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Review Article

REVIEW ON HYDROXYAPATITE-CARBON NANOTUBE COMPOSITES AND SOME OF THEIR APPLICATIONS

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ABSTRACT

Hydroxyapatite (HAP) has been one of the cheapest and well known ceramics for diversified applications including clinical bone graft procedures for more than 25 years. Even though it has many applications, its poor tensile strength, fracture toughness and mechanical properties make it unsuitable for major load-bearing applications. Carbon nanotubes (CNTs) have excellent mechanical properties to strengthen and toughen HAP. Further when incorporated with HAP the range of applications for this material is broadened. Hence, in this review we discuss techniques to synthesize and process HAP–CNT composites. Moreover, the methods to improve the properties along with the applications of the composites are also discussed.

Keywords: Hydroxyapatite, CNT, Composite, Bone tissue engineering

INTRODUCTION

The major mineral phase in bone is made up of Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) with the ratio of Ca/P of 1.67 and embedded as nanocrystalline form in collagen triple helix structure.[1] HAP has hexagonal structure in which calcium cations (Ca^{2+}) and phosphate anions (PO_4^{3-}) are arranged around the column of monovalent hydroxide anion (OH^-). Moreover, this network of phosphate groups gives the skeletal frame work and stability.[2] Several methods are reported for the synthesis of HAP powder including wet chemical precipitation,[3] hydrothermal reaction,[4] mechanochemical–hydrothermal synthesis[5] and sol–gel synthesis.[6,7] The biomedical application of HAP calcium phosphate ceramic is mainly due to their biocompatible, osteoconductive and bioactive properties. The other applications of HAP include drug carrier,[8] protein purification process,[9] replacement of the eye in human,[10] bone defects in orthopedic, maxillofacial surgeries and dentistry.[11-13]

Carbon nanotubes (CNTs) are attractive in the field of material science due to their good mechanical properties including high tensile strength, high resilience, flexibility and other unique structural, electrical and physicochemical properties. [14-16] Even though several papers are available on the preparation and mechanical characterization of composites, its poor dissolution property restricts the applications of CNTs.[17-21] These disadvantages can be avoided to some extent by the organic functionalization developed in recent years. Currently, CNTs are used in several biomedical applications including cancer therapy, treatment of central nervous system disorder and tissue engineering applications including bone tissue engineering, sensing cellular behavior, augmenting cellular behavior, cell tracking and labeling.[22] Further, the formation of composite with CNTs acts as an excellent reinforcement material with enhanced strength, toughness and flexural strength for major load bearing applications. Moreover, the bioactivity of HAP is not affected by the incorporation of CNTs. Thus opening up wide range of clinical applications for these materials.[2]

BIOCOMPATIBILITY AND CYTOXICITY OF CNT

The capacity of a material to perform with in an appropriate host response in a specific application is known as biocompatibility. Biocompatibility without cytotoxicity is an important property of CNT based composite materials for biomedical applications. Carbon based biomaterials are not new for biomedical applications; the biocompatible pyrolytic carbons are used as biomedical implants and coatings for several years. There are also several studies on the biocompatibility of CNTs coatings for orthopedic and cardiovascular applications. The CNTs coated stainless steel rods implanted in sheep were found not to have any adverse effect on bone or muscle tissue even though the unrefined CNTs exhibit some degree of toxicity both in vitro and in vivo. Further, the refinement of CNTs reduced the toxicity.[23] Toxicity in the unrefined CNTs is attributed to the catalytic metals present in the CNTs including iron, cobalt and nickel. The complete removal of catalytic metal present on the CNTs will help identify any toxicity effect responsible only because of CNTs.[23,24] Even though the SWNTs without metal catalyst increased cellular apoptosis/ necrosis in vitro the unrefined SWNTs increased oxidative stress.[24] The metal catalyst in the CNT can be removed by acid treatment. According to Alexander et al., SWNTs at concentration 0-10 µg/ml using serum free medium showed no significant decrease in cell viability at any of the above concentration of CNTs. The Raman spectrum suggests the acid treated of SWNTs increased the number of functional group on the side walls of the CNTs and hence the acid treated SWNTs due to their negatively charged functionalization can easily be localized within cytoplasm of the cell.[25] The size and concentration of CNT play an important role in the cytotoxic effect. When compared with graphene sheet, the tubular shape of the SWNT can easily penetrate into the membrane increasing the uptake by cells and exhibiting strong interaction with various proteins causing more damage to the cells.[26] Whereas acid treated functionalized CNTs are less toxic than the untreated CNTs. The biodistribution and clearance studies have also proved that the functionalized CNTs can be excreted rapidly through kidney and visualized in urine.[3]

SYNTHESIS

The major objective of CNT-HAP composite is the homogeneous dispersion of CNTs and their interaction with HAP which has been achieved through chemical functionalization of CNTs.[2] There are many reports on the synthesis of CNT-HAP composite, we have explained three important way of synthesizing them here.

1. The CNT-HAP composite can be synthesized by ion exchange reaction as follows; its anionic derivative of $[CNT]^+[SbF_6]^-$ can be formed by reacting CNT with $[bmim][Sb_2F_{11}]$. This anion can be exchanged with NaCl to give air stable product of CNT-Cl which can further be reacted with HAP to give the required composite material.[27]

2. The sol-gel process for the synthesis of CNT-HAP can be achieved by any one of the following three methods. In the first method, the HAP sol is prepared from $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ solution of calcium then CNTs and sodium dodecyl sulphate (SDS) are added separately to the HAP sol by mixing. In the second method, HAP sol is prepared and mixed with previously prepared CNTs and SDS sol. In the third method, CNTs-SDS sol is synthesised by adding nitrate and phosphate solution to form CNT-HAP composite. Among the three methods, the third method gave the smaller crystal size and higher crystalline nature.[28]

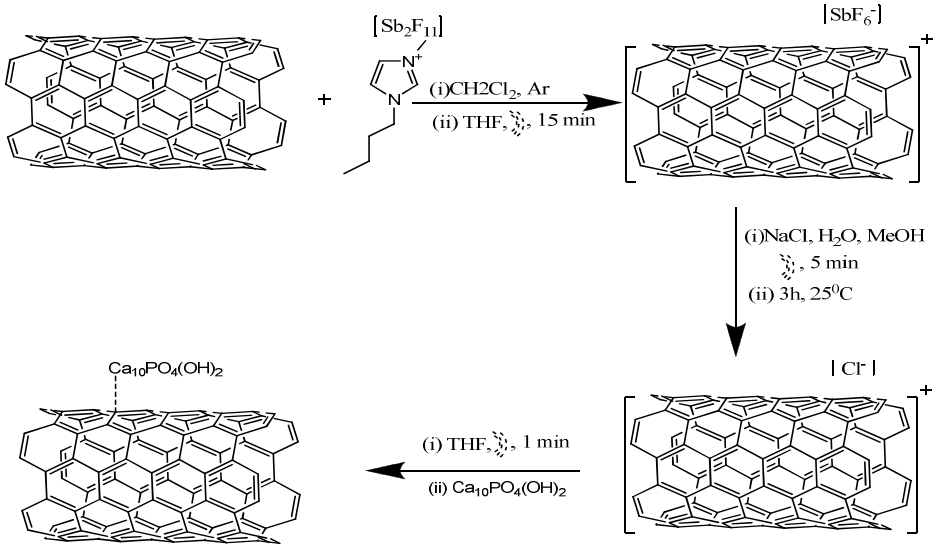

Fig. 1: Synthesis of CNT-HAP composite by ion-exchange reaction.[27]

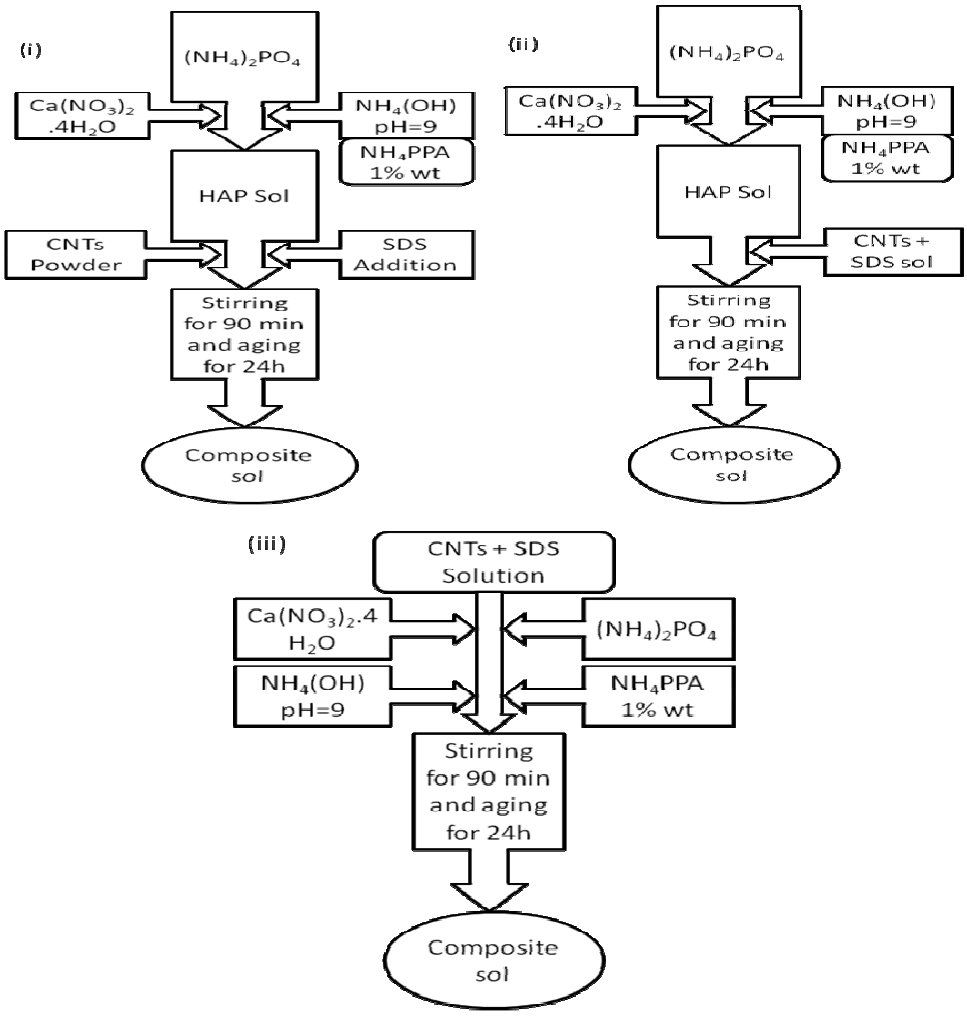

Fig. 2: Schematic representation for sol-gel synthesis of CNT-HAP composite.[28]

3. The CNTs are acid functionalized using 3:1 volume of sulphuric acid to nitric acid and sonicated for 12 h at approximately 30 °C then the acid functionalized CNTs are dispersed in deionised water and added to 0.5M aqueous $Ca(OH)_2$ for 4 h. Further H_3PO_4 is added drop-wise, allowed to stand overnight, filtered and dried.[29]

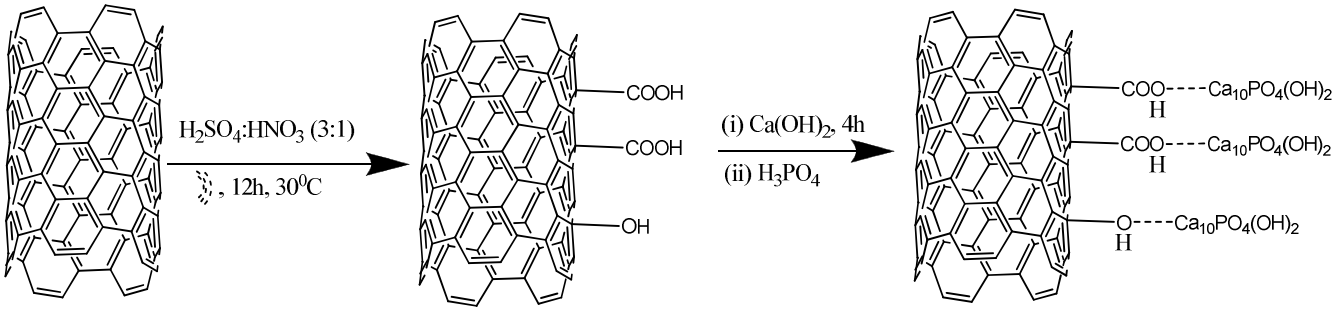

Fig. 3: Synthesis of CNT-HAP composite by functionalization of CNT.[29]

SINTERING

The synthesised composite material of CNT-HAP can be obtained as dense material with high mechanical strength by the sintering process. Sintering is a process in which the synthesized samples are subjected to higher temperature. The higher degree of sintering process can have higher mechanical properties of composite material. There are many difficulties in achieving densification of CNT-HAP composite by sintering process. During the sintering process a high temperature of nearly 1200-1300 °C is normally employed. The problem is that at this temperature CNT can be oxidised and the HAP can undergo dehydroxylation and may decomposes to form tricalcium phosphate as well as tetra calcium phosphate at about 900 °C in the presence of air and at 850 °C in water free atmosphere but the atmosphere required for sintering of CNT-HAP composite should allow the retention of both CNT and hydroxyl group of HAP. Spark plasma sintering is an alternative way used for sintering with its high sintering rate and applied pressure but the limited geometry and cost are the disadvantages. The CNTs because of their property to with stand high temperature in an inert atmosphere can undergo sintering process in an inert atmosphere. Hence pressure less sintering with several atmosphere including vacuum, argon, nitrogen, air, water, air-water, hydrogen (H_2), carbon monoxide (CO), H_2-CO, CO-H_2-H_2O and CO-H_2-H_2O (ice) can be achieved. Among them CO-H_2-H_2O (ice) showed good balance and density of CNTs and is considered to be a optimal way for sintering the CNT-HAP composite. [2,29,30] The composite sintered in Ar showed the interface bonding between CNT and HAP to be weak and with high porosity, where as the composite sintered in vacuum exhibited strong interface bonding between CNT and HAP with low porosity.[30] The pressure less sintering process is widely used because of simple method. There are many other factors that affect the degree of sintering and density of the final sample including powder property, sample preparation, phase purity of raw material, sintering temperature, atmosphere, heating rate and the Ca/P ratio of sintered product. The alternative method of laser surface alloying is used for the sintering of CNT-HAP composite to create a CNT-HAP coating on titanium alloy. Partial matrix melting induced by a laser and spark plasma sintering is also achieved by this method. Moreover, this method is having the advantage of forming highly dense material within few minutes.[2]

BIMEDICAL APPLICATION

In the recent years nanostructure biomaterials have been playing an important role in regenerative medicine. The scaffold biomaterials serve as a temporary 3D substrate for tissue formation and organisation which can mimic the characteristic of extra cellular matrix.[31] The tricomponent composite of CNT-HAP biomaterials are also used for bone tissue engineering, gene delivery, biosensor and biofuel.

BONE TISSUE ENGINEERING

- CNT-HAP composite synthesised by spark plasma sintering method exhibited a higher mechanical strength including high value of modulus (131.3 GPa) and hardness (6.86 GPa). Moreover an increase in the fracture toughness (92%) and elastic modulus (25%) was observed when compared with free HAP matrix. Furthermore an excellent proliferation on human osteoblast cell was also observed. The inclusion of CNT on HAP matrix not only increased the bio-mechanical properties effectively but also promoted cell growth on osteoblast cell and hence can be used in bone tissue engineering.[32,33]

- When CNT-HAP composite is coated on Ti–6Al–4V alloy using electrophoretic deposition method; it exhibited excellent corrosion resistance, high mechanical strength and increased biocompatibility. Further it can be used for biomedical applications including total hip replacement due to the enhanced proliferation and alkaline phosphatase (ALP) activity on the MC3T3-E1 pre-osteoblast cell.[34-38]

- The scaffold material obtained from functionalized CNT-chitosan-HAP composite synthesised by freeze drying method decreased water uptake, retention and degradation ability but increased thermal stability when compared to free chitosan. Further the CNT-chitosan-HAP composite showed a improved cell proliferation on MG-63 than that of pure chitosan and hence can be used as a biomaterial for bone tissue engineering.[39]

- Poly(methyl methacrylate) (PMMA) used as bone cement has low mechanical strength but the reinforced PMMA-HAP with CNT and HAP reinforced with CNT and Al_2O_3 showed higher mechanical properties than the free HAP and can be used for bone tissue engineering because of these improved properties along with biocompatibility.[40,41]

- The guide tissue regeneration (GTR) membrane synthesised by electrospinning suspension of poly(L-lactic acid) (PLLA), CNT and HAP (PLLA/CNT/HAP) showed 30% enhanced adhesion and proliferation on periodontal ligament cells (PDLCs) and 30% inhibited the adhesion and proliferation of gingival epithelial cells compared with control group. This membrane showed excellent dual biological functions and satisfied the requirement of the GTR technique successfully in spite of a monolayer structure. Compared with other conventional GTR membranes, the membrane can not only simplify the manufacturing process and reduce the fabrication cost but also avoid possible mistakes in clinical application. Above all, it needs not be taken out after surgery because of the biocompatibility and hence have great potential for GTR and tissue engineering. [42]

BIOSENSOR AND BIOFUELS

The ascorbate/O_2 biofuel cells are assembled by the laccase/CNT-HAP modified electrode as cathode for the reduction O_2 and the glucose oxidase/CNT-HAP modified electrode as anode for the oxidation of glucose. The enzyme modified CNT-HAP electrode can be used as biofuel cell as well as biosensor.[43]The HAP functionalized CNT is also successfully used for DNA complexation, the formed complex serve for gene delivery as well as biosensor in biomedical applications.[44,45]The haemoglobin immobilised CNT–HAP composite act as a modified electrode when coated with glassy carbon (GC). The modified electrodes of Haemoglobin-CNT–HAP/GC exhibit fast amperometric response with very high sensitivity, good reproducibility, stability and good bioelectrocatalytic activity for electrochemical reduction. Hence, these modified electrodes are useful in the field of bioelectronic nanodevices such as electrochemical biosensor and enzyme-based biofuel cells.[46]

CONCLUSION

The synthesis of HAP-CNT composites and some methods of improving the properties are discussed. The composites are found to exhibit wonderful properties compared to that of HAP and CNTs. Even though CNTs have excellent mechanical properties and strength, they are found to be toxic because of the dissolution property. Though HAP has good biocompatibility it lacks the strength. The composites are found to complement each other and improve the required properties. Therefore this is an attempt to bring out the ideas in this emerging field, to kindle the research in this line, to modify the composition of the composite and to explore the highly potential applications.

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