CHAPTER 6

SUMMARY AND SUGGESTIONS FOR FUTURE WORK

6.1 SUMMARY

Biomineralization is the process in which living organisms provides a chemical environment that controls the nucleation and growth of unique mineral phases. It generally involves, biologically produced materials, such as shells, bone and teeth, and the processes that lead to the formation of these hierarchically structured organic-ionorganic composites. In human body it produces both beneficial and harmful effects. Crystal deposition diseases may be defined as a pathological condition associated with the presence of crystals which contributes to the tissue damage and cause pain. On the other hand beneficial mineralization includes the formation of bone and teeth.

HAp is known to be the major constituent of bones and teeth. As a biomaterial, HAp has excellent properties such as bioactive, biocompatible and osteoconductive. Hence it has been used as a substitute or filling material, for tooth roots and bone defects. It also acts as a potential candidate for drug release. Calcium phosphates like calcium pyrophosphate and dicalcium phosphate dehydrate are also important biomaterials, since they act as precursors for HAp and are applied as coating materials for bones and teeth implants. HAp was partially converted to β-TCP and α-CPP. The degradation nature was high for the mixture of calcium phosphates (HAp, β-TCP and α-
CPP) when compared with pure HAp and hence it can be used as a biomaterial for bone tissue engineering. Due to the poor mechanical property, HAp could not be used for load bearing application. Hence metal ions and polymers are added to enhance the mechanical properties of HAp.

Incorporation of HAp with La$^{3+}$ ion inhibits dental caries and shows higher flexural strength, lower dissolution rate and excellent biocompatibility. Zn$^{2+}$ will promote bone formation when it was doped with HAp. The incorporation of La$^{3+}$ and Zn$^{2+}$ will enhance orthopedic and dental application. Fe$^{3+}$ is one of the most essential elements in the human metabolism and has been used for hyperthermia treatment of bone tumors. Ag doped HAp was used to fight against infections when it was used as an implant.

Nano rods (75-100 nm) of pure and lanthanum doped HAp was prepared by sol-gel method. XRD confirms there was no substitution of La$^{3+}$ into HAp. In addition, increase in crystallinity and crystallite size on doping was also confirmed. The surface area of the samples (~31 %) and hardness (upto 14 %) increased with the incorporation of lanthanum. The porous nature of these samples may enable the circulation of body fluids and nutrient, thereby increase its biocompatibility. The dissolution rate of HAp in PBS was hindered by the presence of lanthanum, which could be used to prevent the dental caries. Doping of lanthanum has extended the sustained release upto 76 h which could be used to prevent infections during surgery (implantation). While sintering, the HAp has partially converted to β-TCP and α-CPP, which will degrade faster than HAp and it can be used as a good biomaterial for bone tissue engineering. Sintering leads to interconnected pores (2-7 µm) which could allow blood capillaries to grow in and facilitate nutrient transportation when used as an implant. Hence the prepared material could be used as an implant in biomedical field due to its excellent properties such as bioactivity, increased surface area, hardness, crystallinity, crystallite size,
drug delivery, high resistance to Gram positive and Gram negative bacteria and lower dissolution rate.

Freeze dried composites of agarose, gelatin and HAp with microporous 3D as well as 2D lamellar structure scaffolds were fabricated without using crosslinking agents. The interaction of polymer backbone with the HAp lattice had shifted the characteristic HAp planes in XRD. In addition the presence of HAp and their interaction with the polymer scaffolds was confirmed by FTIR. Addition of HAp increase the tensile stress (68 %), elongation break (66 %) and Young’s modulus (89 %) for G1H sample. By varying the amount of HAp and polymer (agarose and gelatin), the swelling behaviour and the drug release can be regulated. The agarose scaffolds (A0.5H and A1H) showed extended drug release (5 days) which is more useful to prevent infection during implantation. Gelatin based drug free scaffolds showed efficacy against S. aureus and this may be due to the presence of gelatin. The prepared scaffolds have enhanced properties such as interconnected porosity, high mechanical property, high haemocompatible, extended drug release and resistance against Gram positive bacteria. Due to these properties the prepared scaffolds could find potential application in tissue engineering.

Nanosized HAp with c-axis orientation was mineralized in a photopolymerized polyacrylamide gel matrix. The c-axis oriented growth of HAp would enhance the absorption of acidic proteins. Self assembled laminated structures (which resembled the natural bone) embedded with nano sized HAp sphere and flake like structure on the polymer matrix and interconnected porous network and laminated structures were produced for room temperature dried and freeze dried samples. The enlargement of pores with an increase in pH of the precursor solution was noted. Hence, this process could be used to engineer the pore size by varying the pH of the
solution. The presence of nano pores in the samples could be made use of to design drug delivery systems. The flake like structure showed excellent bioactivity, whereas resorption was noted in laminated structure that augments applicability of the present process to synthesize natural bone like biomaterials. In addition, polyacrylamide gel is an excellent alternative to silicone gel, owing to the minimum risk of fibrosis and migration. Controlled and extended (6 days) drug release was achieved for 0.4Mp8 and 0.4Mp10F samples. The antibacterial efficacy against S. aureus was high for the samples dried by both methods. The presence of various amount of calcium and phosphate have regulated the swelling behaviour of the samples, and it could have a beneficial effect on osteoblast growth and differentiation. The presence of interconnected porous structure on the freeze dried samples could be used as a scaffold for tissue engineering and drug delivery application.

HAp and DCPD was mineralized in the photopolymerized polyacrylamide gel matrix at physiological condition. Increase in iron concentration (0.01-0.1M) leads to the formation of pure HAp. Closely packed rod shaped crystals of length ~ 20 µm and thickness ~ 5 µm as well as laminated structures with pores ~ 15 µm was observed on the control sample. The interconnected pores (2.5-10 µm) along with spheres (1-2.5 µm) were produced on the polymer matrix for doped samples. This could be used as scaffold in tissue engineering and drug delivery system. Controlled and the extended release of 52 h (EG, Fe0.01HAp and Fe0.05HAp) and 73 h (C) was achieved. Presence of Ag, Zn and Fe on the polymer matrix was confirmed by EDX analysis. AgHAp polymer composites showed efficacy against bacterial strain, therefore it can be used as an antimicrobial agent.
6.2 SUGGESTIONS FOR FUTURE WORK

Investigations could be carried out on synthesizing HAp with trace amount of rare earth elements (Eu$^{3+}$, Tb$^{3+}$ etc) to explore their influence as a biological probe and interaction with proteins. Further, in vitro drug delivery may be studied using different combination of drugs (antibiotic and anti-cancer) for local drug delivery system.

Work can be extended to fabricate HAp based polymer composites with and without using cross-linking agent, different combination of polymers and by biomineralization method having excellent mechanical properties, drug release, in vitro biocompatibility, bioactivity and non-toxicity.

The effect of dopants on the mineralization of HAp in polymer matrix may be studied by varying pH, concentration and temperature of the precursor solutions and it can be dried using freeze drier. Their mechanical, drug release, in vitro biocompatibility, bioactivity and non-toxicity was also carried out.