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It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

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ABBREVIATIONS

1393-1Cu (1% CuO incorporated 1393 glass scaffolds)

1393-2Cu (2% CuO incorporated 1393 glass scaffolds)

1393-3Cu (3% CuO incorporated 1393 glass scaffolds)

BBG (Borate based bioactive glass; 1393B3)

BG (bioactive glass/ glass derive scaffold; 1393)

BGs (bioactive glass derivatives; 1393)

CBBGs (Borate based bioactive glass derivatives; 1393B3)

C1BBG, C2BBG, C3BBG [CuO incorporated (0.50, 1.0, 2.0 %) 1393B3 glass glass/ scaffolds]

HA, HAp (Hydroxyapatite)

HCA (Hydroxycarbonate apatite)

L929 (Mouse fibroblast cell line)

mBMSCs (mouse bone marrow stromal cells)

NIH/3T3 (Mouse mbryonic fibroblast cell line)

PBMC (Peripheral blood mononuclear cells)

PU (Polyurethane)

PVA [Poly(vinyl alcohol)]

S1, S2, S3, S4, and S4 (Strontium substituted for CaO (0%, 5%, 20%, 50%, and 100%) in 1393 glass/ scaffolds)

SBF (Simulated Body Fluid)

SCC-25 (human squamous carcinoma cell line)

TEOS (Tetraethyl Orthosilicate)

TEP (Triethyl phosphate)

Tris (Tris-hydroxymethyl aminomethane) buffer

U2OS (Human osteosarcoma cell line)

PREFACE

This thesis reports the preparation of some metallic therapeutic ions (Copper, Zinc, and Strontium) incorporated 1393 or 1393B3 glass based scaffolds and their characterization through in vitro and in vivo investigations. The preparation of 1393 glass and their metallic ions derived scaffolds involve melting of proportionate amount of AR (analytical reagent) grade reagent followed by foam replication (melt-route) or stepwise hydrolysis and polycondensation of the precursors to prepare gels, and impregnation (gels) into PU foams (sol-gel route). The characterizations involve in vitro bioactivity and cytocompatibility, osteogenesis, mechanochemical performances and in vivo evaluations. The in vitro bioactivity was measured through structural (XRD), functional (FTIR) and morphological (SEM-EDX, EDS mapping) changes due to surface modification and behavioral changes (pH) of SBF (simulated body fluid) solution due to ion exchange. The in vitro cytocompatibility of scaffolds were analyzed using various cell lines [e.g. **L929** (mouse fibroblast cell), **U2OS** (Human osteosarcoma cell), **NIH/3T3** (mouse normal embryonic cells), **SCC-25** (human squamous carcinoma cell), **HCT 116** (human colon epithelium cell), **mBMSCs** (mouse bone marrow derived stromal cell) and **PBMC** (mononuclear cell; **lymphocytes** and **monocytes**)] to assess the viability, proliferation, cytotoxicity, hemocompatibility and apoptosis of cells on the scaffolds. Osteogenesis of BMSC after grown onto scaffolds was evaluated by ALP activity and gene expression of osteogenic genes (**OPN**, **OCN** and **GAPDH**). In vivo study was performed in **Albino Wister** rats after the derived scaffolds were implanted in femur bones for 35 days and were analyzed through radiology, histology and serum RFT and LFT. Mechanical properties of the therapeutic ions derived scaffolds along with parent glass based scaffolds were examined using UTM by measuring the compression, flexure and modulus of elasticity. Physical properties like

density, porosities and pore geometry were also analyzed by Archimedes' principle, solvent saturation, and NIH ImageJ software. The in vitro bioactivity of the scaffolds were appeared to be augmented in most cases while minimally affected in few cases. Likewise, the biocompatibility of the scaffolds were also enhanced for the therapeutic ions incorporated scaffolds comparing the parent ones in most cases. Osteogenesis of stromal cells by ALP activity and gene expression was again found improved for the ZnO derived scaffolds. Physicomechanical properties were enhanced as well after incorporation of metallic ions to the parent glass systems. Bone healing and remodeling ability of CuO incorporated 1393B3 glass scaffolds was investigated by radiology and H& E staining histology in rat defects model to assess the osteogenic ability of the materials in vivo.

This thesis comprises of eight chapter as follows

Chapter 1 consists of literature review and a general introduction of the studies. The literature review sought the recent trend of biomaterials and motivated to work on therapeutic metallic oxides substituted scaffolds that induce osteogenesis and angiogenesis to regenerate bones and tissues to heal diseased/ damaged bones. The introduction briefly describes about the therapeutic nature of those metallic ions and their need in human body as supplements or nutrients by means of food, drug or controlled delivery through implant materials.

Chapter 2 discusses objectives of the investigations. It includes brief description about the importance of therapeutic ions (CuO, ZnO and SrO) and purposes of their incorporation into bioactive 1393 and 1393B3 glass scaffolds.

Chapter 3 presents a brief description about materials and methods include reagents, and precursors used and their assay, methodologies of scaffolds and SBF preparation. A brief

description about instruments used, cells/ cell lines isolation and cultures, and characterization techniques.

Chapter 4 describes “ZnO derived bioactive 1393 glass scaffold with enhanced biocompatibility and osteogenesis for neo-bone tissue regenerative application”. Detailed elaboration of cellular responses upon seeding onto the scaffolds. Osteogenic differentiation ability of stromal cells in scaffolds by ALP and osteogenic genes (OPN, OCN and GAPDH) was discussed.

Chapter 5 describes the effect of mechanical, physicochemical and biological performances of 1393 glass scaffolds post CuO incorporation into the pure 1393 glass system. In brief, starting from the raw materials selection (i.e. Quartz, ammonium dihydrogen orthophosphate, magnesium oxide and carbonates of calcium, potassium and sodium) and batch preparation, melting them in electric furnace (around 1400 °C) in platinum crucibles, quenched the glass in water, crushed, ball milled and slurry prepared followed by foam replica are covered in this chapter. Further, the in vitro bioactivity by XRD, FTIR, SEM and pH and in vitro cell culture and viability, proliferation, hemolysis and apoptosis of cells were also elaborated as well in this section.

Chapter 6 reports the “SrO assisted 1393 glass scaffold with enhanced biological compatibility”. In brief, sol-gel derived SrO incorporated 1393 glass based scaffolds (S1, S2, S3, S4 and S5) were assessed for In vitro bioactivity via XRD, FTIR, and SEM-EDX, and pH behavior of the SBF solution during immersion. Cellular metabolic activity, viability and growth and cell attachments in scaffolds were evaluated through MTT, Live/Dead, and SEM by using L929 cells. Mechanical properties of (before and during immersion in SBF) the derived scaffolds were examined for mechanical stability in physiological fluid.

Chapter 7 describes the preparation of CuO assisted 1393B3 glass scaffolds and their in vitro and in vivo characterizations. In brief, the in vitro characterizations include bioactivity of the derived scaffolds and biological and mechanochemical performance post CuO incorporation. The in vitro bioactivity assessment contains characterizations of the BBGs via XRD, FTIR, SEM-EDX, and pH behavior of the SBF solution. Whereas, the biological compatibility and mechanical stability include the study of cell-scaffold interactions via MTT, cell adhesions, and Live/Dead assay, and comparative analysis of mechanical performances of doped and undoped scaffolds as well as their degradation of strengths during immersion in physiological fluid (SBF) for 14 days. The in vivo assessment includes an assessment of bone healing ability of CuO incorporated 1393B3 glass scaffolds, compared to pure BBG via creation and remodeling of bone defects (femur) in rat model. The bone healing ability of the BBGs was assessed via radiography and histology. RFT, LFT, and ALP were also studied to evaluate their health condition caused due to the creation of defects and treatments in comparison to BBG, post-surgical operations.

Chapter 8 Summarizes the general conclusions covering chapter 4 to chapter 7 and future scope of the scaffolds.