CHAPTER 7

SUMMARY AND SUGGESTIONS FOR FUTURE WORK

7.1 SUMMARY

Biomaterials are used to make devices to replace part of a living system or to function in intimate contact with living tissue without being rejected by the body. Biomaterials are used every day in biomedical applications, surgery, and drug delivery. In general, biomaterials are classified into four major groups: metals, polymers, ceramics and composites. All of them play an important role in replacement and regeneration of human tissues. Bioceramics has been recognized to play a significant role in biology for medical applications, mainly for implants in orthopaedics, maxillofacial surgery and for dental implants over a century. Bone is a complex living tissue which has elegant structures at a range of different hierarchical scales. It is a composite of organic and inorganic materials which forms the basis of the skeleton and its functions are numerous and complex. The organic compound is composed mainly of type I collagen fibers and occupy 30-35% of bone by weight. The inorganic portion of bone is calcium phosphate compound which has 65-70% hydroxyapatite.

Hydroxyapatite-based ceramic is the main inorganic component of hard tissues like bone and dental reparation and also for the applications in drug delivery systems. HAp exhibits excellent properties, like high osteoconductivity and osteoinduction when implanted in the human body. HAp is an elective material because it is biocompatible, nontoxic in nature, and has been developed as a drug carrier for treating bone infections at specific sites. Recently, HAp has shown to accelerate the healing of bone fractures upon electrical stimulation. This response to electrical stimulation has been attributed to the porosity and microstructure of HAp. Porosity plays an important role in the osteoconductivity, circulation of body fluids and acts as a barrier for various drugs. Such approaches have been documented in the treatment of osteoarthritis, osteosarcoma and osteonecrosis. The main drawback of this material is the lack of mechanical properties which lead to implant failures. To enhance these properties of HAp composites by doping with different ions and through sintering will give better results for the implant materials.

Osteosarcoma is one of the bone tumors which can be remedied by using magnetic nanoparticles. Magnetic nanoparticles are subjected to oscillating magnetic field that absorbs energy from the electromagnetic wave and may show remarkable heating effects. The absorption of energy is due to several processes like hysteretic losses, relaxation processes and viscous losses. Magnetic induction hyperthermia is a technique to destroy cancer cells by their hysteresis loss when placed under an alternate magnetic field. The temperature can be maintained in a cancerous tissue within the range of 42 - 46 °C for 30 min or more, thus cancer cells are destroyed. In the HAp structure, divalent cation such as calcium ion (Ca^{2+}) may be substituted as Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Cr^{2+}, Mn^{2+}, Co^{2+}, Sr^{2+}, Pb^{2+} and Cd^{2+}, and the anions phosphate $(PO_4)^{3-}$ ions and hydroxyl $(OH)^-$ ions will be replaced by F^-, Cl^-, B^-, CO_3^{2-} and VO_4^{3-}. HAp incorporated with some magnetic ions and exhibiting strong ferromagnetic properties, play an important role in medicine for example, iron (Fe), cobalt (Co), nickel (Ni), etc. By substituting Ca^{2+} in HAp could be useful for biological applications such as magnetic resonance imaging (MRI), cell separation, targeted drug delivery and heat mediator for the hyperthermia treatment of cancers.

Surface modification through ion irradiation is an important tool for the ceramic materials. To improve the bulk properties, sterilization and surface modification of ceramic materials can be used widely through radiation process. The irradiation method enables to us modify the chemistry of HAp in a reproducible way so as to optimize the bone response to the implant and improve bio interaction for cell adhesion and proliferation. It also creates pores, crystallinity, reduces particle size, wettability, surface roughness, densification and mechanical property.

The incorporation of fluoride in dental enamel has been largely practiced to reduce the formation of dental decay. Fluoride has been administered in several forms, through table salt, drinking water, tooth pastes and mouth washes. Fluoride ion in drinking water is known to have both beneficial and detrimental effects on health. If the concentration of fluoride is high in ground water, it causes dental and bone fluorosis. Fluoride concentration between 0.9-1.2 mg/L may result in lack of protection against dental caries, especially for children at the age of 22 to 26 months, while intake between 1.5 and 3.0 mg/L causes dental fluorosis (mottling, yellow and brown stains). Some studies reported that long term exposure levels between 3-6 mg/L of fluoride concentration could result in skeletal fluorosis, whereas level beyond 10 mg/L resulted in crippling skeletal fluorosis (World Health Organization 2004). Among various processes, adsorption process is effective, environmental friendly and economical. Various types of absorbents like cement paste, Iron (III)-Aluminium mixed oxide, montmorillonite, bleaching powder, titanium oxysulphate ($TiOSO_4.xH_2O$), Fe(III) loaded carboxylated chitosan beads and quick lime were investigated to reduce concentration of fluoride in water. Among these sorbents, HAp showed an excellent potential in the process of water treatment. Research works on the removal of uranium, copper, cadmium, lead, nitrate, oxovanadium and zinc using hydroxyapatite were carried out.

Nanosize hydroxyapatite (nHAp) doped with varying levels of Fe^{3+} (Fe-nHAp of average size 75 nm) was synthesized by hydrothermal and microwave techniques. The crystallite size and the crystallinity decreased with an increase in Fe^{3+} content. The functional groups of nHAp were characterized by FT-IR. The spherical nHAp (200 ± 5 nm) is modified on Fe^{3+} doping, into rodlike structures, with a considerable reduction in average size (75 ± 5 nm). The maximum hardness of 0.1Fe showed 38 GPa with a particle size of 75 ± 5 nm, whereas 0Fe showed 16.5 GPa with a particle size of 200 ± 5 nm. These results suggest that the Fe^{3+} doping decreases the particle size along with a significant increase in hardness. The Fe^{3+}-doped samples showed hysteresis loop indicating superparamagnetic behavior, whereas nHAp was diamagnetic. The increasing trend in dielectric constant values on Fe^{3+}-doped nHAp shows the remarkable polarizability of this material. The haemolytic assay proved that Fe^{3+}-doped nHAp is a blood compatible biomaterial. The drug release studies performed with the antibiotic (amoxicillin) and anticancer (5-Fluorouracil) drugs showed that Fe^{3+} doping at a low concentration of about 8 ppm leads to highly prolonged drug release. In addition, the Fe^{3+} doping led to an increase in the bioactivity when tested in

SBF. The growth of the apatite layer on the samples surface has been confirmed by EDS analysis of the deposited layer. The antibacterial efficacy as determined against *E. coli*, *S. aureus*, and *S. epidermidis* by the agar diffusion test showed that this biomaterial will be useful in treatment of bone and joint infections. Hence, the proliferative potential for undoped and doped samples showed noncytotoxic by MTT assay in MG63 osteosarcoma cells. Sustained drug release systems developed in the present study would be a useful approach to treat malignant tumors, because it overcomes the impediments of treatment-induced drug resistance and nonspecific systemic toxicity.

Cobalt (Co^{2+}) doped HAp (Co-HAp) was synthesized using a combination of hydrothermal and microwave irradiation techniques. Incorporation of 57 ppm and 139 ppm of cobalt into HAp leads to the formation of cobalt oxide along with the HAp phase. The monodispersed spherical HAp particles (200 nm) were converted into interconnected porous (average pore size of 421 μm), structures by Co^{2+} (16.69 wt%) incorporation. At higher concentration of Co^{2+} (139 ppm) incorporation hexagonal elongated rods (75 x 24 nm) with spherical crystals (22 nm) were formed. The particle size was found to decrease with an increase in cobalt incorporation from 274.7 nm to 207.4 nm. The net negative surface zeta potential was increased with -10.4 mV to -5.27 mV. Increase in magnetic behavior of Co-HAp such as magnetization (M_s), retentivity (M_r), coercivity (H_c) and magnetic susceptibility (χ_g) for Co^{2+} incorporation. Co doping converts the specimen from diamagnetic to a superparamagnetic one. Increase in the dielectric property of Co-HAp may promote fracture healing. The 0.01Co and 0.2Co respectively showed sustained drug release and fast release with an anti-cancer drug. The Co^{2+} doping sample showed better drug release and exhibits higher electrical, magnetic properties than the iron doped hydroxyapatite sample, reported previously. The haemolytic assay showed that all the samples have good biocompatibility. Hence Co-HAp would be useful for bone growth, fracture healing and to treat the cancer cells due to its excellent magnetic properties.

The pure and Ni^{2+} doped HAp samples were synthesized by a combination of hydrothermal and microwave irradiation techniques. The XRD pattern revealed no phase change. The crystallite size and the degree of crystallinity were decreased with increase in Ni^{2+} concentration. FT-IR spectrum showed an enhancement of water and CO_2 adsorption due to high Lewis acid strength for the metal (Ni^{2+}, Co^{2+} and Fe^{3+}) doped samples. The pure and Ni^{2+} (0.01Ni, 0.05Ni and 0.1Ni) doped HAp samples showed agglomerated spherical morphology with reduction in particle size. Incorporation of Ni^{2+} (33 ppm) modifies the spherical structure to rod like morphology. The Ca/P ratio of the Ni^{2+} doped HAp was similar to that of the biological apatite (1.50 to 1.85). The dielectric constant was found to decline in Ni^{2+} on incorporation. The pure and 5 ppm incorporation of HAp showed diamagnetic behavior. Higher concentration of Ni^{2+} (>5 ppm) HAp sample exhibits paramagnetism. In addition, there was an increase in magnetic behavior for Ni^{2+} (>5 ppm) such as magnetization (M_s), retentivity (M_r) and coercivity (H_c).

The effect of swift heavy Si^{5+} ion (60 MeV) irradiation on nanosize Fe-HAp synthesized by hydrothermal technique was investigated. The crystallite size of Fe-HAp reduced on irradiation. The crystallinity and the lattice parameters decreased with an increase in ion fluences. Surface roughness of the Fe-HAp sample was increased by ion beam irradiation. The porosity was increased (300 to 360 ± 1 nm) with increase in ion fluences. The pores help to flow the body fluid into implants which enhances the cell attachments. In addition, the irradiated samples led to an increase in the bioactivity. The haemolytic assay reveals better blood compatibility for the irradiated samples. The antibacterial efficacy as determined against *S. aureus* by the agar diffusion test showed that this biomaterial will be useful in the treatment of bone and joint infections.

Rapid removal of fluoride ions from NaF solution using the nHAp sorbent was synthesized by hydrothermal followed by microwave techniques and characterized by XRD, confirms the HAp phase. The nHAp of 200 ± 5 nm in size had respective surface area and pore size of 34.37 m^2/g and 24.05 nm. Rapid DC (within 10 min) was observed for nHAp sorbent. The effect of concentration of fluoride on the nHAp was showed to decline in the percentage fluoride removal. Further, there was no significant change in DC in the pH range of 3-11 and in the presence of co-anions like Cl^-, NO_3^-, HCO_3^- and SO_4^{2-}, which might be due to the monodispersed nature of spherical nHAp particles. The nHAp sorbent showed 92% of fluoride removal capacity, 60% of regeneration and 70% of first cycle of fluoride adsorption process. In addition, the fluorinated HAp led to an increase in the bioactivity when tested in SBF. Therefore, the nHAp sorbent can be effectively used to remove the fluoride ions and also it has better efficiency to re-use for removal of fluorine ions. Hence hydrothermal followed by microwave technique is a better method for the synthesis of nHAp in the rapid (10 min) removal of fluoride ions.

7.2 SUGGESTIONS FOR FUTURE WORK

The evaluation of hyperthermia effect of Fe doped superparamagnetic nHAp may be studies. Further *in vivo* investigations would be performed in mice model. The *in vitro* heating efficiency of the material will be carried out by placing the sample in a thermally insulating surface within the excitation coil at room temperature for induction heating may be tested.

Synthesis of biomagnetized nanomaterials which have the potential to treat the malignant tumours could be carried out. The biological properties like blood compatibility, *in vitro* drug release, *in vitro* bioactivity through SBF, antibacterial efficacy and cell proliferation could also be studied.

Work to be extended to synthesize various adsorbents which have high efficiency to absorb toxic elements such as fluorine, arsenic, lead, etc., which are present in the ground water. The synthesized samples could be examined at various pH, temperature, dosage, contact time and the effect of co-anions to commercialize the environmental-friendly sorbents which prevent water pollution.