Volume : 6 | Issue : 4 | April - 2017 ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

Original Research Paper Engineering

HYDROXYAPATITE IN BIOMEDICAL FIELD

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KEYWORDS Hydroxyapatite (HAP), Bioceramic /Biomaterial, Nano-crystals,Dental Applications,Orthopaedics.

INTRODUCTION

Hydroxyapatite, abbreviated as HAP, is a bioceramic material that is frequently applied in biomedical field. Mr. Abraham Gottlob Werner, a German geologist coined the term "Apatite" in the year 1786. Apatite is a mineral that ` often gets mistaken for other minerals. Word Apatite has Greek origin. It is derived from the word 'apatein' meaning deceiving or to mislead. In general, Apatite means a group of minerals that contain phosphates as major component. Apatite group includes three common members referred to as hydroxylapatite, fluorapatite and chlorapatite which carry high concentrations of various ions i.e., OH−, F− and Cl− ions, respectively in their resulting crystals. A relatively rare form of apatite carrying numerous carbonate and acid phosphate substitutions, but minimal or nil hydroxyl groups, is a large component of animal bone material.Hydroxyapatite has a chemical composition as $Ca_{10}(PO_4)6(OH)_2$.

HAP has numerous medical applications in various physical forms i.e., porous, dense and granular states. This being the natural inorganic substance of teeth along with bones, has been widely used in the biomedical field. It is commonly employed in dentistry and in orthopedics because of its similarities to inorganic component of teeth as well as that of bones. Hydroxyapatite is frequently being used for filling of periodontal lesions.

Apatite is produced and used in procedures pertaining to biological micro-environmental systems. One more name for hydroxyapatite is hydroxylapatite. It is found out to be the main component enamel of teeth and bony minerals. The eminent role of HAP is known in medical applications. Hydroxyapatite; a ceramic material is being used for bone reengineering and also in medicine delivery systems because it is biocompatible. (Pandey H.M.et.al.,2012). HAP has major biological and industrial roles. The porous character of HAP allows good binding affinity for pharmacologicals including antibiotics, enzymes, hormones, steroids, antibody fragments. (Sadjadi M.S. et al 2010). The porosity character allows HAP in the treatment of various skeletal diseases such as osteoporosis, osteomyelitis, bone cancers etc. The synthesized HAP has osteoconductive properties. Therefore it is used to replace hard tissues. The role of HPA in biomedical uses is basically measured according to its similarity with body apatite, which comprises the calcified mineral phases of dentine and bone.

Moreover it is usually applied as powder form to supplement Calcium drugs, in both ceramic and composite forms connecting the bones along with their repair. Also it is coated on metals and various alloys forming bone splints and screws. HAP has rapid activity to form direct bonding with the living bone lacking connective tissue as intermediate source. HAP is widely used as a calcium supplement drug, as a ceramic, for connecting the bone, in orthopedics for repairing bone and as filler to replace amputated bone. It is enormously being used in dentistry as coating material to promote growth inside dental implants and for augmentation. In powder form it is used as a drug to supplement calcium; to connect bones it is to be in ceramic or composite form connecting the bone, orthopedic or repairing bone, coating on metals and alloys for bone splins. Nano crystalline HAP is similar to natural bone as far as composition and crystalline structure are concerned. Its controlled reabsorption in the body fluids following implantation makes it an ideal bone graft substitute.

Nano HAP stimulates adhesion in bone cells and assist their proliferation. It promotes deposition of bone on bioceramic material surface. Hence particle size, composition morphology of HAP are important parameters. (Sasikumar S. and Vijayaraghavan R. 2006).

Hydroxyapatite can be synthesized without the need for an ageing step or subsequent heat treatment.Spindle-shaped,20-50nm sized nano-HAP particles serve as an effective agent for occluding dentinal tubules. For the first time in dentistry, the effect of tricalcium phosphate reagent in intra-boney defects in dogs was studied, but later it was demonstrated that it was a mixture of hydroxyapatite and tricalcium phosphate.

PROPERTIES OF HYDROXYAPATITE

Hydroxyapatite ceramics are never toxic. Moreover these do not possess any inflammatory response or pyrogenic response. HAP never leads on to fibrous tissue formation between the placed implant and the host bone tissue. Hydroxyapatite has high bioactivity. Its non-toxicity is a major feature to promote its applications. Hydroxyapatite is approximately seventy percent by weight and fifty percent by volume as present in bones. Biological apatite is different from pure HAP. Variations include:

- Structure
- Composition
- Crystallinity
- Solubility
- Biological reactivity
- 0ther physical and mechanical properties

It has been reported that biological apatite is usually calcium insufficient with less Crystallinity. It is generally carbonated substituted. The amount of carbonate is approximately 3-8% by weight of the hard tissues constituting human body. Besides Ca^{2+} and Po_4^{3-}, Carbonate (CO_3^{2-}) makes the major secondary ions weights in HAP that is to be used biologically. However, HAP has tremendous intrinsic brittleness. It has poor mechanical properties. Such features restrict its approach in respective applications associated with load-bearing.

Calcium hydroxyapatite is the most stable but the least soluble phosphate salt of calcium at pH more than 4.2. HAP powders possess relatively high thermal stability in highly alkaline media and also good phase purity at temperature ranging between 1100-1300^{0}C.It was reported that the synthetic HAP, with a Ca/P ratio near to 1.67, was only stable below 1200^{0}C. It was seen that beyond 1200^{0}C, HAP gets deprived of hydroxyl groups and is modified to oxyapatite ($Ca_{10}(PO_4)6O$). At a temp. beyond 1450^{0}C, it further breaks down into trisodium phosphate, calcium pyroclore and tetracalcium phosphate. Later it was reported that pure HAP powders prepared in pure water may withstand temperatures as high as 1300^{0}C when heated in dry air for a period of hours without getting decomposed.

Hydroxyapatite has rapid bone regeneration ability and creates a direct bond with the host living bone without intermediate connective tissue.It is versatile because of excellent biocompatibility & surface active properties, non-toxicity, non-inflammatory behavior and non-immunogenic characteristics.Nano HAP stimulates proliferation of bony cells and their adhesion. Simultaneously it increases proliferation of bone on bioceramic material surface. It also reduces apoptotic cell death.

Main characteristics of Hydroxyapatite include ratio i.e., Ca/P, size of crystals, morphology, specific surface area, uniform distribution of particles, resorption and associated biological activity.It has been reported as a better replacement in case of graded materials. It provides bioactivity and good mechanical properties.Its physicochemical properties especially solubility and crystallographic details are directly proportional to the mineralization processes in living systems.

The morphology of HAP can be varied by varying pH of the solution. The needle like structure change to flower like structure on performing SEM. At pH above 10 followed by calcinations,the structures at microscopic level are more dense and homogeneous than at pH below 10. EDTA acts as a capping or chelating agent. It helps to prevent agglomeration of particles. Other parameters include initial ingredients, physical & chemical conditions, calcination temperature, microwave power and doping with a non-reacting metal. These lead to variation in the structure, shape, size, morphology and bonding pattern of HAP crystals along with its optical characteristics.

HUMAN SKELTON AND HAP

The biomaterials are being most extensively found so that they can be used as alternative material. Hydroxyapatite, is one of the biomaterial among the others. The major reason for choosing the hydroxyapatite as a biomaterial is because of its close similarity to the natural bone. Due to these abilities, it has been extensively employed for tissue engineering of the bones.As we observe now a days, large amount of people are suffering from various orthopedic abnormalities. There are many reasons that lead to these defects; which either be due to accident or ageing. The orthopaedic injuries majorly make the person suffer due to the associated trauma. As such hydroxyapatite is mostly used in treating the bone defects. As it has various advantages such as biocompatibility, high porosity and many others, now our target is to use this material in treating the dental problems.

Our human skeleton is made up of around 206 bones. Bones not only provide strength to our body but also enable us to walk. The longest bone which is present in our body is called femur which is also known as thigh bone; whereas the smallest bone is present in the ear and has been named as step bone. Even our hand consists of 26 bones. In Our body, only ear and nose are the organs which are made up flexible material called cartilage, and is not hard like bone. Bones are interconnected to each other by joints. There are several types of joints, comprises of stagnant joints (which is found in skull, and comprises of many bones), pivoted joints (which are found in the fingers as well as toes), and other type of joint is ball-and-socket (which are found in the shoulders and hips). Bones is made up of calcium and helps in manufacturing of blood cells as well as also help in storing important minerals.Bone modelling is generally done in small children or adolescents so that bone growth can take place in length as well as in cross-sectional area. The development of bones by the aggregation of material onto endosteum or periosteum region which facilitate in the modelling process, and can occur for lifetime. Bone remodeling generally includes removal or replacement of bone. Thus, it facilitates us for the continuous recycling of the bones whereas healthy tissue stay away from micro-cracks that may lead to fatigue seizure of that structure. This type of process is commonly seen during the healing process of fracture.

Hydroxyapatite (HAP) is an artificial biomaterial which is commercially used in repairing of bone tissue. HAP, general formula is $Ca_{10}(PO_4)6(OH)_2$, that is primarily made up of calcium and phosphorous interrelated by hydroxide ions which gets eliminated at high temperature. HAP among the other Ca/P minerals have been extensively employed as material for implant from long time since it has exceptional biocompatibility and adhesiveness because of its structural as well as the similarity in the composition with the mineral phase material of the hard tissue which is present in the human bones. HAP coatings have virtuous ability to be employed due to its biocompatibility assisted with bone bonding capability similar to that of the ceramic, whereas additionally using the mechanical properties of other substrates and various other alloys that are biocompatible. These metallic materials which are used with HAP not only contribute in the mechanical properties, but these also provide the osteoconductive surface for the accumulation of the material for the bone growth, implant anchoring and relocating burden over the skeleton; and additionally also help in combatting during atrophy of the bone.

Ratio of Ca/P is around 1.52±2.0 that makes it an exceptional choice in dentistary and orthopedics as it can be used as a bio-ceramic coating. The prominence of a coating is largely dependent on its overall qualities and features of the powder which has been synthesized. Thus, attributes consist of phase composition, clarity, crystallinity, size of the particle, distribution of the particle-size, and surface area of the particle, density of the particle and morphology of the particle. These important features that determines the success rate of HAP coating aggregated onto implanted teeth with the plasma thermal spraying. The chemical quality of HAP, however, contributes itself as a substitution, which means that synthesis of nonstoichiometric HAP is there. The most usual substitutions are CO_3^{-2}, F^- and Cl^- that replace the hydroxyl groups, whereas deficiency in HAP causes the developing of certain defects.

The reason for selecting the HAP is because of its versatile nature and properties as enumerated below:

1. Excellent Biocompatibility and Surface active ability
2. Non-toxic nature
3. Non-inflammatory behavior.
4. Non-immunogenic response
5. Similar to mineral constituent of natural bone
6. Promotes regeneration of bones at targeted site

Over the last few decades, the use of bone substitutes in human surgery has inclined enormously. Such materials have been used to guide as well as to expand the healing of bone tissue and thereby to get integrated within that. It resembles remodeling process in the actual bone. Hydroxyapatite (HAP) rods were rapidly synthesized by a continuous hydrothermal three pump mechanism using a water feed. The product obtained is a highly crystalline and phase pure material. Silver (Ag):HAP nanoparticles are obtained by an inexpensive technology. These nanoparticles work to protect against macrophages.These also take care against cytotoxicity. Their activity is to be monitored to get rid of unwanted immune responses in the human body. So optimization of doses is essential.

PREPARATION OF HAP

There are many methods to synthesize HAP. These include:-

- Wet Chemical Deposition
- Biometric Deposition
- Sol Gel Route (Wet Chemical Precipitation)
- Electrodeposition
- Hydrothermal Technique
- Ultrasonic
- Spray Drying
- Microemulsion

- Macheno-Chemistry
- Co-Precipitation
- Chemical Vapour Deposition

Two commonly used methods for the preparation of HAP are - :

(1) Chemical Methods: These include wet precipitation processes and hydrothermal technologies.

(2) Solid State Reaction Method: Dry method. HAP has been synthesized in the presence of polyacrylic acid by precipitating calcium nitrate tetra hydrate with ammonium dibasic phosphate. This is followed by hydrothermal treatment. HAP is found to be sable thermodynamically at physiological pH, as well as osteoconductive.

Hydroxyapatite (HAP) nanocrystals have been synthesized by chemical precipitation technique. Advantages of chemical synthesis include simple equipment, low cost, ability to obtain nanosized HAP powder, large scale production and associated high purity. The reaction involved is shown below:-

$10\ Ca(OH)_2 + 6\ H_3PO_4 \quad Ca_{10}(PO_4)6(OH)_2 + 18\ H_2O$.

The best method for synthesis of HAP remains sonication followed by precipitation.

Preparation modifications

A more recent approach to developing porous, ceramic fibers is by use of electrospinning. In this case, the inorganic solution (precursor to the ceramic) is mixed with a polymer solution followed by electrospinning at voltage of 10 to 30 kV. Some methods need high temperature for processing.There is high raw material cost and synthesis process remains complex.Interaction of antimicrobials with HAP nanopowder synthesized by chemical precipitation method has been studied. The respective zones of inhibition produced thereof are to be analysed.

Titanium dioxide (TiO_2) have been investigated extensively for the killing or growth inhibition of bacteria.. Hence HAP and TiO_2 in combination form a composite material which can decompose bacteria and organic materials and is considered to be good in antibacterial applications and photocatalytic decomposition of biomaterials, such as proteins,lipids etc. In the field of biomedical, many failures in the implantation are may be due to the formation of microbes in the implanted site. If the implant material has the capability of antimicrobial activity within them, then the problem of failure will be reduced. Moreover, microbes which cause a wide variety of infections in humans and other animals can spread through common places like bathroom tiles, doorknobs, packing materials etc., can be controlled by the antimicrobial materials and coatings. The present work is mainly focused on the biocompatibility and antimicrobial activity of the hydroxyapatite/ TiO_2 nanocomposites which was synthesized by combined high gravity and hydrothermal treatment of colloidal HAP and TiO_2 solutions. Different concentrations of HAP and TiO_2 were employed to prepare the composites. A model animal cell was used to study the cell compatibility of various HAP/TiO_2 nanocomposite powders. Bioactivity of HAP material depends on many factors during process of synthesis. These include reagents used, impurities present, size of crystals with morphology status, concentration of reagents with their mixture order, temperature of procedure and pH value. On gross examination HAP is seen to be white in colour and is of crystalline nature with high porosity.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity is tested by well-diffusion method against pathogenic bacteria such as Escherichia coli (E-coli) and Staphylococcus species.{Baur et.al.,1966).

NANOSTRUCTURE CONFIRMATION

Structural and morphological analyses are carried out in order to confirm the composite and nanostructure formation.These include Fourier Transform Infrared Spectroscopy (FTIR),Scanning Electron Microscopy (SEM), **Transmission Electron Microscopy (TEM) and X-Ray Diffraction (XRD).**

LITERATURE REVIEW

Hydroxyapatite (HAP) is the most effective calcium salt as far as clinical applications are concerned. HAP is known for its excellent biocompatibility. It has such surface active properties that make it compatible with the living tissues. [Mohandes,F.1,M.et.al.2013].

There is very high prevalence of dental hypersensitivity in many regions of the world. Treatment of such disorder is too costly. Rarely it may show a are very life threatening outcome. Using, By fabrication of natural polymer i.e., polyphosphate, microparticles in amorphous form were prepared. These were used to treat the hypersensitivity. I t is done by resealing the dentinal tubules that got exposed.[Werner E.G.et,al.,2016]

Surface-modification of HAP was done by the addition of beta alanine. A inorganic-organic nan composite was successfully prepared. In the process of HAP modification, the morphology was altered from rod to sheet and subsequently changed from flake to needle. The primary amino-terminated HAP has been proved to be an excellent initiator for the ring-opening polymerization of amino acid named N-carboxyanhydride. This form could even be dissolved in chloroform. HAP-PLGA with surface carboxyl groups could be obtained by the catalytic hydrogenation of HAP-PBLG. [Yukai S et.al.,2013]

Chemical precipitation, hydrothermal as well as emulsion techniques can be followed using surfactants (ionic or nonionic) as pore template. Mesoporous HAP nanoparticles obtained thereby are useful because these have sustained release properties. [Mohammad N.F.et.al.,2014].

TEM means Transmission Electron Microscopy and it is a basic tool to investigate microstructure of HAP. It provides crystallographic information along with nanometeric scale composition.Specimen preparation includes thinning of the sample up to the extent of electron transparent thickness which could result in formation of artifacts and those could be briefly reported.[Sridhara et. al., 2010] It is notable that the aqueous temperature assumes a key part in the controlling the crystallite estimate, level of crystallinity and the stoichiometric proportion However, in recent years, nano-sized hydroxyapatite (nHAP) with appropriate stoichiometry, morphology and purity have stimulated great interest in scientific research.[J. Anita Lett 2015]

The vast majority of the earthenware productions require the utilization of exactness techniques. Savvy shear blending system can be used to produce CNT–HAP composites[Mukherjeea, S. et all 2014].

All in all, HA nanorods have been effectively incorporated by usage of snail shells as a calcium source by means of simple microwave light technique in quick way utilizing EDTA as a chelating operator. The acquired item was recognized to be B type carbonated HA with hexagonal precious stones. The arrangement of nanorods is expected to the anisotropic development propensity for HA crystallites under microwave light. Subsequently it can give new prospects in the improvement of biomaterials for orthopedic applications. [Suresh KumarG.et.al., 2015].

BoneSource™-hydroxyapatite concrete is another calcium phosphate concrete biomaterial which is self-setting and is equipped with exceptional biocompatibility and physical properties. So it is used in craniofacial skeletal recreation.[Craig D.et.al., 1998]

At the demand of restorative groups from the maxillofacial part, an exceedingly permeable artistic bolster based hydroxyapatite powder prepared by directly reacting $(NH_4)_2HP0_4$ with $CaCI_2$ in an aqueous solution; the microemulsion processing route led to a significant refinement in the particle size, particle size distribution and the degree of particle agglomeration. [Fabbri M et.al.,1995].

The mixing procedure determines the final stoichiometry of the precipitated HAP [Ca_{10}(PO4)6$(OH)_2$].Particles with controlled stoichiometry were precipitated from solution by forced hydrolysis.[Lopez,M.et.al.,1998].

HAP and dicalcium phosphate dihydrate nanoparticles were prepared by co-precipitation method; HAP was formed in a needle shape and rice shape depending on varying pH values. [Mansour1,S.et.al.,2015].

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