Chapter 1

# Introduction and literature review

Materials used as medicine was reported to be recognized after the first successful aseptic surgery by Dr. Joseph Lister in the 1860s (Kuhn, 2012, Worboys, 2013, Cartwright, 1963, Stanton, 2012). However, the biomaterials used in biomedical applications in recent times were not known to people about 80 years ago (Ratner, 2004), yet some materials in the form of crude biomaterials with poor results have been reported to be used (Ratner, 2004).

Biomaterials are any natural or synthetic substances that have been engineered to interact with the biological systems in order to treat diseased or damaged tissues, organs or functions. According to Williams in 'definitions in biomaterials' (Williams, 1986) and 'dictionary of biomaterials' (Williams, 1999), biomaterials are nonviable materials used in medical devices intended to interact with biological systems.

Biomaterials can be purely ceramic, polymer or metal based, and also composites based (Raghavendra et al., 2015, Axinte et al., 2019, Ali et al., 2018). Prof. L. L. Hench, classified materials broadly into four categories according to their material tissue interactions and biocompatibility i.e.; (i) Toxic (ii) Bioactive (iii) Inert and (iv) Resorbable (Hench et al., 1971). However, according to Prof. Hench, there are no such materials as 'absolute inert': all elicit a response from the host tissue upon implantation (Hench et al., 1971). Due to the high mechanical strengths and nearly bioinert nature, some metallic implants were initially considered as natural bone grafts substitute (Schalock and Thyssen, 2013), yet the material-tissue incompatibility related complications like hypersensitivity, allergy, and inflammation (Teo and Schalock, 2017, Basko-Plluska et al., 2011, Rostoker et al., 1987, Merle et al., 1992, Munro - Ashman and Miller, 1976, Wang et al., 1997) restricted them from conventional (i.e., ceramic and polymeric) biomaterials (Wang et al., 1997, Kazantzis,

1978, Puleo and Nanci, 1999). Even the so-called biocompatible titanium implants have provoked unwanted immune responses in metals on metals (MOM) joint prostheses, and total hip arthroplasty (THA), are a few of the examples of the drawbacks of metallic implants (Wang et al., 1997, Kazantzis, 1978, Black et al., 1990). Nevertheless, polymeric biomaterials have enormous contributions in tissue engineering (TE) applications, but repairing or replacing hard tissues with polymeric materials is very challenging since they always lack in mechanical strengths due to their high degradable tendency.

Nevertheless, ceramic biomaterials, particularly bioactive glasses apart from *Autografts* and *Allograft (*gold standard for bone grafts) (Liu et al., 2013, Bi et al., 2013), are so far the best synthetic grafting material because they are biologically compatible and can be converted to hydroxyapatites, the mineralogical component of 'bone and teeth' and also can be tailored to the architecture of the physiological organ to mimic natural tissues (bone, skin, etc.) (Ali et al., 2018, Ali et al., 2020a, Ali et al., 2020b, Liu et al., 2013). A revolution has occurred during the last 60 years to improve human life quality using ceramic based biomaterials. Since then, ceramics have continuously been contributing to repairing diseased and damaged parts of the body. Such ceramics, which have gained significant trust in the betterment of human lives by their considerable contribution to tissue engineering applications, are known as bioceramics. Some clinical achievements of bioceramics include successful repairing and augmentation of the skeletal system, bone joints, damaged teeth, and other hard and soft tissues. Ceramics were also used to replace parts of the cardiovascular system, especially heart valves. Special formulations of bioglasses were also used therapeutically for the treatment of tumors.

When implanted, bioceramics show material-tissue interactions followed by the formation of a strong, compliant interface between the host tissue and the material. The quality of the

interface indicates the biocompatibility of the bioceramics. The soft and hard tissues attachment to the materials is governed by the quality of the interface formed due to material tissue interactions (Hench, 2013).

However, bioactive glasses are the most important bioceramics for tissue engineering applications. The discovery of bioactive glass, was a breakthrough for the ceramic-driven biomaterials. The need for bioactive glasses was perceived when a Vietnam war returnee and Prof. Hench had a conversation about the necessity of such a material that acts like normal bones (Hench, 2006) to avoid the complexity of polymeric and metal implants and their substantial rejection. After almost two years of research, Prof. Lary L. Hench and the team discovered 45S5® bioactive glass, also known as Hench glass at the University of Florida, US, in 1969. Since then, bioactive glasses/ scaffolds have triggered a revolution in tissue engineering applications. However, bioactive glasses are known for their ability to elicit a specific response from the host tissue and form a strong, compliant bond at the material-tissue interface. The bioactivity of such biomaterials is ascribed by the formation of biocompatible HCA (hydroxycarbonated apatite) layer on the surface by a series of reactions while remain in contact with the physiological fluid.

The reactions involve rapid ion exchange (generally $Ca^{2+}$, $Na^{+}$ etc.) from the glass surface to the solution and increase the basicity of the solution. The basic solution easily attacks on the glass network to form silanols (SiOH) (for silicate glasses). Condensation and polymerization of silanols to form amorphous silica rich layer. Accumulation of the species (i.e., $Ca^{2+}$ and $PO_4^{3-}$) on the silica rich layer forms ACP (amorphous calcium phosphate). Gradual crystallization of ACP turns to crystalline HCA (hydroxycarbonated apatite). The reactions are as follows

(i) Ion exchange:
$Si - O - Na^+ + H^+ + OH^- \rightarrow Si - OH + Na^+ (solution) + OH^-$

(ii) Formation of silanols:
$Si - O - Si + H_2O \rightarrow Si - OH + OH - Si$

(iii) Condensation and polymerization:
$OH - Si - OH + OH - Si - OH \rightarrow OH - Si - O - Si - OH + H_2O$

(iv) Accumulation of species (i.e., $Ca^{2+}$ and $PO_4^{3-}$) on $SiO_2$ rich layer:
$Ca^{2+} + PO_4^{3-} \rightarrow Ca_3(PO_4)_2$

(v) Crystallization of ACP by incorporation of $OH^-$, $CO_3^{2-}$ anions from the solution to form crystalline HCA layer:
$Ca_3(PO_4)_2 + OH^- + CO_3^{2-} \rightarrow Ca_{10}(PO_4)_{6-x}(OH)_2(CO_3)_x$

This HCA is the mineralogical component of bone. However, the HCA can be in other forms when calcium combined with other elements i.e., $[(Ca, M)_{10} (PO_4, CO_3, Y)_6 (OH, F, Cl)_2]$ (where, M=Sr, Fe, Zn etc).

In addition to Hench glass (45S5®), the other important bioactive glasses which are widely used in soft and hard tissue reconstruction and regeneration are 1393 and 1393B3 bioactive glasses.

Although both 45S5® and 1393 are silicate-based bioactive glasses, the 1393 bioglass contains higher percentage of silica with two additional network modifiers and some other preloaded qualities. 1393 glass typically composed of 53% $SiO_2$, 20% CaO, 6% $Na_2O$, 4% $P_2O_5$, 12% $K_2O$ and 5% MgO (wt%). Unlike 45S5®, 1393 cannot be easily transformed into a crystalline form from their glassy state, due to their lesser crystallization tendency during sintering. The tendency of retention of glassy nature in 1393 is due to the presence

of MgO into the glass network. Nonetheless, 1393 can be pulled easily into particles or short to long range fibers without devitrification and it has better bioactivity than that of 45S5® (Fu et al., 2008). Literatures also suggest that 1393 bioactive glass is one of the most commonly used bioglasses for clinical trials (Baino et al., 2018). Both 1393 and 45S5® bioglasses have been approved for the in vivo use respectively in Europe and US (Rahaman et al., 2011).

However, the 1393B3, also known as cotton candy (traded name DermaFuse™/ Mirragen™), has excellent wound healing ability (Baino et al., 2018). The cotton candy name was given after the incident when a registered nurse Peggy Taylor, who was also a wound care specialist, accidentally applied 1393B3 onto a stubborn wound due to their cotton-like appearance (Wray, 2011). Miraculously, the stubborn wound had been healed after applying the 1393B3 bioglass.

The $B_2O_3$ is the glass network former for borate based 1393B3 glass like $SiO_2$ in the silicate glasses. While both being network formers, they differ by their viscosity (as melt) and crystallization tendency. Borate glasses have relatively facile viscous flow behavior than silicate glasses at liquidus temperature; therefore, they have a higher devitrification tendency than silicate based glasses (Schmelzer et al., 2005). Whatsoever, both the glasses different compositions and properties, and therefore, they have different application areas.

The typical chemical composition of 1393B3 is $SiO_2$-56.6%, CaO-18.5%, $Na_2O$-5.5%, MgO-4.6%, $K_2O$-11.1%, $P_2O5$-3.7% (wt. %) (Rahaman et al., 2011). The 1393B3 glass follows the reaction kinetics of 45S5 and converts to glass-ceramics when sintered (Fu, 2009). Unlike the silicate based 1393, the borate 1393B3 cannot be easily drawn to glass fibers and form scaffolds until it is converted to crypto-crystalline form by means of control

heat treatment (Jung and Day, 2009). Due to the faster degradation and conversion to bone minerals (HA; $Ca_{10}(PO_4)_6(OH)_2$), borate based 1393B3 is considered as another crucial bioceramic for tissue engineering (TE) application (Rahaman et al., 2011, Huang et al., 2006). Besides, the 1393B3 glasses leave no leftover like silica rich residual layer (like silicate glasses) when converted to bone minerals (Bi et al., 2013, Huang et al., 2006, Yao et al., 2007).

Bioactive scaffold is, however, a porous enough template that allows bone and tissue to grow inside the pores (osteogenesis and angiogenesis) through 3D interconnected porous struts by means of osteoinduction, osteoconduction, and osseointegration. An ideal bioactive glass scaffold prepared for bone tissue regeneration, therefore, should mimic the porous architecture of the bones (e.g. trabecular bone). According to Prof. Jones and Prof. Hench, an ideal scaffold for bone-tissue regeneration should possess the following qualities (Jones and Hench, 2003)

(i) Scaffold material is biodegradable so the conversion to bone minerals is possible

(ii) Scaffold material is bioactive and biocompatible, and not cytotoxic

(iii) Macro-porous network containing interconnected pores with having minimum pore diameter greater than 50μm (Loh and Choong, 2013) for tissue ingrowth, vascularization, and nutrients transportation.

(iv) Scaffold materials should be osteoconductive, and support osseointegration (Jones and Hench, 2003).

(v) Outer surface morphology of the scaffolds should be supportive to cell adhesion to ensure biological fixation at the material-tissue interface.

(vi) Mechanical reliability of the scaffolds.

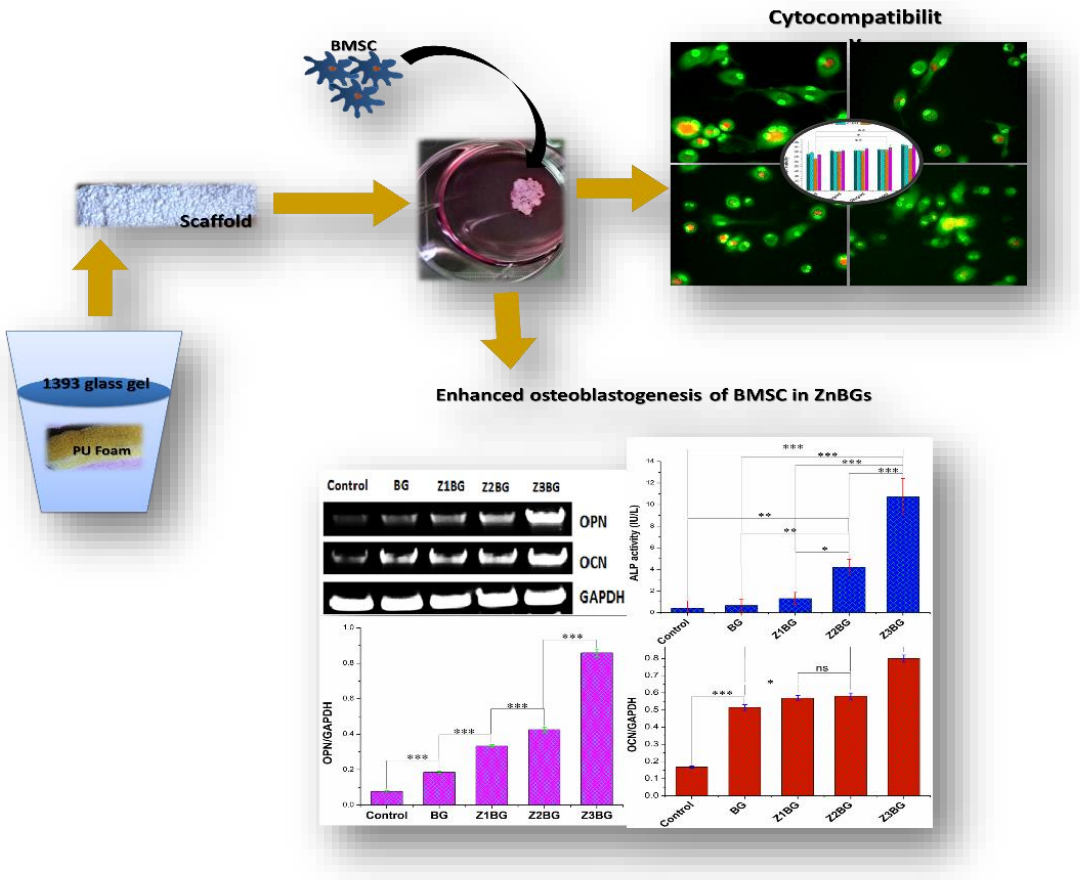
(vii) Scaffold materials having osteogenic and angiogenic potential is an added features.

Herein, the properties of some metallic therapeutic ions (copper, zinc, and strontium) incorporated 1393 or 1393B3 glass-based scaffolds have been evaluated to ensure their scope as future biomaterials.

The ***abstracts*** of the study are as follows

**Chapter 4: ZnO derived bioactive 1393 glass scaffold with enhanced biocompatibility and osteogenesis for neo-bone tissue regenerative application**

*Zinc is an essential trace elements for our skeletal system and is accountable for the formation, development, and maintenance of healthy bones. Here, the ZnO substituted sol-gel derived 1393 glass scaffolds were assessed through a series of in vitro investigations to examine the bioactivity, biocompatibility, neo bone formation ability, and mechanical stability. Results demonstrate that the ZnBGs, particularly Z3BG showed an improved biocompatibility compared to BG while assessed through carcinogenic (U2OS), normal (NIH/3T3), and stromal (mouse bone marrow stromal cells; mBMSc) cell lines. Furthermore, a multifold increase in ALP activity and osteogenic gene expression (OCN, OPN and GAPDH) confirms enhanced bone formation ability of the ZnBGs than BG.*

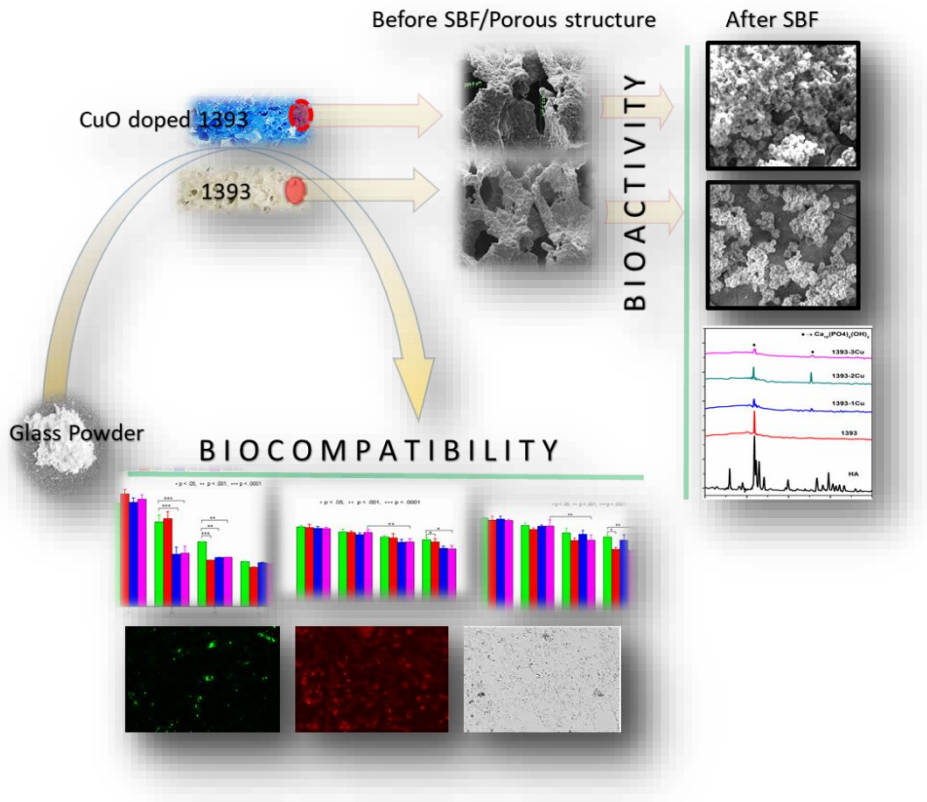



**Graphical abstract:** Sol-gel derived glass scaffolds were seeded with cells. ZnBGs exhibit enhanced osteogenesis and biocompatibility

## Chapter 5: Studies on effect of CuO addition on mechanical properties and in vitro cytocompatibility in 1393 bioactive glass scaffold

*In the present study, we have synthesized CuO substituted 1393 glass scaffolds (namely1393, 1393-1Cu, 1393-2Cu and 1393-3Cu) with the general formula (54.6-X)$SiO_2$. 6$Na_2O$. 7.9 $K_2O$. 7.7 MgO. 22 CaO. 1.74 $P_2O_5$. XCuO (all are in mole%; where X=0,1,2,3) through traditional melt-quench route. Polymer foams have been used on later stage to prepare 3D interconnected porous struts. The addition of CuO in glass system was to enhance the mechanical and biological performance of the scaffolds. However, the results indicate that the increasing trend of CuO in the 1393 glass scaffold has increased the compressive and flexural strength and elastic modulus of the scaffolds.*

*In-vitro cellular growth inhibition and cell viability assay of CuO incorporated 1393 glass scaffolds demonstrated that it did not inhibit the proliferation and viability of human squamous carcinoma cell (SCC-25) at low materials concentration. The materials caused moderate level of apoptosis at higher concentrations and were also tolerated by human RBC as studied by hemolytic assay.*

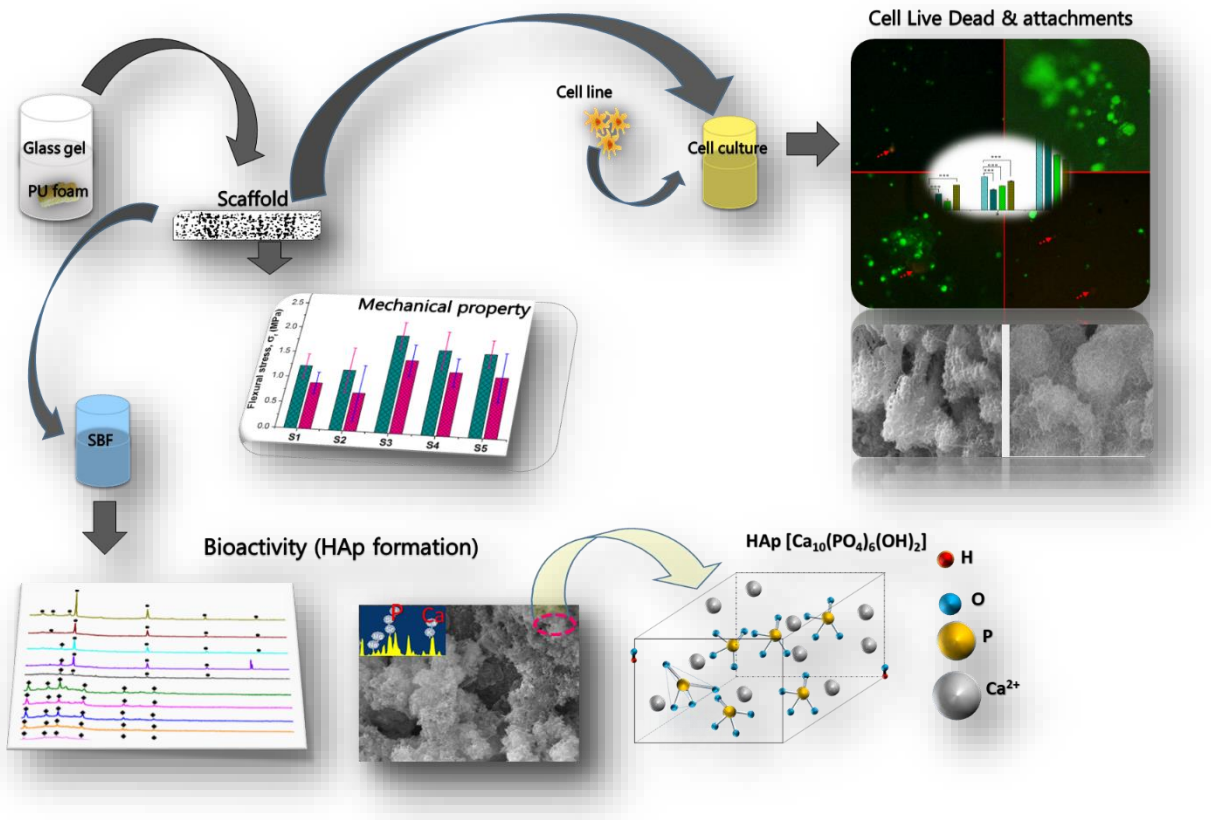



**Graphical abstract:** Melt-routed glass powder were foam replicated to scaffolds. Post SBF glass samples showing optimal bioactivity and biocompatibility for the CuO incorporated scaffold.

## Chapter 6: SrO assisted 1393 glass scaffold with enhanced biological compatibility

*Being therapeutic ion strontium is often substituted for calcium to confer enhanced therapeutic potential in bioactive glasses. The present study deals with the evaluation of desired physico-chemical and biological compatibilities of SrO derived bioactive 1393 glass scaffolds. Herein, the SrO substituted for CaO (0, 5, 20, 50 and 100%) in 1393 glass based scaffolds (namely S1, S2, S3, S4 and S5) were prepared through the most versatile sol gel route followed by foam replica*

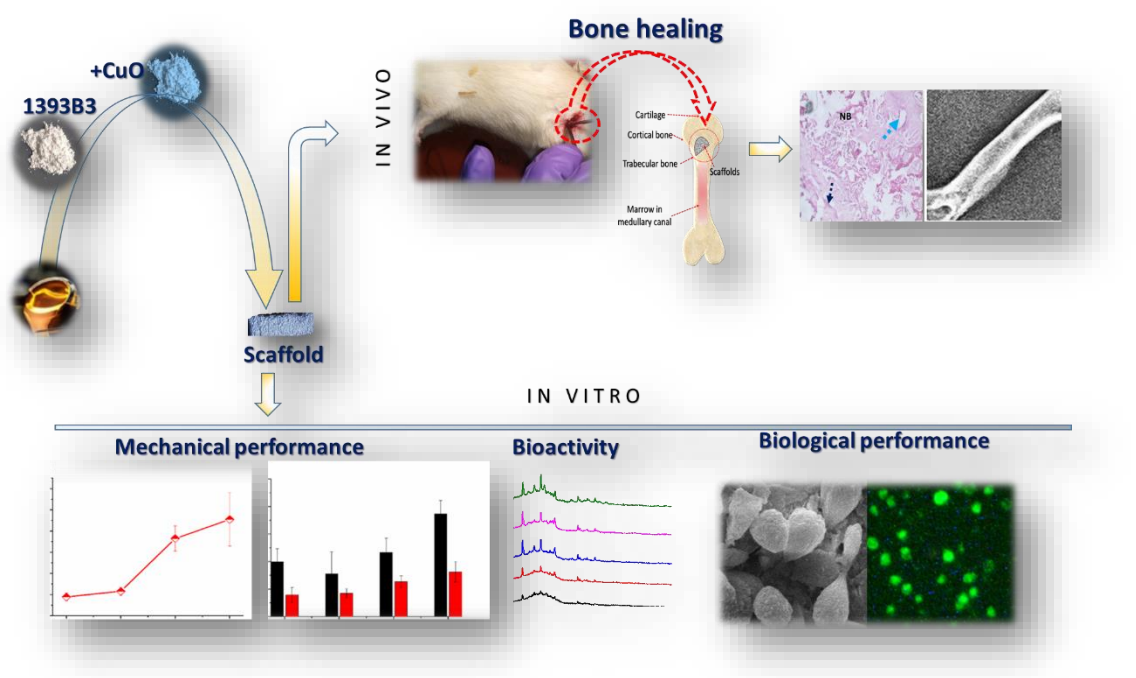
*technique. The In-vitro bioactivity of the scaffolds characterized by XRD, FTIR, SEM-EDX, and pH behavior was enhanced by increasing SrO percentage in parent 1393 glass system. Physicomechanical properties were also considerably improved in SrO derived scaffolds than that of the pure glass system in both 'as prepared' and 'during soaking in SBF'. Further, the formation of strontium hydroxycarbonated apatite [Sr-HCA; $Ca_{(10-x)}Sr_x(PO_4)_{6-y}(CO_3)_y(OH)_2$] layer on the SrO derived scaffolds have improved the cell-scaffold interactions and augmented the biocompatibility in SrO substituted scaffolds.*




**Graphical abstract:** SrO assisted 1393 scaffolds exhibit enhanced biological performance.

## Chapter 7: Assessment of CuO assisted 1393B3 on in-vitro biological and mechanochemical performance, and in-vivo bone healing potentiality in rat defects model

*The borate-based porous 1393B3 glass (BBG) scaffold and its CuO derivatives (C1BBG, C2BBG, and C3BBG) were prepared by conventional melt-quench route following the foam replica techniques. . The biological and physicomechanical performance of the glass derived scaffolds were evaluated by a series of in vitro studies. Furthermore, the bone regeneration ability of the scaffolds was assessed by the bone defects model in Wister rats. However, the results illustrate that CBBGs (CuO derived 1393B3) showed optimal biological compatibility and augmented mechanical performance in comparison to BBG. The in vivo bone healing study also exhibits a better bone regeneration potency of CBBGs as compared to both control and BBG.*




**Graphical abstract:** CuO assisted 1393B3 glass based scaffolds exhibit enhanced biological performance