Chapter 6 Effect of magnesia substitution on bioactivity, biocompatibility and physico-mechanical behavior of bioactive glass

6.1 Introduction

Orthopaedic and dental applications so often encounter difficulty during surgery of large bone defects which are caused due to fracture, tumours and bone infections in the body. Although autologous bone grafting is a better technique of treating these defects. Bioactive glasses (BG) and glass-ceramics have been studied extensively due to their high bioactive nature and ability to form an apatite layer on the surface of the substrate upon immersion in body fluids [13][144]. Moreover, the 45S5 bioglass® is being used in clinical applications since its discovery by Hench [31][145]. The 45S5 bioglass has been shown to stimulate osteogenesis and bond with living hard bone as well as to soft tissues [146]. Hench and Polak had reported that third generation biomaterials are those which are simultaneously biodegradable and bioactive [38]. The biomaterials should be able to activate a gene expression that stimulates tissue regeneration. Further, the bioactive glasses have demonstrated higher biodegradation rate than A-W glass ceramics and hydroxyapatite (HA)[147]. Moreover, the glass system containing SiO_2–Na_2O–CaO–P_2O_5 has exhibited higher bioactivity in comparison to hydroxyapatite [8][9].

Magnesium (Mg) is one of the main trace metal alkaline earth element being present in the bone, muscle and soft tissues of human body and it is one of the important divalent cations in the human bone (bone and dentin contains Mg of 0.47 and 1.11 wt%, respectively) [120]. Magnesium was found to show a significant role in stimulation of osteoblastic cells and it reduces bone resorption [148]. Moreover, Mg plays an

important role in numerous biochemical reactions in the human body as it involves in production of protein and nucleic acid synthesis, cytoskeletal integrity and cell cycle. Furthermore, it has been demonstrated that magnesium encourages phagocytosis and regulates active calcium transportation[149]. In addition, the lack of the element (Mg) in the body can adversely affect on bone metabolism which may lead to osteoporosis and bone fragility [150]. This indicates that magnesium is an important element in the bone mineral, the substitution of Mg in the silicate glass influences on the structure and properties of the bone. Watts et al had substituted MgO for CaO in the system $49.5SiO_2$ – $1.1P_2O_5$ – (23.0(1-x)) CaO – xMgO –$26.4Na_2O$ (mol %), where $0 \leq x \leq 1$. They had reported that MgO acts as a network modifier depolymerising the silicate structure, whereas some part of it behaves as an intermediate oxide forming MgO_4 tetrahedra and polymerising the silicate structure [151]. Furthermore, earlier studies have also demonstrated that MgO acts as a glass network modifier and intermediate oxide [152][153]. Therefore, Mg plays a dual role in silicate glass network structure and which significantly influences on the glass structure and physico-chemical properties. Earlier authors had noticed a decrease in the rate of glass dissolution by substitution of MgO for CaO at different concentrations in the glass composition. They had reported the change in concentrations of Ca^{2+}, Mg^{2+}, P^{5+}, and Si^{4+} ions in Dulbecco's Modified Eagle Medium (DMEM), Tris–HCl and SBF solutions [154][21]. It has been also reported that MgO significantly affects on the bioactivity and ion release behavior of bioactive glasses due to its dual role as a network modifier and an intermediate. Previous authors had demonstrated the presence of MgO less than 7.0 mol% in the glass which minimises the rate of glass dissolution and retards the HA layer formation in SBF and DMEM [154][21]. Moreover, several authors had reported that substitution of MgO decreased the glass dissolution and delayed the formation of HA layer

[61][120][62]. Network connectivity (NC) is defined as the average number of bridging oxygen (BO) bonds in the silicate network. It was demonstrated that NC can be used to predict the number of properties like glass structure, durability, mechanical, thermal, bioactivity and biological etc. [119][25,26]. Generally, the NC of the glass tends to decrease with increasing the network modifying ions in the glass system.

It is still needed to develop new bioactive glasses with substitution of Mg as it has got its potential applications. However, several research reports are available on the substitution of MgO for CaO in bioactive glasses [120][154][21][61][62]. The new bioactive glasses have been designed, where MgO was substituted on molar basis for SiO_2 and compared with the reference glass containing MgO for CaO. The new bioactive glasses (low NCB glasses) are expected to show faster HA layer formation and the work systematically reports the Mg substitution on the change in bioactivity, degradability, physico mechanical properties and cell culture studies.

6.2 Materials and methods

6.2.1 Formulation of bioactive glass composition

Four bioactive glass compositions were formulated in a five component system (Na_2O–CaO–MgO–P_2O_5–SiO_2) along with 45S5 bioglass® which was also prepared for comparison as shown in **Table 6.1**. The bioactive glass (Mg-1) was prepared as a reference sample where MgO was substituted for CaO. The new formulations contained partially substituted MgO for SiO_2 on the molar basis. Network Connectivity (NC) was calculated on the basis of the following general equation (6.1) [25,28] assuming that SiO_2 forms the network structure in the glass whereas, P_2O_5 remains in orthophosphate phase.

$$NC = \frac{4 \text{ x } SiO_2 + 6 \text{ x } P_2O_5 - (2 \text{ x } CaO + 2 \text{ x } MgO + 2 \text{ x } Na_2O)}{SiO_2} \quad - - - (6.1)$$

6.2.2 Preparation of the bioactive glasses

The bioactive glasses shown in **Table 6.1** were prepared by melting analytical reagent grade calcined quartz (purity 99.9%), sodium carbonate (99.5%), calcium carbonate (99.0%), magnesium carbonate (99.5%) and ammonium dihydrogen orthophosphate (99.0%) as a source of SiO_2, Na_2O, CaO, MgO and P_2O_5, respectively. The weighed batches were mixed for 30 minutes in an agate mortar and pestle and melted in a 100 ml platinum crucible at 1400°C for 2 h in an electrical furnace. In order to ensure homogeneity, the glass melts were taken out of the furnace, poured on a preheated aluminum plate, cooled, crushed and re-melted in the furnace for an another period of 2 h. The bulk glass samples were annealed in a pre-heated muffle furnace at 450 °C and after 1 h of annealing, the furnace was cooled to room temperature. The bulk glass samples were cut, ground and polished into required dimensions. The polished glass samples were ultrasonically cleaned in an acetone bath. The densities of the glasses were determined by ASTM B962-14 method.

Table 6.1 Chemical composition of the bioactive glasses (mol %).

Glass code	SiO_2	P_2O_5	CaO	Na_2O	MgO	NC
Mg-0 (45S5)	46.10	2.60	26.90	24.40	0.00	2.11
Mg-1	46.10	2.60	24.22	24.40	2.00	2.11
Mg-2	45.10	2.60	26.90	24.40	1.00	2.03
Mg-3	44.10	2.60	26.90	24.40	2.00	1.94
Mg-4	43.10	2.60	26.90	24.40	3.00	1.84

6.3 Results and Discussion

6.3.1 Effect of MgO on bioactivity in SBF

A. pH behavior of SBF

Figure 6.1 represents the change in pH of SBF after immersion of the samples for different time periods. The mechanism of HCA formation can be assessed by pH behavior of SBF after immersion of the samples [1,31,131]. The pH behavior of all the bioactive glasses has shown similar trends. The pH of SBF was found to increase significantly from an initial value of 7.38 to 8.37, 8.12, 8.41, 8.29 and 8.23 for Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4, respectively after 4 days of immersion. The increase in pH demonstrates the dissolution of cations in the solution from the surface of the glass. Higher values of pH leads to attack on silica network and results in formation of silanols. Later, the pH of the SBF solution decreased in all the samples, which is due to the absorption of calcium and phosphate ions from the SBF to promote the HCA layer formation on the surface of the samples [31,132][130]. The results demonstrate that the substitution of MgO for SiO_2 in the present study did not alter the mechanism of bioactivity in SBF. While reference sample Mg-1 has exhibited lowest pH when compared to other bioactive glasses and this could be due to substitution of MgO for CaO. The Mg-O has greater bond strength as compared to Ca–O which could decrease the glass dissolution kinetics as well as retard the ability to exchange of Mg^{2+} with H^{+} ion in SBF and hence a lower pH value was reported[21][61]. In the presence of Mg-2, Mg-3 and Mg-4, these low NC bioactive glasses (low NCB glasses), the pH values were reported more than Mg-1 on day 4 which confirmed the release of ions from the glasses. It can be attributed due to disruption in network connectivity of the glass by substitution of MgO for SiO_2. These results suggest that the substitution of MgO for

SiO_2 is beneficial in releasing the ions from the bioactive glasses. It is noteworthy that the pH plays an important role in the deposition of calcium and phosphate (Ca-P) layer and a higher pH leads to the calcium carbonate precipitation [133]. Moreover, an appropriate pH is required for HCA layer formation on the surface of the samples. Importantly, when the bone forms, the cross linking of the collagen chains and the subsequent precipitation of hydroxyapatite are pH dependent during this process [28,37].

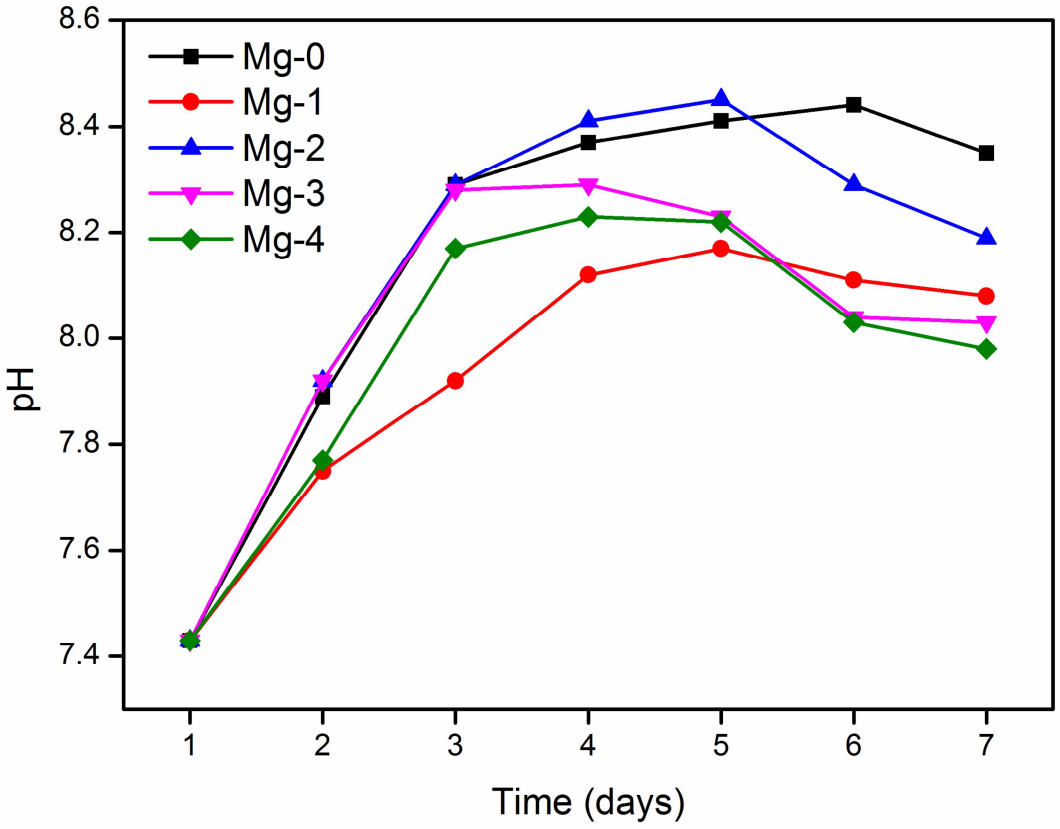

Figure 6.1 pH behaviour of SBF after immersion of the bioactive glasses for different time periods.

B. FTIR spectral analysis before and after soaking in SBF

Figure 6.2 shows the FTIR absorption spectra of the Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4 bioactive glass samples recorded in the wavenumber range of 400–4000

cm^{-1}. The FTIR bands were found at 454, 702, 1010, 1430 and 1590 cm^{-1}. The band centered at around 454 cm^{-1} is associated with a Si–O–Si symmetric bending mode of vibration in silicate network. An another minor band at around 702 cm^{-1} corresponds to Si–O–Si symmetric stretching of non-bridging oxygen atoms between SiO_4 tetrahedra. The major peak observed at 1010 cm^{-1} is attributed due to Si-O-Si asymmetric stretching mode of vibration with one non-bridging oxygen (Si-O-1NBO) per SiO_4 tetrahedron [115,134]. It was observed that the intensity of absorption band centred at 1010 cm^{-1} has increased in Mg-3 sample in comparison to others and this increase is due to the formation of more number of non-bridging oxygens (NBOs) (Si-O-NBO). Another bands at 1403 cm^{-1} and 1590 cm^{-1} correspond to C-O stretching mode of vibration which might have appeared due to reaction between the glass and carbon dioxide present in the atmosphere [115].

It is well known that the molecular structure of glasses plays an important role in deciding their bioactivity. After immersion in SBF for 7 days the new bands emerged at 570, 883, 1024, 1188, 1431 and 3750 cm^{-1} as shown in **Figure 6.3**. The bands centred at 578 cm^{-1} and 1188 cm^{-1} are attributed due to the P-O bending mode of vibrations. The major peak observed at 1024 cm^{-1} is attributed due to the presence Si-O-Si asymmetric stretching mode of vibration. The new bands were marked at around 883 and1458 cm^{-1} corresponding to C-O stretching mode. A broad band at around 3750 cm^{-1} is assigned due to the presence of O-H groups. These characteristic bands represent the formation of hydroxyl carbonate apatite (HCA) layer on the surface of the bioactive glass samples [95,155].

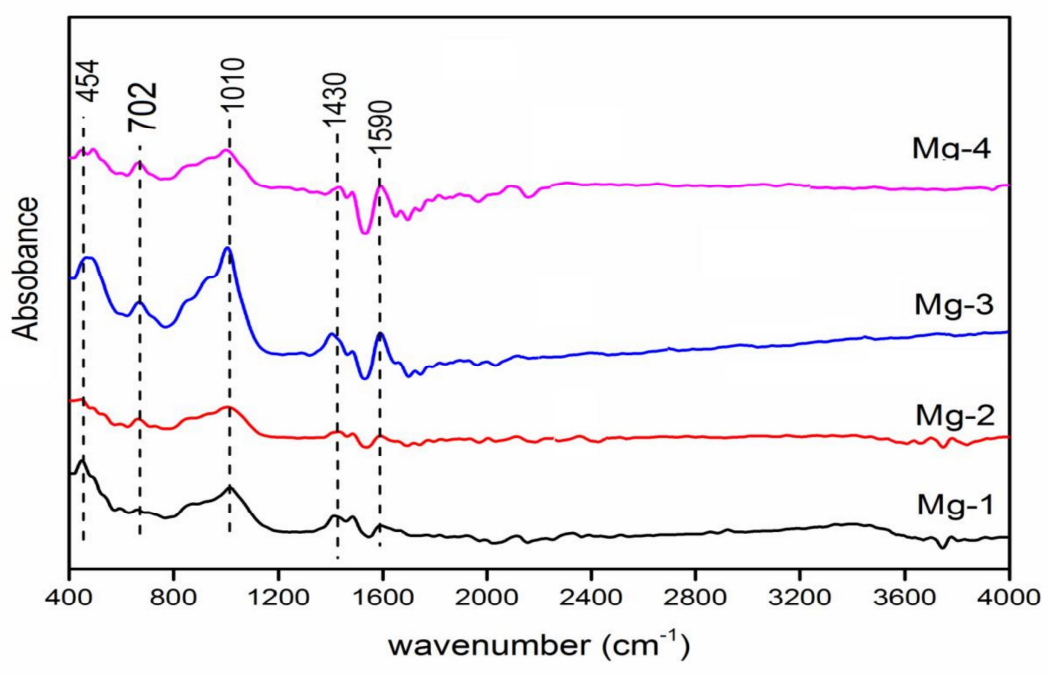

Figure 6.2 FTIR spectra of the bioactive glass samples (Mg-1, Mg-2, Mg-3 and Mg-4) before immersion in SBF.

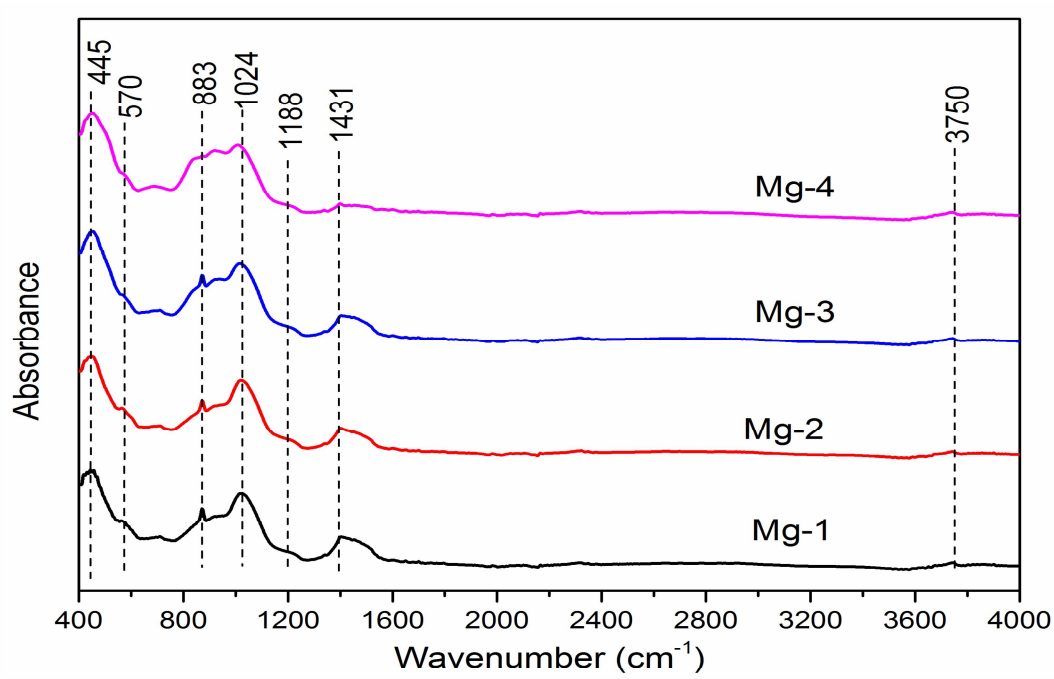

Figure 6.3 FTIR spectra of bioactive glasses ((Mg-1, Mg-2, Mg-3 and Mg-4) after immersion in SBF for 7 days.

C. XRD analysis of the samples before and after soaking in SBF

Figure 6.4 shows the XRD pattern of the prepared bioactive glass samples (Mg-0, Mg-1, Mg -2, Mg -3 and Mg -4). It is evident from the XRD data that all the bioactive glasses were found to be amorphous in nature. The bulk glasses were optically transparent after melting and casting. Notably, the amorphous scattering of a broad hump at $2\theta \simeq 32°$ was found in all the glasses. This hump is more intense in Mg-2 (1 mol%) and it could be due to MgO acting as modifier and hence narrowing the silicate network. However, it can be seen from the figure that due to further addition of MgO for SiO_2 in Mg-3 and Mg-4 samples there is slight deviation in the trends and the hump has broadened which might be attributed to the change in the role of MgO as it shifts from modifier to network intermediate oxide[156].

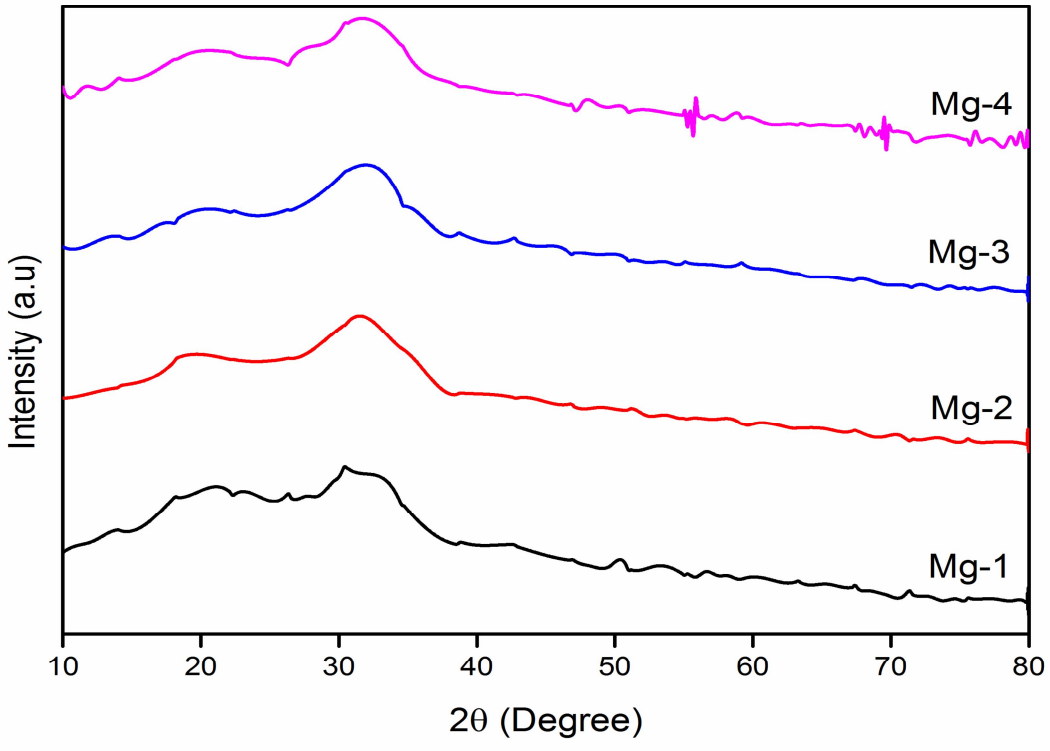

Figure 6.4 XRD pattern of the bioactive glass samples (Mg-1, Mg-2, Mg-3 and Mg-4) before immersion in SBF.

Figure 6.5 shows the XRD patterns of the bioactive glasses after immersion in SBF for 7 days and the results show the formation of crystalline phases after SBF treatment. In general, *in vitro* bioactivity of the sample is associated with the ability of hydroxy apatite (HA) layer formation on their surface in SBF under physiological conditions. The *hkl* planes (211), (203) and (313) are located at 2θ corresponding to crystalline phase of hydroxy apatite [$Ca_{10}(PO_4)_6(OH)_2$] and the diffraction peaks were matched with the standard PCPDF#: 74-0565 [95,131]. Therefore, all the samples confirm the HA phase formation after immersion in SBF for 7 days. It was observed from the XRD patterns that the intensities of the peak (211) differed with each other and this difference is attributed due to the varying amount of the phase formed in each sample. It is interesting to discuss here that the reference bioactive glass (Mg-1) exhibited the less intense HA peak formation. The present trend is well supported by earlier reports that the substitution of MgO for CaO has decreased the glass dissolution and reduced the HA layer formation on the surface of the samples in SBF [61]. This is due to the greater bond energy of Mg–O as compared to Ca–O which decreases the release of Mg^{2+} ions from the glass network. Consequently, it decreases the exchange ability of Mg^{2+} with H^{+} ion from the solution which is in good conformity with results of Mg-1 sample (**Figure 6.5**). This is also in good agreement with the pH data which had shown a lower rate of ion release (**Figure 6.1**). Further, it is to emphasize that the low NCB glasses exhibit a significant growth of HA crystallinity in SBF in comparison to reference glasses. This improvement may be attributed to the substitution of MgO for SiO_2, which lowers the glass network connectivity. This is also in good conformity with previous results which have shown that the bioactivity of glasses increased with decreasing in network connectivity [119] [25,29,132]. During contact with body fluids the leaching of alkali and alkaline earth ions would be much easier and faster in these

bioactive glasses as a result in leaving silica rich layer (Si-OH groups). Subsequently, this layer plays an important role in adsorption of Ca^{2+} and PO_4^{3-} phosphate ions from the solution [29,95]. Therefore, more calcium and phosphate ions might have deposited on surface of these bioactive glasses forming amorphous (Ca-P) layer. Moreover, the peak intensities are found to be more in Mg-3 and Mg-4 samples. This clearly suggests that substitution of MgO for SiO_2 has resulted in an increase in bioactivity of the samples as compared to CaO in the glass.

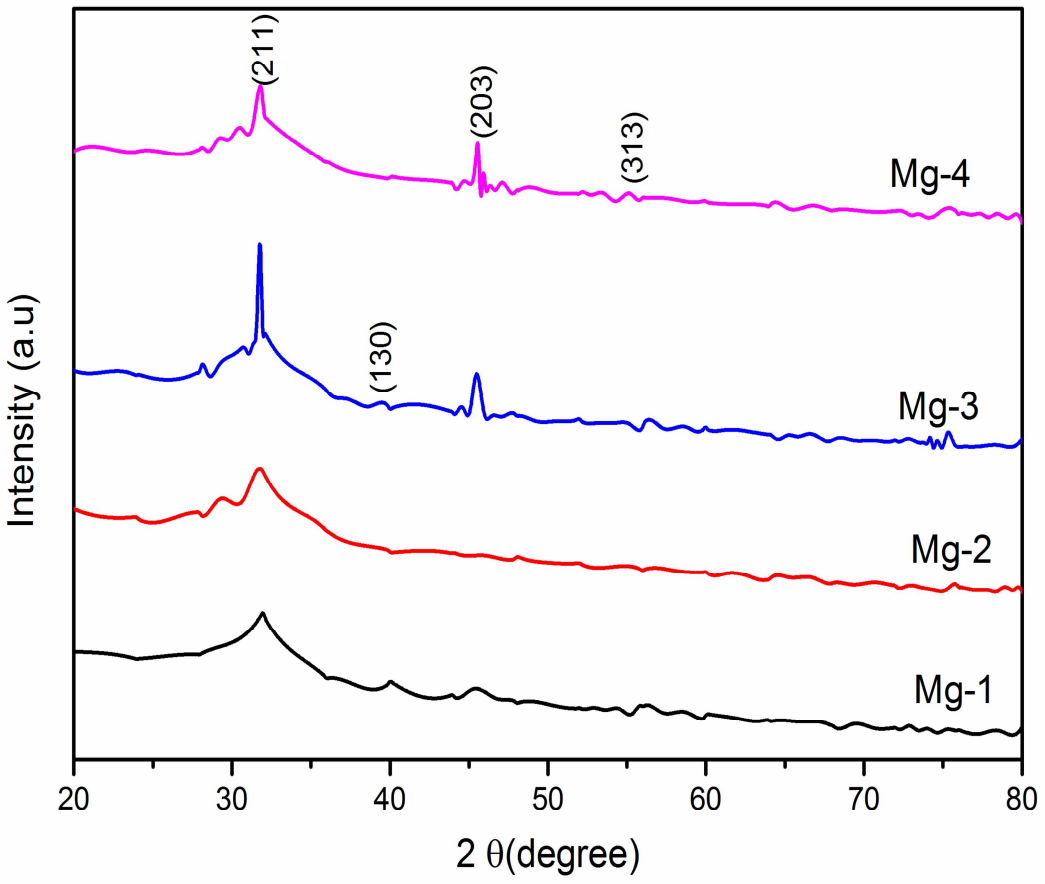

Figure 6.5 Figure 7.5 XRD patterns of the bioactive glasses (Mg-1, Mg-2, Mg-3 and Mg-4) after immersion in SBF for 7 days.

D. SEM and EDS analysis of the samples before and after soaking in SBF

Figure 6.6 (A-D) shows the SEM images of Mg-1, Mg-2, Mg-3 and Mg-4 bioactive glass samples, respectively and all represent the glassy nature before immersion in SBF. **Figure 6.6 (E-F)** shows the EDS spectra of Mg-1 and Mg-3 both the samples which contain same amount of 2 mol% MgO and also indicate the presence of Si, Ca, Na, P and Mg elements on the surface of the sample. **Figure 6.7(A-B)** shows the SEM micrographs of the Mg-1 and Mg-3 bioactive glasses after the immersion in SBF for 7 days. A change in surface morphology is seen when it is compared with the initial surface of the samples. The SEM micrographs demonstrate that spherical particles have covered the surface of the bioactive glass samples with variable shape and size. The EDS analysis was also done on the surface of Mg-1 and Mg-3 samples after immersion in SBF for 7 days as shown in **Figure 6.7 (C-D**). The result shows that the spherical particles contained C, Ca, P and Mg with high intensity and Si with low intensity peaks when compared with the initial surface as in **Figure 6.6 (E-F**). This significant increase in C, Ca, and P as well as a decrease in Si intensities has indicated the formation of hydroxy carbonate apatite (HCA) layer [26]. It was also observed that the number of HCA crystals is more on the surface of Mg-3 as compared with Mg-1 reference bioactive glass. This significant development of HCA crystals might be associated with a considerable deposition of Ca-P layer which is in conformity to the XRD analysis (**Figure 6.5**).

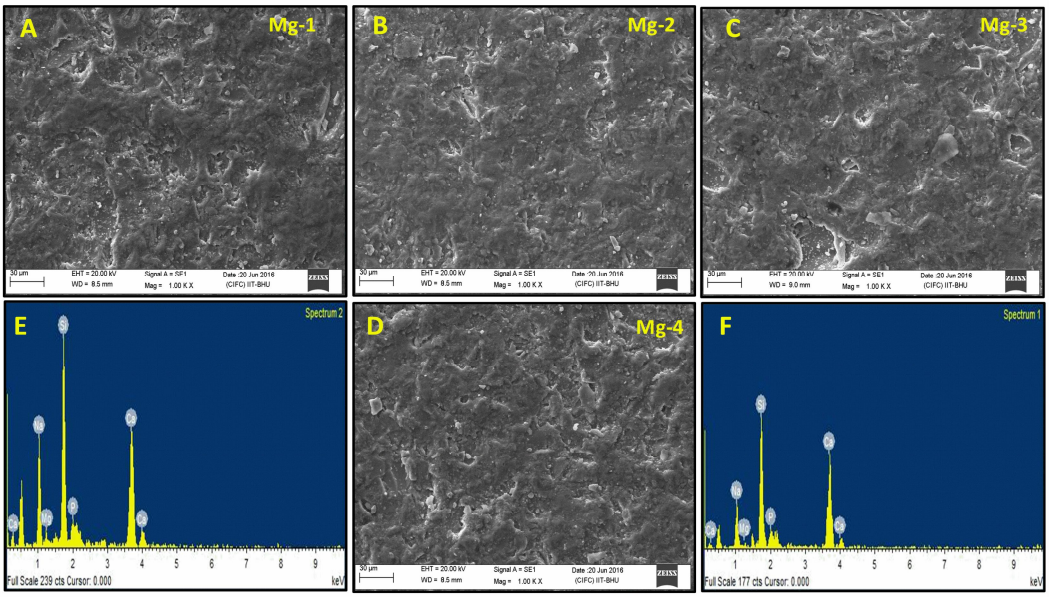

Figure 6.6 SEM images of Mg-1 (A), Mg-2 (B), Mg-3 (C) and Mg-4 (D) bioactive glass samples and EDS of bioactive glasses Mg-1 (E) & Mg-3 (F).

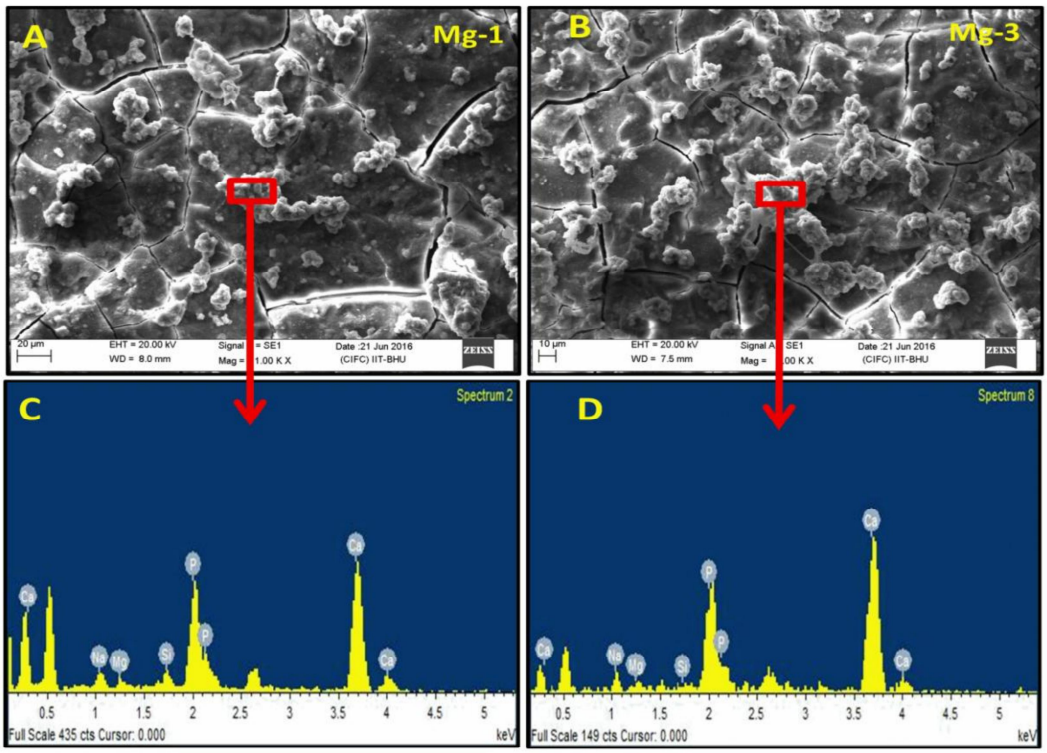

Figure 6.7 SEM images of Mg-1 (A) and Mg-3 (B) bioactive glass samples after soaking in SBF for 7 days and respective EDS of Mg-1 (C) & Mg-3 (D) bioactive glasses.

6.3.2 Effect of MgO on mechanical behavior

A. Compressive strength of the bioactive glasses

In order to investigate the mechanical behavior, the bulk glass samples were cut into required dimensions, ground and polished well to get an optically transparent. The compressive strengths of the bioactive glasses (Mg-0, Mg-1, Mg-2, Sr-3 and Sr-4) have been presented in **Figure 6.8**. It is found that compressive strength of Mg-1 is higher as compared to Mg-0 besides the same amount of the modifiers contained in both the glasses as MgO which was substituted for CaO. This could be due to the Mg^{2+} ion acting as network modifier which has increased its contribution in the glass network. Thus, it leads to an increase in compactness of the glass structure which might have resulted in a better strength. Further, it is observed that the strength has increased significantly with increasing concentration of MgO for SiO_2 in Mg-2 and Mg-3 bioactive glass samples. Further addition of MgO (Mg-4) was observed to cause a drastic fall in the strength. High concentration of MgO might have entered in to the silica network to form Si-O-Mg which possesses a brittle nature. Mostly, the modifiers like Mg^{2+}, Ca^{2+}, Sr^{2+} and etc. occupy the interstitial positions in the glass structure [112]. This is in good conformity with the density results, which showed an appreciable increase with increasing the concentration of MgO up to 2.0 mol% and further addition caused a decrease in compressive strength in these series of glasses as shown in **Figure 6.9 (D)**. This confirms the close packing of atoms which increased the compactness of the glass structure and might have resulted in significant improvement in compressive strengths.

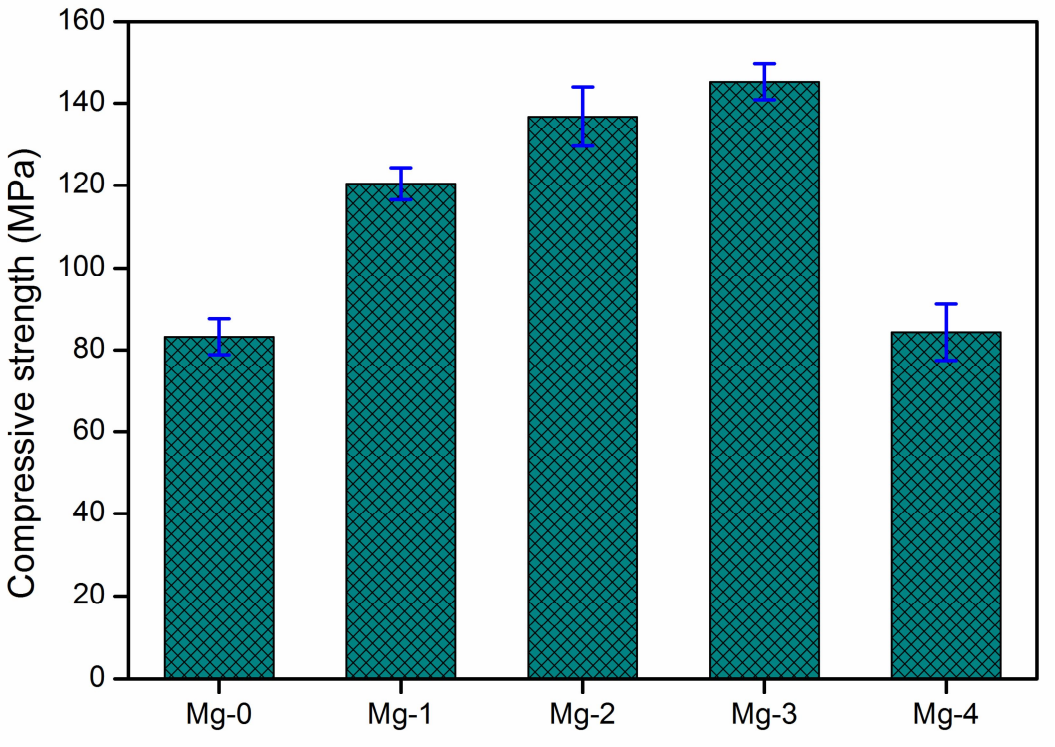

Figure 6.8 Compressive strength of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4).

B. Elastic modulus of the bioactive glasses

The mechanical behavior of the samples in non-destructive model namely, Young's modulus (E), shear modulus (S) and bulk modulus (K) has been also presented. In order to investigate the mechanical behavior, the bulk glass samples were cut, ground and polished. Elastic moduli such as Young's, bulk and shear modulus of the bioactive glasses (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4) have been presented in **Figure 6.9 (A-C)** respectively. Similarly, the Young's, shear and bulk modulus were found to increase with increasing MgO content upto 2.0 mol% in both cases as MgO was substituted for CaO and SiO_2 as compared with MgO-free sample (Mg-O) and then it started decreasing with further addition of MgO for SiO_2. This results confirms that MgO acting as a network modifier up to certain concentrations resulting in an increase

in the packing density of these bioactive glasses. Hence, the average number of the network bonds per unit volume has increased in the glass structure [99,112,114]. Therefore, the propagation of sound wave in a compacted glass might be much faster which has resulted in the improvement in elastic moduli

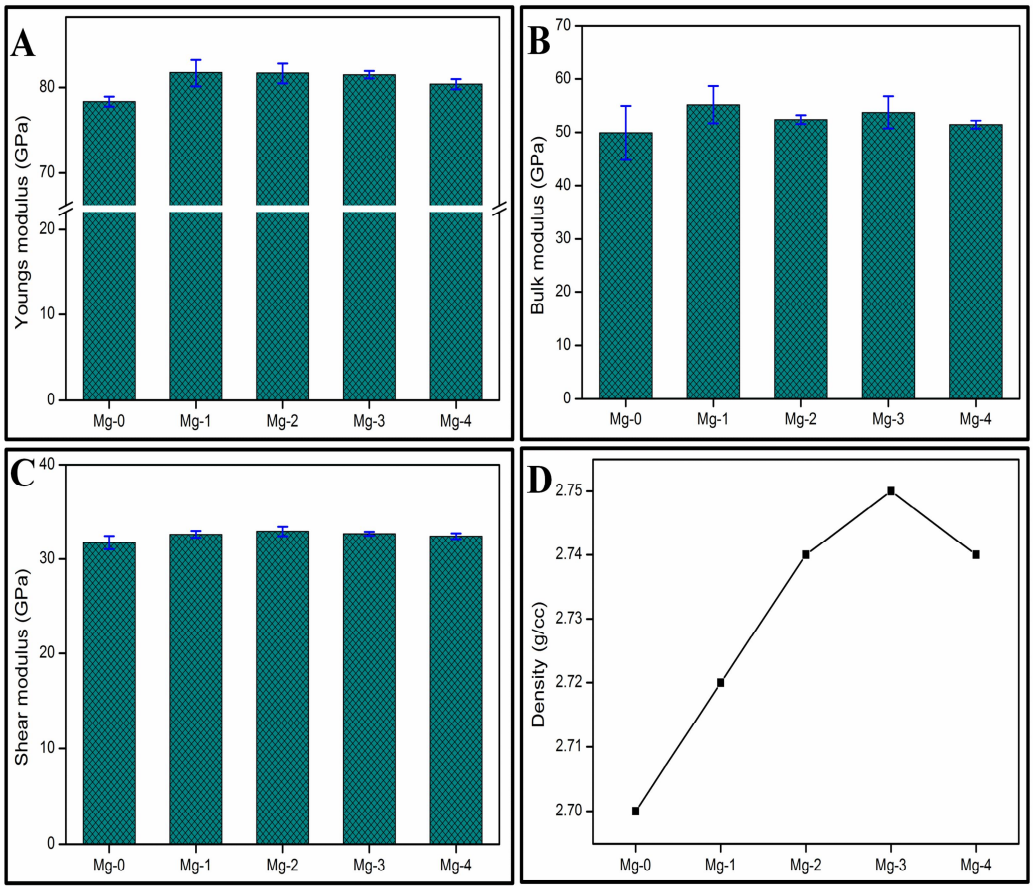

Figure 6.9 (A)Young's modulus, (B) bulk modulus, (C) shear modulus and (D) density of the of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4).

6.3.3 Effect of MgO on biocompatibility

A. Cell Viability

The *in vitro* cell culture studies were carried out using human osteosarcoma U2OS cell lines and assessed the cell viability, proliferation and blood compatibility. In general, most of the biomaterials at lower concentrations can exhibit biocompatibility

during *in vitro* cell culture studies. The higher concentration of the sample can furnish better information and conclusions for the compositional effect. Cell viability of the Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4 samples was assessed against U2OS cell lines by XTT viability assay. The percentage cell viability at different concentrations incubated for 48 h and also with increasing time at a constant concentration (50 mg/ml) of the samples at 37^{o}C in 5% CO_2 has been presented in **Figure 6.10 (A – B)**, respectively. The percent viability was calculated by considering the viability of tumour cell cultured in complete medium only as 100%. It was observed from the results that the cell viability has increased with increasing the concentration (mg/ml) of the sample (**Figure 6.10A**). The results of new the bioactive glasses are comparable with standard and reference samples with all the concentrations studied in the experiment. It is noteworthy that these bioactive glasses have exhibited higher extent of ions release rate from the glass surface as reported in pH measurement. However, the amount of ions released from these bioactive glasses did not affect the cell survival [62][157]. Therefore, the substitution of MgO for SiO_2 did not harm the cell viability significantly. The same is evident in both the experiments with increasing concentration and prolonged time periods. This is also in good conformance with earlier investigations that the Mg^{2+} ion substituted bioactive glasses do not affect on the cell compatibility [120] [148][157]. Hence, the results have demonstrated that all the new bioactive glass samples are cytocompatible with U2OS cells and are comparable with the standard as well as reference bioactive glasses.

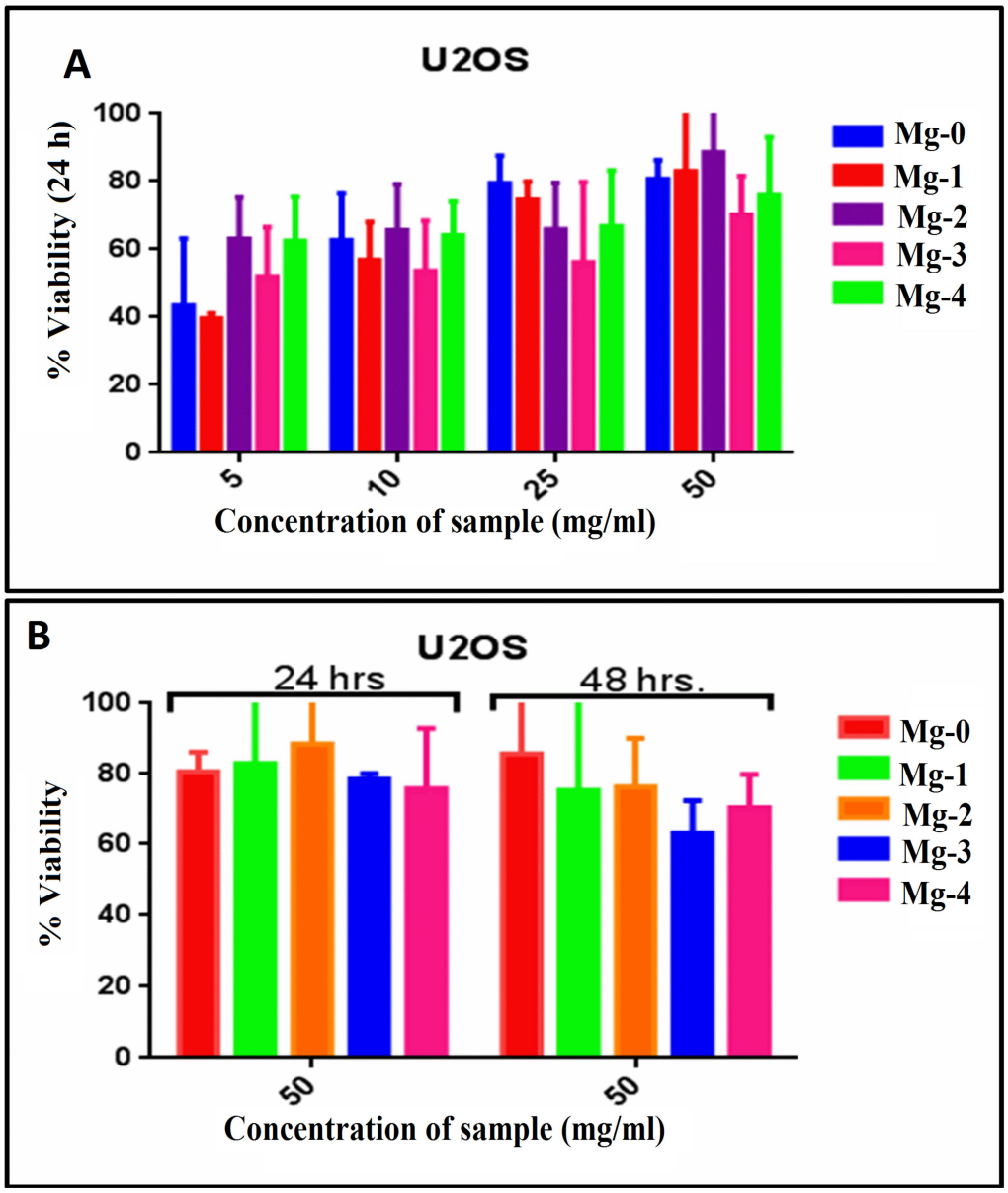

Figure 6.10 Cell viability (A) increasing concentration and (B) increasing time of the bioactive glass samples (Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4).

B. Cell Proliferation and inhibition

Cell proliferation and growth inhibition assay was carried out using U2OS cell lines cultured with different concentrations (5, 10, 25 and 50 mg/ml) of the bioactive glass samples for 48 h as shown in **Figure 6.11 (A & B)**, respectively. The cells were found to proliferate in the presence of all the samples after incubation of 48 h at all the

concentrations (5, 10, 25 and 50 mg/ml) as shown in **Figure 6.11 (A)**. It was observed that the cell proliferation increased significantly with increasing the samples concentrations and it is analogous to cell viability data. Whereas, the growth inhibition data shows that as the concentration of the sample increases the growth decreases (**Figure 6.11 B**). This could be due to that the Mg^{2+} ions significantly influence on metabolic processes which stimulate the bone growth [145][151]. Therefore, the MgO substitution for SiO_2 did not harm the cell proliferation and inhibition significantly. It has been reported earlier that network connectivity of the glass played a significant role in HCA development [25,29]. The high rate of release of ions from the new bioactive glasses as compared to reference sample into biological fluid was found to play a vital role in cell survival and growth. Thus, the results signify that the present bioactive glasses have a potential scope for bone regeneration.

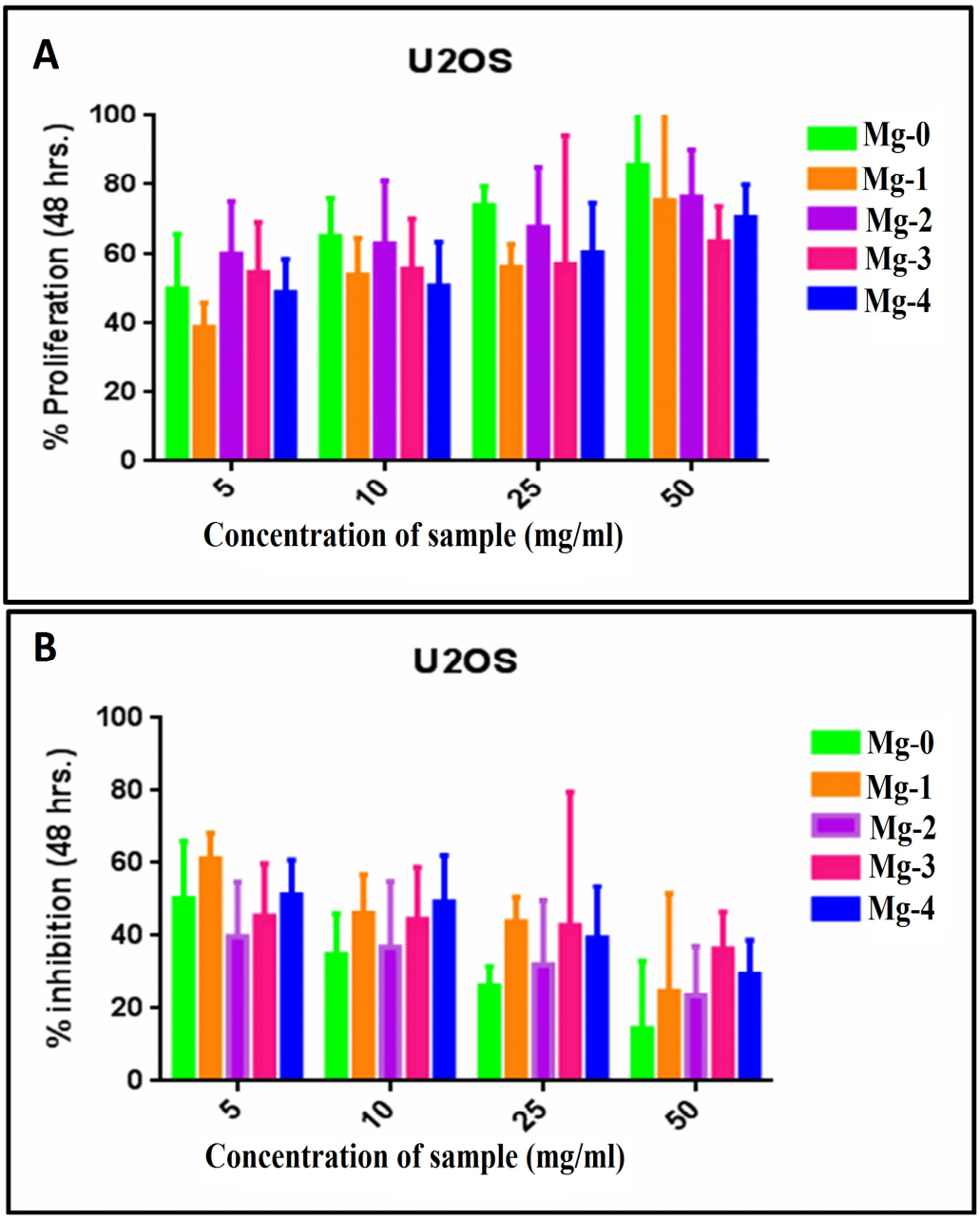

Figure 6.11 (A) Cell Proliferation and (B) Growth inhibition after 48h of culture of bioactive glasses of different concentration of the samples.

C. Cell attachment

Figure 6.12 (A-E) shows SEM images for the cell attachment and growth of Mg-0, Mg-1, Mg-2, Mg-3 and Mg-4 samples after cell culture for 5 days, respectively as well as **Figure 6.12 (G)** shows the bone like structure at higher magnification of

Mg-2 sample and **Figure 6.12 (F & H)** shows the EDS spectra of Mg-1 and Mg-3 bioactive glass surfaces after cell culture. It can be seen clearly from SEM images that there is an excellent attachment and spreading of cells on the surfaces of all the bioactive glasses. The significant change on surface morphology is clearly differentiated when compared with the initial surfaces of the samples. Similarly, the EDS analysis was done after culture for 5 days of the Mg-1 and Mg-3 samples. The EDS results show that the newly formed layer contain C, Ca, P and Mg with high intensity and Si with low intensity peaks as compared with the initial spectra (**Figure 6.12 F & H**). This further indicates the formation of hydroxy carbonate apatite layer on the surfaces of the samples. It is noteworthy that substitution of MgO for SiO_2 not only decreases the network connectivity but also decreases the surface energy. The surface energy of a material is generally defined by its charge density and the net polarity of the elements present in the system [141]. It was reported earlier that the decrease in surface tension increases the hydrophilic nature of the sample and thus increases the cell recruitment [40]. Furthermore, it important to mention herewith that the formation of new layer is more on Mg-3 sample in comparison to Mg-1 as evidenced from their SEM images. Although both the samples contain equal amount of MgO content but with change in their network structure. Hence, the NC plays an important role in cell attachment and growth on the bioactive glass samples.

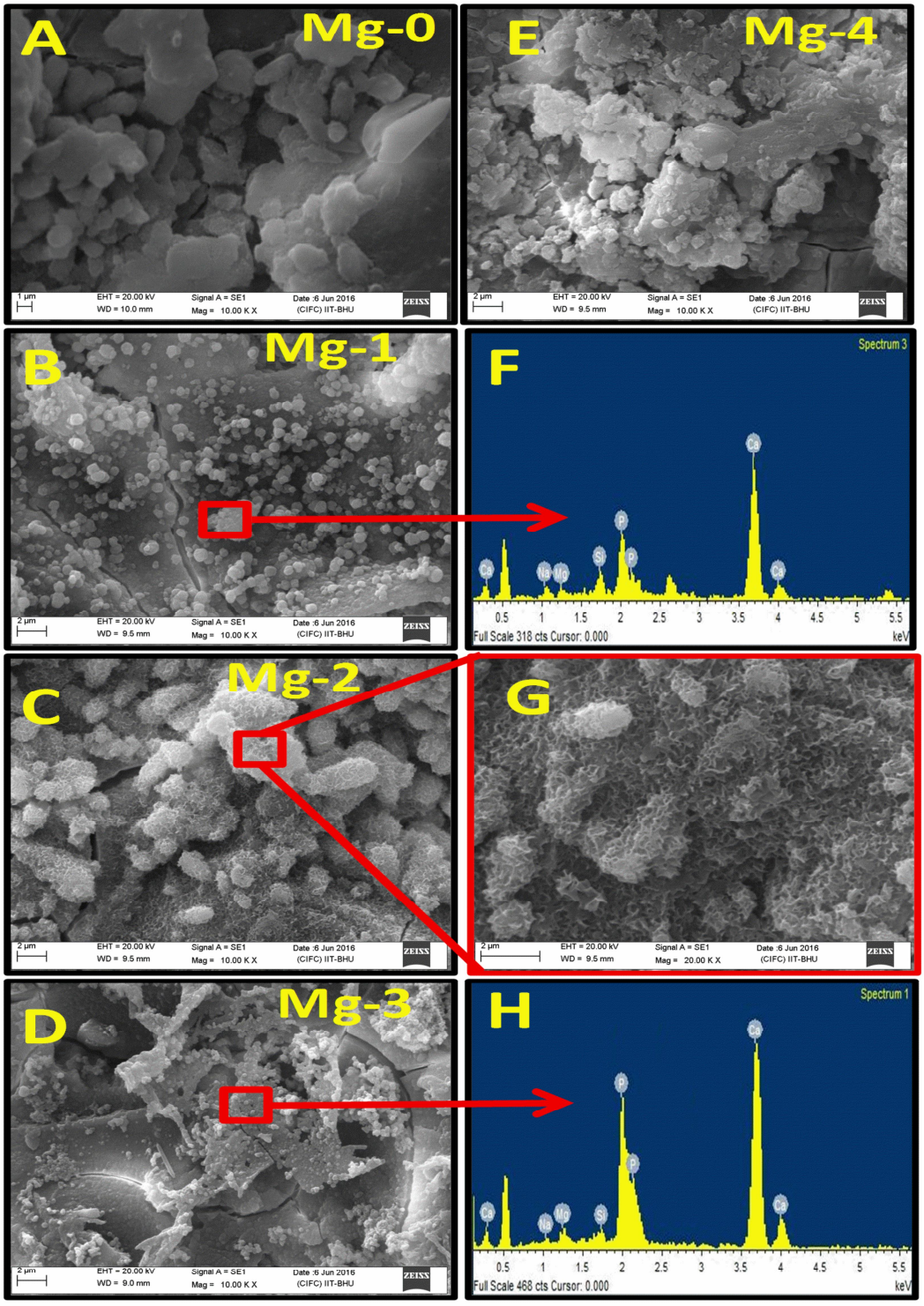

Figure 6.12 SEM images show cell attachment and growth on (A) Mg-0, (B) Mg-1, (C) Mg-2, (D) Mg-3 and (E) Mg-4 bioactive glasses after 5 days culture and (F) & (H) EDS spectra of Mg-1 and Mg-3 samples, respectively and (G) shows the bone like structure at higher magnification of Mg-2 sample.

D. Hemolysis assay

Hemolysis takes place when the red blood cells (RBC) come in contact with implant materials and it is very important to check before their clinic trials. Hence, *in vitro* blood compatibility of the bioactive glasses was determined by % hemolysis. The experiment has been carried out for the prolonged time periods (0.5, 1, 2 and 4 h) at 50 mg/ml concentration. Hemolysis (%) caused by the bioactive glass sample is presented in **Figure 6.13.** These results give a clear understanding of the effectiveness of glass compositions on blood compatibility. The bioactive glass samples did not affect RBC with respect to prolonged time periods and these results are well within the limit of less than 5% [143]. However, it was found that the % hemolysis has increased considerably with increasing time period but still it was within the limit. Thus, the substitution of MgO for SiO_2 in bioactive glass did not affect on blood and the samples are tolerant to the RBC and may be considered as non-haemolytic.

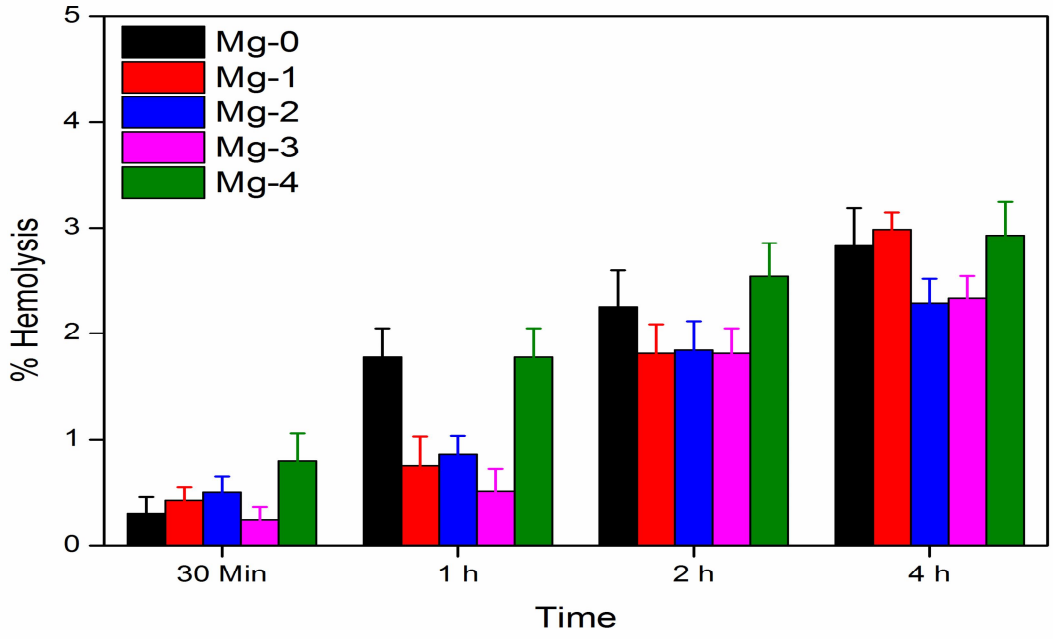

Figure 6.13 Blood hemolysis caused by Mg-0 Mg-1, Mg-2, Mg-3 and Mg-4 bioactive glass samples after different incubation time periods.

6.4 Conclusions

It can be concluded from the results that the substitution of MgO for SiO_2 has significantly effected on glass network. Further, the compressive strengths of the bioactive glasses were found to increase with increasing concentration of magnesia for silica. All the bioactive glasses have possessed the HCA layer formation in SBF as confirmed by pH behavior, FTIR, XRD, SEM and EDS analysis, but it was more prominent in the new bioactive glasses where SiO_2 was partially replaced by MgO. The low NCB glasses exhibited better cell compatibility and growth of human osteosarcoma U2OS cells as well as human blood compatibility as compared to reference glass sample (Mg-1). Furthermore, the cells were found to attach and grow on the surface of the bulk samples significantly. The present work demonstrated that the substitution of MgO for SiO_2 has a significant benefit over CaO. Thus, these bioactive glasses are proposed herewith to be potential material for bone regeneration.