration from cuttlefish", Bone 37: pp 850–857
- [30] Dajana Milovac et al (2014) "PCL-coated hydroxyapatite scaffold derived from cuttlefish bone: Morphology, mechanical properties and bioactivity", Materials Science and Engineering C 34:437–445
- [31] Jun lee et al (2013) "Improvement of the compressive strength of the cuttlefish bone-derived porous hydroxyapatite scaffold via polycaprolactone coating", J Biomed Mater Res Part B 2013:101:1302-1309
- [32] Mohammad Shakir et al (2015) "Synthesis and characterization of a nano hydroxyapatite/chitosan/ polyethylene glycol nanocomposite for bone tissue engineering", Polym. Adv. Technol., 26: 41–48
- [33] Aminatum et al (2013) "The effect of sintering process on the Characteristics of Hydroxyapatite from Cuttlefish Bone", Research journal of Pharmaceutical, Biological and Chemical Sciences, pg.no 1431
- [34] Sang-Jin Lee et al (2010) "Sintering Behavior and Biocompatibility of Calcium Phosphates Fabricated by Cuttlefish Bone and Phosphoric Acid", Tissue Engineering and Regenerative Medicine, Vol. 7, No. 5, pp 556-560
- [35] Fangfang Sun et al (2011) "Various preparation methods of highly porous hydroxyapatite/polymer nanoscale biocomposites for bone regeneration", Acta Biomaterialia 7:3813–3828
- [36] C. P. Dhanalakshmi et al (2012) "Synthesis and preliminary characterization of polyethylene glycol (PEG)/hydroxyapatite (HAp) nanocomposite for biomedical applications", International Journal of Physical Sciences Vol. 7(13), pp. 2093 – 2101.
- [37] Huang L-Y et al (2000) "A study of the process and kinetics of electrochemical deposition and the hydrothermal synthesis of hydroxyapatite coatings" J Mater Sci Mater Med 11:667–673
- [38] Baddiel C et al (1966) "Spectra structure correlations in hydroxy and fluorapatite" Spectrochim Acta 22:1407–1416
- [39] Muhammad Kusumawan Herliansyah et al (2012) "Natural Bioceramics Bone Graft: A Comparative Study of Calcite Hydroxyapatite, Gypsum Hydroxyapatite, Bovine Hydroxyapatite and Cuttlefish Shell Hydroxyapatite", Proceedings of the Asia Pacific Industrial Engineering & Management Systems Conference 2012.
- [40] Hui P et al (2010) "Synthesis of Hydroxyapatite Bio-Ceramic Powder by Hydrothermal Method", Journal of Minerals & Materials Characterization & Engineering, Vol. 9, No.8, pp.683-692
- [41] Arunseshan Chandrasekar et al (2013) "Synthesis and characterization of nano-hydroxyapatite (n-HAP) using the wet chemical technique" Vol. 8(32), pp. 1639-1645.
- [42] Sergey V. Dorozhkin (2013) "Calcium Orthophosphate-Based Bioceramics", Materials, 6, 3840-3942
- [43] Liao C J, Lin F H, Chen K S and Sun J S 1999 Biomaterials 20 1807
- [44] Ferraz MP et al (2004) "Hydroxyapatite nanoparticles: A review of preparation methodologies" J. Appl. Biomater. Biomech. 2:74-80.
- [45] Ravi Sharma et al (2012) "X-ray diffraction: a powerful method of characterizing nanomaterials", Recent Research in Science and Technology 4(8): 77-79
- [46] Silverstein, R.M, (1981) "Spectrometric Identification of Organic Compounds", 4th edition New York: John Wiley and Sons, QD272.S6 S55.





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