

Chapter 8

Summery and future scope

This thesis deals with the preparation of some therapeutic ions (copper, zinc, and strontium) incorporated silicate-based 1393 and borate-based 1393B3 glass scaffolds mimicking trabecular bones' architecture and the in vitro and in vivo evaluations aiming their applicability for bone tissue regenerative applications.

Herein, a summary of **Chapter 1** to **Chapter 7** has been delineated in brief.

In Chapters 1, 2, and 3, a comprehensive literature survey, objectives, and detailed materials and methods were elaborated.

In chapters 4-7, the silicate and borate-based bioactive 1393 and 1393B3 glass scaffolds were prepared by incorporation with ZnO, CuO and SrO in order to introduce the therapeutic properties into the scaffolds. The derived scaffolds were investigated by a series of experimentation (both in vitro and in vivo) to ensure any improvements in properties. The ZnO and SrO derived 1393 glass scaffolds (BG and ZnBGs; chapter 4 and chapter 6) were prepared by the most versatile sol-gel route and foam replication by using PU foams as sacrificial template materials, whereas, CuO derived 1393 and 1393B3 glass scaffolds (chapter 5 and chapter 7) were prepared my melt quench route, followed by foam replica. All of the derived scaffolds after immersion into simulated body fluid were evaluated by their structural (XRD), functional (FTIR), and morphological (SEM and EDX, and EDS mapping) characterizations to assess HA like layers formation. Also, the mechanical performance of these derived scaffolds comparing the base systems were evaluated in both 'dry (as prepared)' and 'wet (immersed in SBF)' state (for SrO derived 1393 and CuO derived 1393B3) to ensure their mechanical reliability as implant materials. The

biocompatibility of the derived scaffolds was assessed by various cell lines i.e., mouse fibroblast **L929**, human osteosarcoma **U2OS**, normal embryonic cells (mouse) **NIH/3T3**, human squamous carcinoma **SCC-25**, mouse bone marrow derived stromal cell **mBMSCs**, and **lymphocytes** and **monocytes** to ensure their (scaffolds) minimal cytotoxicity, hemolysis and apoptosis.

Additionally, the ZnO derived scaffolds were analyzed for active bone formation capability by ALP activity and osteogenic gene expressions in vitro. Further, the in vivo bone healing investigations were performed in CuO derived 1393B3 (CBBGs) glass scaffolds in rat bone defect model. However, the conclusions of the individual chapter followed by summary table that includes salient features have been described as follows.

Summary of chapter 4

Herein, a comparative analysis between the ZnO incorporated 1393 bioactive glass scaffolds (ZnBGs) and the BG (pure 1393 bioactive glass scaffolds) was performed to examine the effect of ZnO (in ZnBGs) on the mechanical and biological properties. We observed significant improvements in cellular viability, cell metabolism, and a distinctive cell adhesion morphology in Z3BGs than the pure BG. Moreover, a multifold increase in ALP activity, and osteogenic gene expression suggests enhanced osteogenic differentiation of the BMSc (mouse bone marrow stromal cells) in ZnBGs. However, the Z3BG showed optimal compression and flexural strengths, and bending modulus in comparison to other BGs.

Summary of chapter 5

The in vitro biological and mechanical properties of the copper containing 1393 bioactive glass scaffolds were examined and compared with the pure 1393 bioactive glass system.

The cell culture study illustrates enhanced cellular proliferation and viability up to 2% CuO incorporated glass (1393-2Cu). Moreover, the CuO-assisted 1393 glass system exhibited enhanced blood compatibility and minimal cell lysis up to 2% CuO in the glass system. Therefore, the 1393-2Cu seems an excellent biomaterial that could be used in hard tissue reconstructive applications after further elucidations.

Summary of chapter 6

Herein, the intense XRD peaks in the SrO substituted bioactive glasses indicate the strontium apatite formation [Sr-HCA; $\text{Ca}_{(10-x)}\text{Sr}_x(\text{PO}_4)_{6-y}(\text{CO}_3)_y(\text{OH})_2$] on the soaked (in SBF) samples. However, the Sr apatite layer seems to have augmented the biological compatibility (cellular metabolism, and live/dead assay, cell adhesion) of the scaffolds. The SrO derived scaffolds exhibited significant improvements in compressive and flexural property as well, compared to the S1. However, the enhanced cellular metabolism, cell survivability and cell adhesion were observed up to 5% SrO substituted glass (S2), than the pure 1393 glass (S1) or other SrO derived glasses (S3, S4, and S5). Henceforth, considering their negative side effects and global concern, a tolerable limit could be introduced in the bioactive glasses to promote therapeutic potential of the biomaterials.

Summary of chapter 7

The fabricated borate based 1393B3 glass derived scaffolds (CBBGs and pure BBG) were characterized and analyzed to determine the property enhancements after CuO addition into the BBG. However, the results illustrate that, the CBBGs exhibited enhanced cellular

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metabolic activity, cell adhesion, and cell viability and minimal cell lysis than that of BBG. Radiology and histology of bone also suggests augmented bone healing potential of BBGs compared to pure BBG.

Summary table

Title of the investigation	Salient features
<p>Chapter 4: ZnO derived bioactive 1393 glass scaffold with enhanced biocompatibility and osteogenesis for neo-bone tissue regenerative application</p>	<ol style="list-style-type: none"> 1. ZnO incorporated 3D porous 1393 bioactive glass scaffolds (ZnBGs, and pure BG) were successfully prepared by sol-gel technique followed by foam replica 2. The Z3BG exhibit significantly enhanced cellular viability and cell metabolism, and a distinctive cell adhesion morphology than the pure BG. 3. Optimal mechanical properties (compression and flexure, and bending modulus) was also observed In Z3BG. 4. A multifold increase in ALP activity, and osteogenic gene expression suggests enhanced osteogenic differentiation of the BMSc (mouse bone marrow stromal cells) in ZnBGs.
<p>Chapter 5: Studies on effect of cuo addition on mechanical properties and in vitro cytocompatibility in 1393 bioactive glass scaffold</p>	<ol style="list-style-type: none"> 1. The CuO incorporated glass, specially (1393-2Cu) showed optimal cellular proliferation and minimal cytotoxicity. 2. The 1393-2Cu exhibited enhanced blood compatibility and minimal blood cell lysis in comparison to other glass system 3. CuO addition in the glass has significantly enhanced the mechanical properties.
<p>Chapter 6: SrO assisted 1393 glass scaffold with enhanced biological compatibility</p>	<ol style="list-style-type: none"> 1. Enhanced bioactivity was observed due to plausible strontium apatite [Sr-HCA; $\text{Ca}_{(10-x)}\text{Sr}_x(\text{PO}_4)_{6-y}(\text{CO}_3)_y(\text{OH})_2$] formation on the soaked (in SBF) samples 2. Augmentation in cellular viability, and live/dead assay, cell adhesion indicates enhanced cytological compatibility in the SrO incorporated 1393 bioactive glasses, particularly in S2. 3. A significant improvements in compressive and flexural property in the SrO assisted system (S2, S3, S4, and S5) compared to the S1 (pure 1393 bioglass)
<p>Chapter 4: Assessment of CuO assisted 1393B3 on in vitro biological and mechanochemical performance, and in vivo bone healing potentiality in rat defects model</p>	<ol style="list-style-type: none"> 1. The CBBGs, especially C2BBG exhibited enhanced cellular metabolic activity, cell adhesion, and cell viability and minimal cell lysis than that of BBG 2. C2BBG showed better bone healing than the control or pure BBG, confirmed by the radiological and histological examination of the bones in the bone defect model.

Future Scope and area of development

- ➔ Additional in vivo studies of these derived scaffolds for better assurance of their bone tissue regenerative potentiality.
- ➔ Novel approach to further improve mechanical performances of the scaffolds.
- ➔ Investigation of osteogenesis and angiogenesis potential of the scaffolds.
- ➔ Clinical trials

We observed in these investigations that the 1393/1393B3 bioactive glass (BG) is an excellent biomaterial and they can be converted to hydroxyapatite (the mineralogical component of bone and teeth) and tailored to the natural bones. However, due to the brittleness, we cannot achieve the desired mechanical strengths just by using bioglasses only. Therefore, the 1393/1393B3 BG if combined with biopolymers (like collagen, silk fibroin etc.), their synergistic efforts shall reinforce the composite structure (due to anisotropic behavior of polymers and enhance the mechanical properties, especially the fracture toughness. It is noteworthy to mention that the bone is also a composite of bone minerals and collagen. Furthermore, the therapeutics elements (i.e., trace elements found in our body; in drug-laden biomaterials the vancomycin hydrochloride could be considered) will also facilitate in promoting osteoblastogenesis (neo bone formation) and angiogenesis (blood vessel formation), and enhance physicochemical properties in the biomaterials. Moreover, both the bioactive glasses (1393/1393B3) and biopolymers (collagen/ silk fibroin), due to their pro-osteogenic characteristics, shall provide an optimal microenvironment to promote differentiation of the stromal (multipotent mesenchymal stem) cells towards the required lineage. Furthermore, the BG/polymer composite fabrication by advanced fabrication techniques like additive manufacturing (3D printing by FDM-fused deposition modelling; or SLS-selective laser sintering) will ensure desired microstructure and enhance mechanical strengths due to their precise, controllable computer aided fabrication processing. Upon successful preparation and in vitro characterizations, the BG/ polymer composite biomaterials shall be subjected to in vivo studies followed by clinical trials.