Preface

A major challenge is posed for regeneration of bone defects generally caused due to infections, trauma, accidents, tumors or genetic malformations in the human body. It necessitates the need for effective materials of bone regeneration and tissue engineering capability. Bioactive glasses are able to form interfacial bonds with living organisms of hard and soft tissues by their degradation in physiological solutions and formation of a stable hydroxyl-carbonate apatite (HCA) layer on the glass surface. The SiO_2–Na_2O–CaO–P_2O_5 glass system has higher bioactivity in comparison to HA. Moreover, the bioactive glass (45S5 bioglass®) has been studied extensively and is being used as bone regenerative material in orthopaedic and dental applications. This made me to use the SiO_2–Na_2O–CaO–P_2O_5 glass system with substitution of some therapeutic ions to enhance their regenerative properties.

Chapter 1 consists of a brief introduction and literature review about the regions of bioactive glass compositions and types of bioceramics according to tissue attachments. Further, bioactive glasses are paying more attention towards dental and orthopaedic applications as well as tissue engineering, I also understood about the general glass composition and its structure as well as the role of network formers, modifiers and intermediates in glass and how these parameters play an important role in bioactive glasses. The relation between glass network connectivity and bioactivity was clearly understood. The mechanism of bioactivity, HA formation and bone bonding in the bioactive glass was discussed. Moreover, the detailed literature review was done about the bioglass® and the substitution of barium, strontium, magnesium and silver oxides in the bioactive glass as well as how these oxides influence on the bioactivity and biological performances

Chapter 2 presents the experimental procedures which were used in the present research work such as preparation of bioactive glasses through melting and sol-gel routes as well as preparation of SBF. The prepared bioactive glasses were characterized by different techniques like DTA/TGA, XRD, SEM and EDS. The physico-mechanical properties such as density, flexural and compressive strengths were performed using the samples. The *in vitro* bioactivity and the formation of HCA layer was assessed by immersing the samples in SBF for various time periods under physiological conditions. Further, the *in vitro* cell culture studies like cell viability, cytotoxicity and proliferation and apoptosis were performed. The in vivo implantation of the samples was carried out in a rat femur bone**.**

Chapter 3 reports the objective of my research work as it was observed from the literature that the substitution of MgO and SrO in the bioactive glasses retard the HCA formation in SBF. The formation of HCA is highly dependent on kinetic dissolution and precipitation of calcium and phosphate ions. The MgO and SrO are bone seeking ions which play a vital role in bone remodelling. Further, the formation of biofilm (bacteria) on the implants surface and its integrity with tissue cells led to failure of implant. Therefore, it was aimed to contribute towards better understanding of different bioactive glass systems and to explore the new bioactive glass compositions. It was also aimed to study the process of reactivity and assess their physico-chemical, bioactivity, biological and mechanical behaviours of the present bioactive glass systems.

Chapter 4 demonstrates about the barium substitution in BG. Barium with low concentration in the glasses acts as a muscle stimulant and also it was found in human teeth. I have made a preliminary study by substituting barium in the bioactive glass. The prepared glasses were characterized to determine their use in biomedical

applications. The nucleation and crystallization regimes were determined by DTA and the controlled crystallization was carried out by suitable heat treatment. The crystalline phase formed was identified using XRD technique. Bioactivity of these glasses was assessed by immersion in simulated body fluid (SBF) and formation of HCA for various time periods. The formation of hydroxy carbonate apatite (HCA) layer was identified by FTIR spectrometry, scanning electron microscope (SEM), EDS and XRD which showed the presence of HCA as the main phase in all bioactive glass samples. Mechanical strengths and densities of bioactive glasses have been measured and found to increase with increasing the barium content. *In vitro* cell culture studies such as viability and cytotoxicity, proliferation and cell apoptosis of human osteosarcoma U2-OS cell lines were carried out in presence of these samples at different concentrations which were found to be biocompatible. Further, it was demonstrated that there was a significant cell attachment and growth on the surface the samples as confirmed by SEM and AFM techniques. The human blood compatibility like hemolysis, RBC and WBC was assessed. The blood platelet aggregation study was also evaluated. The samples were incubated with human macrophages to determine the phagocytosis behavior and it was found to be more active in barium contained bioactive glasses. In addition to this, the substitution of barium in the base bioglass® has an advantage that it improves the radiopacity of the glasses. The *in vivo* implantation of the samples in rat femur bone exhibits the bone healing with increasing time as confirmed by X-ray radiographic images. The *in vivo* complete blood analysis (CBC) has also been evaluated at different time periods. Therefore, the prepared barium contained bioactive glasses can be used in orthopedic and dental applications.

Chapter 5 Strontium contained biomaterials have been reported as a potential bioactive material for bone regeneration, as it reduces bone resorption and stimulates

bone formation. In the present investigation, the bioactive glasses were designed to partially substitute SrO for SiO_2 in Na_2O–CaO–SrO–P_2O_5–SiO_2 system. This work demonstrates that the substitution of SrO for SiO2 has got significant benefit than substitution for CaO in the bioactive glass. Bioactivity was assessed by the immersion of the samples in simulated body fluid for different intervals. The formation of hydroxy carbonate apatite layer was identified by X-ray diffractometry, scanning electron microscopy (SEM) and energy dispersive spectroscopy. The elastic modulus of the bioactive glasses was measured and found to increase with increasing SrO for SiO_2. The blood compatibility of the samples was evaluated. In vitro cell culture studies of the samples were performed using human osteosarcoma U2-OS cell lines and found a significant improvement in cell viability and proliferation. The cell attachment and growth on the bioactive glass was performed using U2-OS cell lines. The investigation showed enhancement in bioactivity, mechanical and biological properties of the strontia substituted for silica in glasses. Thus, these bioactive glasses would be highly potential for bone regeneration

Chapter 6 depicts the effect of magnesium substitution in BG. Mg plays an important role in human skeletal system as it stimulates the bone formation and reduces bone resorption. Magnesium has been substituted into SiO_2–Na_2O–CaO–P_2O_5 bioactive glass. In the presents work, the bioactive glasses were designed to enhance bioactivity and the bioactive glasses were prepared by melting route as well as analyzed for their use in biomedical applications. Bioactivity of these glasses was assessed by the immersion of the samples in simulated body fluid (SBF) for different time periods. The formation of hydroxy carbonate apatite (HCA) layer was identified by FTIR spectrometry, scanning electron microscopy (SEM) and X-ray diffractometry (XRD) which had shown the presence of HCA as the main phase in all the samples. Human

blood compatibility of the samples was evaluated by hemolysis which demonstrated better compatibility with blood. *In-vitro* cell culture investigations, viability, cytotoxicity, proliferation and apoptosis of the sample were studied using human osteosarcoma U2-OS cell lines. The Young's modulus and compressive strength of glasses were measured and found to increase with increasing MgO content at lower concentration. Therefore, the new bioactive glasses containing magnesium have demonstrated improved bioactivity and better biocompatibility and hence proposed to use in bone regeneration applications.

Chapter 7 reports the multifunction properties of silver contained bioactive glasses. The bioactive glass (BG) samples containing chemical composition (19-X) Na_2O –23CaO –5SrO –50SiO_2 –3P_2O_5 (where X= 0.0, 1.0 and 3.0 mol% of Ag_2O) were prepared by sol-gel route. The particle size and distribution of the prepared BG powders were studied and reported that the size of particles increased with increasing Ag_2O in the bioactive glass. The thermal behaviors of the prepared samples were analyzed by DTA/TG techniques and these samples were sintered at different temperatures. The formation of crystalline phases was analyzed using XRD and sodium calcium silicate and silver phases were found. Further, the 3 D scaffold was fabricated with an addition of sucrose as pore forming agent. The morphology and porous structure as well as pore size of the scaffolds were characterized by SEM. The compressive strength of scaffolds was found to increase with increasing the concentration of Ag_2O from 2.4 to 18.3 MPa. The porosity of the scaffolds was found in the range of 68.33 to 57.45% for Ag-0 and Ag-2 samples. Bioactivity of the scaffold samples was assessed by the immersion of the sample in SBF. The mechanism of bioactivity and formation of HCA layer was confirmed by pH behavior, FTIR, SEM and XRD techniques. *In vitro* cell culture studies such as viability and cytotoxicity,

proliferation and cell apoptosis of human osteosarcoma U2-OS cell lines were carried out in presence of these samples which were found to be biocompatible. Further, it was also found that there was significant cell attachment and growth on the surface and porous area of the Ag-2 scaffold. The human blood compatibility of major components like RBC and WBC with the samples was assessed and found that these samples were non hemolytic. The blood platelet aggregation study was also evaluated. The scaffolds were subjected for antibacterial effect against *E.coli* bacteria. A major surgery was performed in rat femur bone where Ag-2 scaffold was implanted and the X-ray radiographic images were taken at different time periods to assess the healing. The prospect of the above properties of the scaffold makes it a good potential in the area of bone tissue engineering.

Chapter 8 consists of the overall conclusion of the entire work done as mentioned chapter wise. Further, it has been finally added with the references and list of publications.

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