

---

---

## Contents

Table of Tables .....	xx
Table of Figures .....	xxi
Preface.....	xxxii
Chapter 1 Introduction and Literature review.....	1
1.1 ...Introduction .....	1
1.1.1 Regions of bioactive glass composition.....	2
1.1.2 Types of bioceramics according to tissue attachments .....	4
1.1.3 Nearly inert bioceramics: .....	5
1.1.4 Porous bioceramics: .....	5
1.1.5 Bioactive bioceramics: .....	5
1.1.6 Resorbable bioceramics:.....	6
1.2...Application of Bioceramics.....	6
1.3...Hydroxyapatite: .....	8
1.4...Bioactive glass.....	9
1.5...Glass composition and structure.....	10
1.5.1 Role of network former in glass .....	10
1.5.2 Role of network modifiers in glass.....	11
1.5.3 Role of stabilizers in the glass.....	12
1.5.4 ‘Q <sup>n</sup> ’ structure .....	12
1.5.5 Relation between network connectivity and bioactivity .....	13
1.5.6 Mechanism of bioactivity, HA formation and bone bonding.....	15
1.6...Literature review.....	19

---

1.6.1	Bioglass .....	19
1.6.2	Barium oxide (BaO) as an additive .....	22
1.6.3	Strontium oxide (SrO) as an additive .....	23
1.6.4	Magnesium oxide (MgO) as an additive .....	27
1.6.5	Silver oxide (Ag <sub>2</sub> O) as an additive.....	29
1.6.6	Porous scaffold.....	31
Chapter 2	Statement of problem .....	35
Chapter 3	Materials and Experimental design .....	37
3.1	...Preparation of bioactive glasses .....	37
3.2	...Preparation of SBF .....	37
3.3	...Thermal behavior (DTA/TGA) .....	39
3.4	...Heat-treatment process for converting glass to glass-ceramics .....	39
3.5	...pH measurement.....	39
3.6	...Powder X-ray diffraction analysis.....	40
3.7	...FTIR spectrometric analysis.....	40
3.8	...In vitro bioactivity study of bioactive glass .....	40
3.9	...SEM and EDS analysis.....	41
3.10	Density and flexural strength of glasses.....	41
3.11	Compressive strength .....	42
3.12	Elastic modulus measurement by ultrasonic technique.....	42
3.13	<i>In vitro</i> cell culture studies .....	43
3.13.1	Cell lines and cell culture .....	43
3.13.2	In-vitro Cell viability assay .....	43

---

---

3.13.3 In-vitro Cytotoxicity assay .....	44
3.13.4 Cell proliferation assay.....	44
3.13.5 Detection of apoptosis .....	45
3.13.6 Cell attachment.....	45
3.14.Human blood compatibility.....	46
3.14.1 Hemolysis assay .....	46
3.14.2 Blood PBMC viability assay .....	46
3.15.Statistical analysis.....	47
Chapter 4 Influence of barium substitution on bioactivity, biocompatibility and physico-mechanical properties of bioactive glass..	48
4.1 ...Introduction .....	48
4.2 ...Materials and methods.....	50
4.2.1 Preparation of bioactive glasses .....	50
4.2.2 Heat-treatment of the bioactive glass samples .....	51
4.2.3 U2OS cell line attachment and growth.....	51
4.2.4 Atomic force microscopy (AFM) study .....	52
4.2.5 Phagocytosis assay .....	52
4.2.6 Opacity of the bioactive glass samples.....	53
4.2.7 <i>In vivo</i> animal studies .....	53
4.2.8 <i>In vivo</i> blood analysis .....	54
4.3 ...Results and Discussion:.....	54
4.3.1 Differential thermal analysis curves of bioactive glasses .....	54
4.3.2 Phase analysis of heat treated bioactive glasses.....	55
4.3.3 Structural analysis of bioactive glasses by FTIR spectrometry .....	57

---

---

4.3.4	<i>In vitro</i> bioactivity and HCA formation in SBF .....	58
A.	pH behavior of SBF after immersion of the samples .....	58
B.	<i>In vitro</i> bioactivity of bioactive glasses by FTIR spectrometry .....	60
C.	Surface morphology of bioactive glass samples by SEM .....	66
D.	<i>In vitro</i> bioactivity of the bioactive glasses by X-ray diffractometry.....	68
E.	Thickness of HCA layer formation on bioactive glass blocks .....	72
4.3.5	Physico -mechanical properties .....	74
A.	Density of bioactive glasses .....	74
B.	Compressive strength of bioactive glasses .....	75
C.	Flexural strength of bioactive glasses.....	77
D.	Modulus of elasticity .....	78
4.3.6	Cell culture studies .....	80
A.	Effect of bioactive glasses on cell viability, proliferation and cytotoxicity .....	80
B.	Detection of cell apoptosis .....	82
4.3.7	Cell attachment and growth on bioactive glasses.....	84
A.	Live cell attachment and growth by light microscopic study.....	84
B.	SEM & EDS analysis .....	86
C.	AFM analysis.....	89
4.3.8	Human blood compatibility assessment .....	91
A.	Hemolysis, WBC viability, RBC integrity and size distribution.....	91
B.	Blood platelet aggregation and thrombus formation .....	93
4.3.9	Phagocytosis of bioactive glasses by human macrophage .....	95
4.3.10	Opacity of the bioactive glasses by X-ray imaging.....	96
4.3.11	In vivo animal study .....	98

---

---

A.	In vivo radiographic analysis.....	98
B.	In vivo blood analysis.....	100
4.4...	Conclusions .....	102
Chapter 5	Enhanced bioactivity, biocompatibility and mechanical behavior of strontium substituted bioactive glasses .....	104
5.1 ...	Introduction .....	104
5.2 ...	Materials and methods:.....	106
5.2.1	Formulation of bioactive glass composition .....	106
5.2.2	Preparation of the bioactive glasses .....	106
5.3 ...	Results and Discussion:.....	108
5.3.1	Thermal behavior of glass .....	108
5.3.2	Assessment of bioactivity in SBF .....	110
A.	pH Behavior of the SBF .....	110
B.	FTIR studies .....	112
C.	X-Ray diffraction analysis.....	114
D.	Surface morphology of the bioactive glasses by SEM and EDS.....	118
5.3.3	Mechanical properties .....	121
A.	Compressive and flexural strength of bioactive glasses.....	121
B.	Modulus of elasticity .....	123
5.3.4	Assessment of biocompatibility .....	126
A.	Cell Viability .....	126
B.	Cell cytotoxicity .....	128
C.	Cell Proliferation .....	130
D.	Detection of cell apoptosis .....	132

---

---

E.	Cell attachment .....	134
F.	Hemolysis assay, RBC integrity and size distribution .....	135
5.4...	Conclusions .....	138
Chapter 6	Effect of magnesia substitution on bioactivity, biocompatibility and physico-mechanical behavior of bioactive glass	139
6.1 ...	Introduction .....	139
6.2...	Materials and methods:.....	141
6.2.1	Formulation of bioactive glass composition .....	141
6.2.2	Preparation of the bioactive glasses .....	142
6.3...	Results and Discussion .....	143
6.3.1	Effect of MgO on bioactivity in SBF .....	143
A.	pH behavior of SBF .....	143
B.	FTIR spectral analysis before and after soaking in SBF .....	144
C.	XRD analysis of the samples before and after soaking in SBF .....	147
D.	SEM and EDS analysis of the samples before and after soaking in SBF .....	150
6.3.2	Effect of MgO on mechanical behavior .....	152
A.	Compressive strength of the bioactive glasses .....	152
B.	Elastic modulus of the bioactive glasses .....	153
6.3.3	Effect of MgO on biocompatibility .....	154
A.	Cell Viability .....	154
B.	Cell Proliferation and inhibition .....	156
C.	Cell attachment .....	158
D.	Hemolysis assay .....	161

---

---

---

6.4...Conclusions .....	162
Chapter 7 Multifunctional silver contained bioactive glass-ceramic scaffold for bone tissue engineering.....	163
7.1 ...Introduction .....	163
7.2...Materials and methods.....	165
7.2.1 Sol-gel synthesis of bioactive glasses .....	165
7.2.2 DTA/TG analysis of the sample.....	166
7.2.3 The particle size and size distribution .....	166
7.2.4 Preparation of porous scaffold and characterization .....	167
7.2.5 In vitro bioactivity study .....	168
7.2.6 Compressive strength .....	168
7.2.7 Cell line growth and cell attachment.....	169
7.2.8 Antimicrobial study.....	169
7.2.9 Platelet aggregation study .....	170
7.2.10 <i>In vivo</i> implantation of scaffold in rat femur bone and X-ray imaging.....	170
7.3...Results and Discussion.....	171
7.3.1 Thermal analysis of the gel glass samples.....	171
7.3.2 Particle size and distribution .....	173
7.3.3 XRD Analysis of the bioactive glasses .....	174
7.3.4 Porous scaffold and microstructure of the samples.....	177
7.3.5 Porosity and pore size.....	179
7.3.6 <i>In vitro</i> bioactivity assessment .....	182
A. pH behavior and mechanism of HA formation .....	182
B. FTIR spectrometry analysis.....	183

---

C.	XRD analysis .....	186
D.	Surface morphology before and after SBF treatment of the samples...	188
7.3.7	Compressive strength .....	190
7.3.8	<i>In vitro</i> cell culture studies .....	191
A.	Cell viability, cytotoxicity and proliferation .....	191
B.	Detection of cell apoptosis .....	193
C.	Cell attachment and growth .....	195
7.3.9	Blood compatibility studies.....	198
A.	Hemolysis, WBC viability, RBC integrity and size distribution.....	198
B.	Thrombus formation (blood platelet aggregation).....	200
7.3.10	Antibacterial study .....	202
7.3.11	In vivo radiograph .....	203
7.4...	Conclusions .....	205
Chapter 8	Over all conclusions .....	207
References.....		211
Future scope of work .....		225
List of publications .....		226

---

---

## Table of Table

Table 1.1	Some bioactive glass compositions (wt %) containing constant P <sub>2</sub> O <sub>5</sub> [1]. .....	3
Table 3.1	Ion Concentrations in Simulated Body Fluid (SBF) and Human Blood Plasma.....	38
Table 3.2	Reagents for used for preparation of SBF .....	38
Table 4.1	Chemical composition of the bioactive glasses (mole %) and network connectivity of the glasses.....	51
Table 4.2	Heat treatment temperatures used for nucleation and crystal growth of bioactive glasses .....	57
Table 4.3	Young's modulus (E), shear modulus (S) and bulk modulus (K) of the Ba-1, Ba-2, Ba-3 and Ba-4)bioactive glasses .....	79
Table 5.1	Chemical composition of the bioactive glasses (mol %)......	107
Table 5.2	Elemental analysis of Sr-3 sample before and after SBF treatment by EDS.....	121
Table 5.3	Glass density, oxygen density and elastic modulus (Young's, Bulk and Shear modulus) of bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4). .....	126
Table 6.1	Chemical composition of the bioactive glasses (mol %)......	142
Table 7.1	Chemical composition of the sol-gel bioactive glasses (mol %)......	166

---



---

## Table of Figures

Figure 1.1	Ternary phase diagram of SiO <sub>2</sub> -CaO-Na <sub>2</sub> O containing constant P <sub>2</sub> O <sub>5</sub> [1]. .....3
Figure 1.2	The spectrum of bioactivity for various bioceramic implants. (A) The reactivity take place between the implant and the tissue and (B) The time dependence of bone bonding at an implant interface for the different groups of biomaterials [13].....4
Figure 1.3	Wide range of clinical applications of bioceramics [14].....7
Figure 1.4	Structure of hydroxyapatite, Red: Oxygen, Yellow: Calcium, Purple: Phosphate PO <sub>4</sub> tetrahedra, and Channel OH sites circled.....9
Figure 1.5	Schematic diagram for the glass structure with bridging and non bridging oxygens.....11
Figure 1.6	Schematic diagrams of a SiO <sub>4</sub> tetrahedra possessing (A) Q <sup>2</sup> structure and (B) Q <sup>1</sup> structure.....13
Figure 1.7	Relation between network connectivity of bioactive glasses and the amount of new bone formed in vivo [29].....14
Figure 1.8	Order of interfacial reactions involved between bioactive glasses and bone during bone bonding mechanism [31]. .....16
Figure 4.1	DTA curves of bioactive glass samples (Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4). .....55
Figure 4.2	XRD pattern of the Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples sintered at different temperatures as given in Table 4.2. ....56
Figure 4.3	FTIR transmittance spectra of Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples .....58

---

Figure 4.4	pH behavior of SBF after immersion of the Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples for 7 days.....	60
Figure 4.5	FTIR transmittance spectra of the Ba-0 bioactive glass sample before after immersion in SBF for 1, 3, 7, 14, and 30 days.....	61
Figure 4.6	FTIR transmittance spectra of the Ba-1 bioactive glass sample before after immersion in SBF for 1, 3, 7, 14, and 30 days.....	62
Figure 4.7	FTIR transmittance spectra of the Ba-2 bioactive glass sample before after immersion in SBF for 1, 3, 7, 14, and 30 days.....	63
Figure 4.8	FTIR transmittance spectra of the Ba-3 bioactive glass sample before after immersion in SBF for 1, 3, 7, 14, and 30 days.....	64
Figure 4.9	FTIR transmittance spectra of the Ba-4 bioactive glass sample before after immersion in SBF for 1, 3, 7, 14, and 30 days.....	65
Figure 4.10	(A-D) Scanning electron micrographs of the Ba-1, Ba-2 Ba-3 and Ba-4 bioactive glasses before immersion in SBF, respectively .....	67
Figure 4.11	(A-D) Scanning electron micrographs of the Ba-1, Ba-2 Ba-3 and Ba-4 bioactive glasses after immersion in SBF for 14 days, respectively .....	68
Figure 4.12	XRD pattern of Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples before and after SBF treatment for 14 days and Hydroxyapatite .....	70
Figure 4.13	XRD pattern of Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples after immersion in SBF for 14 days.....	71
Figure 4.14	(A-C) HCA layer formation on the surface of the Ba-0, Ba-3 and Ba-4 bioactive glasses after immersion in SBF for 14 days, respectively, elemental mapping on (D) Ba-0 and (E) Ba-4 bioactive glasses.....	74
Figure 4.15	The variation in density with varying the BaO content in the bioactive glasses (Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4).....	75

---

---

Figure 4.16	Compressive strength of the Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples .....	77
Figure 4.17	Flexural strength of the Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples .....	78
Figure 4.18	(A) Cell viability, (B) growth inhibition and (C) cytotoxicity of the Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4. ....	82
Figure 4.19	Microscopic analysis of induction of apoptosis. U2-OS cells were given indicated treatment with bioactive glass samples at a concentration of 5.0 $\mu$ M, in complete RPMI 1640 medium for 8 h at 37 °C. The FITC-conjugated Annexin V and Propidium iodide (PI) stained apoptotic cells were visualized under a fluorescence microscope (Nikon Eclipse 80i, Nikon, Japan) with Plan Fluor, 40 $\times$ , NA 0.75 objective equipped with green and red filters for FITC and PI, respectively. n=4.....	83
Figure 4.20	(A-F) Microscopic images of growth of U2-OS cells on bioactive glass blocks of control, Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 after 2 days of culture at 37 °C with 5% CO <sub>2</sub> . U2-OS cells were grown in complete medium over the bioglasses in 24 well plates. Images were taken using inverted microscope (Nikon). n=2. BG written on the images represent the sample. ....	85
Figure 4.21	(A-F) Microscopic images of growth of U2-OS cells on bioactive glass blocks of control, Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 after 7 days of culture at 37 °C with 5% CO <sub>2</sub> . BG written on the images represent the sample. ....	86
Figure 4.22	Cell attachment and growth on the bioactive glass blocks of Ba-0 (A) and Ba-3 (B) and their EDS analysis Ba-0 (C) and Ba-3(D), respectively ...	88

---

Figure 4.23	Elemental mapping on Ba-3 bioactive glass sample after cell culture for 5 days representing the presence of Ca, P, Ba, Si and Na ions .....	89
Figure 4.24	(A-C) AFM images of cell deposition on the Ba-0, Ba-3 and Ba-4 bioactive glass samples, respectively and (D-F) their height profile, respectively .....	90
Figure 4.25	(A-C) Blood hemolysis induced in RBC by Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples with increasing time periods (A), Viability of PBMC in presence bioactive glasses at varying concentrations for 18 h and Mean $\pm$ SD, n=3 (B) and Photomicrographs demonstrate the effect of Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples on RBC morphology which was compared to untreated control (C). .....	93
Figure 4.26	<i>In vitro</i> blood platelet aggregation behavior after incubation of supernatant of SBF of Ba-0 and Ba-4 samples with PRP for 8 min and the change in % light transmittance with respect to time (control is without sample).....	94
Figure 4.27	Human macrophages were cultured with Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glasses in complete medium at 37 °C, 5% CO <sub>2</sub> for 24h. The cells were washed, fixed in ethanol and stained with Giemsa stain and observed under microscope. Intracellular particles were counted and percent phagocytosis was calculated for each treatment. Mean $\pm$ SD, n=3. ....	96
Figure 4.28	X-ray radiographic image of Ba-0, Ba-1, Ba-2, Ba-3 and Ba-4 bioactive glass samples .....	97
Figure 4.29	Photographic images of <i>in vivo</i> implantation of bioactive glass sample in rat femur bone.....	99

---

Figure 4.30	(A-D) X-ray radiograph images after implantation of Ba-0 sample for 0, 15, 30 and 45 days, respectively and (E-H) Ba-3 sample for 0, 15, 30 and 45 days, respectively in rat femur bone.....	99
Figure 4.31	Hematology analysis after surgery at different time periods like 0, 10, 30 and 60 days. (A) Hemoglobin, (B) red blood cell count (RBC), (C) mean corpuscular volume (MCV), (D) Platelet, (E) mean corpuscular haemoglobin (MCH), (F) hematocrit, (G) mean corpuscular hemoglobin concentration (MCHC) and (H) white blood cells (WBC) and. Random blood sugar (RBS). .....	101
Figure 5.1	(A) DTA curves of the bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4) and (B) Glass transition, crystallization temperatures and sintering window of bioactive glasses (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4). .....	109
Figure 5.2	pH behaviour of the SBF after immersion of the bioactive glasses (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4) for different time periods .....	111
Figure 5.3	FTIR spectra of the bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4) before immersion in SBF. ....	113
Figure 5.4	FTIR spectra of the bioactive glasses after immersion in SBF for 7 days (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4). ....	114
Figure 5.5	XRD pattern of the bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4) before immersion in SBF. ....	115
Figure 5.6	XRD pattern of the bioactive glass samples after immersion in SBF for 7 days with HA crystallite size (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4) .....	118

---

Figure 5.7	SEM micrographs of the bioactive glasses before immersion in SBF (A) Sr-0, (B) Sr-1, (C) Sr-2, (D) Sr-3, (E) Sr-4 and (F) EDS spectra of Sr-3. .....	120
Figure 5.8	SEM micrographs of the bioactive glass surfaces after immersion in SBF for 7 days (A) Sr-0, (B) Sr-1, (C) Sr-2, (D) Sr-3, (E) Sr-4 and (F) EDS spectra of Sr-3.....	120
Figure 5.9	Compressive and flexural strengths of the bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4).....	123
Figure 5.10	Young's Modulus, shear modulus and bulk modulus of the bioactive glass samples (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4.....	125
Figure 5.11	The variation in density and oxygen density of the bioactive glasses (Sr-0, Sr-1, Sr-2, Sr-3 and Sr-4).....	125
Figure 5.12	(A) Viability of Sr-contained bioactive glasses against U2-OS cells was studied following MTT viability assay (Promega, USA). $5 \times 10^3$ cells were plated in 96 well plates and were cultured in complete medium in presence of Sr-contained bioactive glasses with different concentrations for 24 h.....	128
Figure 5.13	Cytotoxicity of Sr-1, Sr-2, Sr-3 and Sr-4 bioactive glasses against osteosarcoma U2-OS cells determined by 18h LDH release assay. Data presented as mean $\pm$ SD of triplicate determination.....	130
Figure 5.14	(A) Sr-contained bioactive glasses response on cell proliferation and growth of U2-OS cells following co-culture for 48h with different concentrations of the samples. (B) Co-culture with samples for longer duration at constant concentration (100 mg/ml). Data presented as mean $\pm$ SD, n = 4.....	132

---

---

Figure 5.15	Microscopic analysis of induction of apoptosis. U2-OS cells were given indicated treatment with Sr-contained bioactive glasses at a concentration of 5.0 $\mu$ M, in complete RPMI 1640 medium for 8h at 37°C. The FITC-conjugated Annexin V and Propidium iodide (PI) stained apoptotic cells were visualized under a fluorescence microscope (Nikon Eclipse 80i, Nikon, Japan) with Plan Fluor, 40X, NA 0.75 objective equipped with green and red filters for FITC and PI, respectively. n=4 .....	133
Figure 5.16	SEM images showing osteosarcoma U2OS cells attachment and growth on (A) Sr-1 and (B) Sr-3 bioactive glasses after 5 days culture and their respective EDS spectra of (C) Sr-1 and (D) Sr-3 bioactive glasses (region marked on the images taken for EDS).....	135
Figure 5.17	Hemolysis induced by indicated treatment in whole human blood at a fixed concentration (A) or with increasing concentrations (B), and is expressed as percent whole blood hemoglobin content. Mean $\pm$ SD, n = 3. Photomicrographs demonstrate the absence of detrimental effect of Sr compounds on RBC morphology compared to untreated control (C)..	137
Figure 6.1	pH behaviour of SBF after immersion of the bioactive glasses for different time periods.....	144
Figure 6.2	FTIR spectra of the bioactive glass samples (Mg-1, Mg-2, Mg-3 and Mg-4) before immersion in SBF. ....	146
Figure 6.3	FTIR spectra of bioactive glasses ((Mg-1, Mg-2, Mg-3 and Mg-4) after immersion in SBF for 7 days.....	146
Figure 6.4	XRD pattern of the bioactive glass samples (Mg-1, Mg-2, Mg-3 and Mg-4) before immersion in SBF. ....	147

---

Figure 6.5	Figure 7.5 XRD patterns of the bioactive glasses (Mg-1, Mg-2, Mg-3 and Mg-4) after immersion in SBF for 7 days. ....	149
Figure 6.6	SEM images of Mg-1 (A), Mg-2 (B), Mg-3 (C) and Mg-4 (D) bioactive glass samples and EDS of bioactive glasses Mg-1 (E) & Mg-3 (F).....	151
Figure 6.7	SEM images of Mg-1 (A) and Mg-3 (B) bioactive glass samples after soaking in SBF for 7 days and respective EDS of Mg-1 (C) & Mg-3 (D) bioactive glasses. ....	151
Figure 6.8	Compressive strength of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4). ....	153
Figure 6.9	(A)Young’s modulus, (B) bulk modulus, (C) shear modulus and (D) density of the of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4). ....	154
Figure 6.10	Cell viability (A) increasing concentration and (B) increasing time of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4).....	156
Figure 6.11	(A) Cell Proliferation and (B) Growth inhibition after 48h of culture of bioactive glasses of different concentration of the samples. ....	158
Figure 6.12	SEM images show cell attachment and growth on (A) Mg-0, (B) Mg-1, (C) Mg-2, (D) Mg-3 and (E) Mg-4 bioactive glasses after 5 days culture and (F) & (H) EDS spectra of Mg-1 and Mg-3 samples, respectively and (G) shows the bone like structure at higher magnification of Mg-2 sample.....	160
Figure 6.13	Blood hemolysis caused by Mg-0 Mg-1, Mg-2, Mg-3 and Mg-4 bioactive glass samples after different incubation time periods. ....	161
Figure 7.1	(A-B) DTA/TGA curves of sol-gel bioactive glass samples of Ag-0 (A) and Ag-2 (B), respectively.....	173

---

---

Figure 7.2	(A-C) Particle size and its distribution of sol-gel powdered bioactive glass samples containing 0.0, 1.0 and 3.0 mol% of Ag <sub>2</sub> O, respectively. ....	174
Figure 7.3	(A-C) Sol-gel bioactive glasses sintered at different temperatures (A) Ag-0, (B) Ag-1 and (C) Ag-2, respectively.....	177
Figure 7.4	Photomicrographs of the bioactive glass samples sintered at 1000 °C (A) Ag-0, (B) Ag-1 and (C) Ag-3. ....	178
Figure 7.5	SEM micrographs of the Ag-2 bioactive glass sample sintered at 1000 °C (A) plane surface, (B) higher magnification of strut area, (C) higher magnification of pore area, (D) EDS analysis of plane surface and (E) EDS analysis of pore area of the sample as shown by arrow mark.....	179
Figure 7.6	Porosity of the Ag-0, Ag-1 and Ag-2 BGC samples after sintering at 1000 °C .....	181
Figure 7.7	SEM micrographs of Ag-2 BGC sample sintered at 1000 °C (A) Plane view (B) Cross section area.....	181
Figure 7.8	pH behavior of the SBF solution after incubation of BGC samples (Ag-0, Ag-1 and Ag-2) for different time periods .....	183
Figure 7.9	FTIR spectra of (A) Ag-0 and (B) Ag-2 bioactive glass-ceramic samples before and after immersion in SBF for different time periods .....	185
Figure 7.10	XRD patterns of (A) Ag-0, (B) Ag-1 and (C) Ag-2 bioactive glass-ceramic samples before and after immersion in SBF for different time periods .....	188
Figure 7.11	SEM micrographs of the surface of bioactive glass-ceramic sample surface before immersion in SBF (A) Ag-0 BGC, (B) Ag-2 BGC and after immersion in SBF for 7 days (C) Ag-0 BGC and (D) Ag-2 BGC representing the HA crystals and metallic silver.....	189

---

---

Figure 7.12	Compressive strength of Ag-0, Ag-1 and Ag-2 samples after sintering at 1000 °C .....	191
Figure 7.13	<i>In vitro</i> cell culture investigation results of the Ag-0, Ag-1 and Ag-2 bioactive glass-ceramic samples with different concentrations (50, 100, 250 and 500 mg/ml) (A) Cell viability, (B) Cell cytotoxicity and (C) Cell proliferation .....	193
Figure 7.14	Florescence microscopic analysis of induction of cell apoptosis caused by Ag-0, Ag-1 and Ag-2 bioactive glass-ceramic samples after 8 h of incubation .....	194
Figure 7.15	(A) Cell attachment and growth on the porous Ag-2 sample (B) higher magnification, (C) Pore area and (D) Pore wall area at higher magnification as well as (E) EDS spectra of the sample.....	196
Figure 7.16	Elemental mapping of Ag-2 scaffold sample after cell culture exhibiting the concentration and distribution of Ca, P, Si, Na, Ag and Sr ions. ...	197
Figure 7.17	(A) Percent hemolysis caused by Ag-0 and Ag-2 bioactive glass-ceramic samples after incubation for different time periods, (B) Viability of blood peripheral mononuclear cell (PBMC) after incubation at different concentrations and (C) Microscopic images of Ag-0 & Ag-2 on RBC after 4 h incubation with 10mg/ml concentration (20X magnification) .....	199
Figure 7.18	<i>In vitro</i> blood platelet aggregation behavior after incubation of supernatant of SBF of Ag-0 and Ag-2 BGC samples as well as control (A) the change in % light transmittance with respect to time and (B) the % inhibition of platelet aggregation after 8 min incubation. ....	201
Figure 7.19	Antibacterial effect of Ag-0 and Ag-2 bioactive glass-ceramic scaffold samples against <i>E.Coli</i> bacteria .....	203

---

---

---

Figure 7.20	Photographic images during implantation of Ag-2 scaffold sample in rat femur bone .....	204
Figure 7.21	X-ray radiographic images before and after implantation for 15 and 30 days of Ag-2 scaffold sample in rat femur bone. ....	204