Chapter 8 Over all conclusions

- The main spirit of the research work is to enhance the properties of parent bioactive glass (45S5) without major alteration of the base glass composition. The bioactive glass system SiO_2–Na_2O–CaO–P_2O_5 was substituted with barium oxide in small quantities at the cost of silica and it has been successfully prepared. The bioactive glasses showed a decrease in glass nucleation and crystallization temperatures with increasing BaO content. Thermally treated base bioactive glass and barium substituted glasses have shown the main crystalline phases as $Na_2Ca_2Si_3O_9$. The XRD analysis of the bioactive glass before immersion in SBF revealed the amorphous nature of the glass. The FTIR spectrometry confirmed the presence of SiO_4 tetrahedra in the silicate glass network. It has been demonstrated that the substitution of barium decreased the glass network connectivity and thus increased the glass dissolution which caused for the higher pH. The *in vitro* bioactivity in SBF had shown the formation of hydroxyl carbonate apatite layer on the surface of the base and barium contained bioactive glasses as confirmed by FTIR transmission spectrometry, pH behavior, XRD, SEM and EDS analysis. Moreover, the barium contained bioactive glass samples had shown superior bioactivity and higher HCA layer formation in comparison with reference to control sample. The density, compressive and flexural strengths were improved with an increase in BaO content in the base bioactive glass. Further, the Young's, bulk and shear modulus of the samples have also shown similar trends. The *in vitro* cell culture studies like cell viability and cytotoxicity results have shown that the substitution of barium in the bioactive glass is compatible with human osteosarcoma U2OS cell lines and did not cause for cell apoptosis. It was also observed that the cells were proliferating. The U2OS cells were found to be significantly attached and grown on the blocks of barium contained

glasses as compared to control sample which was confirmed by SEM, EDS and AFM techniques. Moreover the barium contained glasses have not caused for the blood hemolysis of RBC and they were also compatible with WBC. It was demonstrated that these glasses did not cause for blood platelet aggregation and hence do not form thrombus. It has been demonstrated that the amount of ions released from the glasses was phagocytosed by human macrophages but it was more prominent in barium contained bioactive glasses. Further, the substitution of barium has improved the radiopacity of the glasses which has a significant advantage during clinical surgery. The *in vivo* implantation of Ba-0 and Ba-3 samples in rat femur bone had exhibited the bone healing with increasing time but it was more active in barium contained BG as confirmed from X-ray radiographic images. The *in vivo* complete blood analysis (CBC) has also revealed that the base and barium contained BG did not affect on blood significantly. In view of the above results, the barium oxide can easily be substituted in base bioactive glass. The prepared barium contained bioactive glasses are promising for bone substitutes in biomedical applications.

- The present work states that the substitution of SrO for SiO_2 has a major advantage over CaO. The density of the glasses increased with increasing concentration of SrO for SiO_2 while the network connectivity decreased in glass. It has shown significant effect on bioactivity, cytocompatibility and mechanical behavior. All the bioactive glasses exhibited HCA layer formation on their surfaces in the presence of SBF as confirmed by XRD, SEM and EDS. Whereas, the low NCB glasses have shown a significant enhancement in HCA crystallinity as compared to that of SrO substituted for CaO in reference bioactive glass. The elastic moduli of the bioactive glasses have increased significantly as the concentration of strontia increased for silica. The cell culture studies in the presence of low NCB glasses were

found to exhibit better cell compatibility, significant cell growth and enhanced human blood compatibility as compared to reference glass. Thus, it is expected that these bioactive glasses are potentially applicable to clinical trials in bone regeneration and tissue engineering.

- It can be concluded from the results that the substitution of MgO for SiO_2 has significantly effected on glass network. Further, the compressive strengths of the bioactive glasses were found to increase with increasing concentration of magnesia for silica. All the bioactive glasses have possessed the HCA layer formation in SBF as confirmed by pH behavior, FTIR, XRD, SEM and EDS analysis, but it was more prominent in the new bioactive glasses where SiO_2 was partially replaced by MgO. The low NCB glasses exhibited better cell compatibility and growth of human osteosarcoma U2OS cells as well as human blood compatibility as compared to reference glass sample (Mg-1). Furthermore, the cells were found to attach and grow on the surface of the bulk samples significantly. The present work demonstrated that the substitution of MgO for SiO_2 has a significant benefit over CaO. Thus, these bioactive glasses are proposed herewith to be potential material for bone regeneration.
- It can be concluded for the results that the bioactive glasses has been successfully prepared by sol gel route with an introduction of Ag_2O. The particle size and distribution was found to increase with an increase in Ag content in the bioactive glass samples. All the sintered samples have exhibited the sodium calcium silicate phase while Ag-1 and Ag-2 samples revealed an additional phase of metallic Ag which indicated that the metallic silver was successfully embedded in the glassy matrix as confirmed by XRD and SEM-EDS techniques. During substitution of Ag_2O in the bioactive glass, the Ag was found to present in both the forms as in metallic and ionic states. Further, the porous scaffold was fabricated successfully using sucrose as

pore forming agent. The scaffolds have shown good porosity of 68.33 to 57.45% for Ag-0 and Ag-2 samples, respectively and the pores size was found in range of 140 – 700 μm. The compressive strength of the scaffold was found to increase from 2.4 to 18.3 MPa with increasing concentration of Ag_2O in the BG. The *in vitro* bioactivity in SBF demonstrated the formation of HCA layer on the surface of the samples as confirmed by FTIR spectrometry, SEM, XRD and pH behavior. Moreover the HCA layer was more prominent in Ag contained samples. The *in vitro* cell culture studies like cell viability, cytotoxicity and proliferation demonstrated that the scaffold samples were tolerant to the human osteosarcoma U2-OS cell lines and thus the substitution of Ag2O did not cause for cells cytotoxicity and allowed the cell growth. Further, the cells were significantly attached and grown on the surface and porous area as well as pore walls of the Ag-2 BGC sample. The elemental mapping on the Ag-2 scaffold had shown the presence of Ag and its distribution in the sample even after culture for 5 days. All the samples demonstrated good human blood compatibility with RBC and WBC while Ag-2 BGC sample diminished the blood platelet coagulation as compared to Ag-0 BGC sample. Moreover, the Ag-2 scaffold sample exhibited good antibacterial activity against *E.coli* bacteria which could be due to the release of Ag^+ ions from the sample. The *in vivo* X-ray radiographic results of Ag-2 scaffold had exhibited the bone healing after 30 days of implantation in rat femur bone. The Ag-contained bioactive glass-ceramic scaffold had demonstrated the multifunctional properties as described above. Therefore, in view of all respects, the prepared scaffold can be used as an implant for bone tissue engineering