6 Zirconia substituted 1393 bioactive glass Zinc Oxide composite system

6.1 Introduction

Bioactive glasses are the category of bio-materials that has wide application in the biomedical industry such as orthopaedics and surgery. The main characteristic of bioactive glass is the formation of an apatite-like layer on the surface after immersing in simulated body fluid (Hench et al., 2010). SBF has ion concentration and pH similar to human blood plasma. On implantation, some kinetic reactions were leading to the formation of an amorphous calcium phosphate layer that crystallizes into a biologically active hydroxycarbonate apatite (HCA) phase. This HCA layer is responsible for interfacial bonding and chemically and structurally equivalent to the mineral phase of bone (Kokubo et al., 2006). Conventional melting and sol-gel method have been used for bioglass synthesis. In the melting method, all oxide precursors were ball milled, melted in a Pt-crucible, and quenched in water to get an amorphous bioglass. The evaporation of P_2O_5 occurs at high temperatures in the melting method therefore the sol-gel method has increasingly been used than the melt method. The advantage of this method is that it requires lower processing temperature and materials produced by this method are homogeneous with high purity. The glasses formed by this method have higher apatite formation ability and controlled bioactivity and biodegradability. Due to higher surface, Si-OH groups, glasses show higher functionalizing ability (Bellucci et al., 2015). Several modifications in the composition of bioglasses were applied to enhance bioactivity and degradation properties and to import some biological properties such as blood vessel formation and wound healing characteristics. Yadav et al., 2017 synthesized 1393 bioglass by substituting ZrO_2 in1.5 mol% in a glass system and found improved bioactivity and mechanical properties. The changes in glass composition and nature import some biological properties such as bacteriostatic, cariostatic, etc. which makes it applicable for clinical applications (Black et al., 2013). Linati et al., 2005 have reported that the thermal and mechanical properties of silicate and borosilicate glasses were improved by adding zinc oxide, while the chemical durability of phosphate glasses was improved with zinc addition. Zinc is known as an essential trace element and stimulates bone formation in both in-vitro and in-vivo. Despite the established roles of Zn in bone metabolism, the feasibility of Zn-containing biomaterials in clinical applications still relies on many factors, especially safety issues associated with the zinc content. Zinc promotes bone proliferation, differentiation, and apatite formation in bioglass (Ali et al., 2020).

This chapter focuses on the evaluation of the biocompatibility and bioactive properties of biocomposite by adding ZnO up to 5.0 wt% in zirconia substituted 1393 bioactive glass prepared by sol-gel method. Biocompatibility was measured by cell viability on MG 63 cell lines. In all experiments, the zinc-containing composites were compared with parent bioactive glass.

6.2 Materials and Method:

6.2.1 Preparation of substituted bioactive glass/ ZnO composites

ZrO_2 substituted 1393 bioactive glass was synthesized by the sol-gel method. The Tetraethyl Orthosilicate was mixed with 0.1N HNO_3 solution for the hydrolysis process. After 45 min stirring, Triethyl phosphate was mixed into the prior solution and stirred for 45 min. Further, Calcium nitrate tetrahydrate, Magnesium nitrate hexahydrate, Sodium nitrate, Potassium nitrate, and Zirconium oxynitrate hydrate were added and stirred until complete sol formation has occurred. Obtained sol was aged and gel dried at 60°C for 24 hr. The dried gel was sintered at 600°C to get the amorphous structure of glass. To prepare composites, the substituted 1393 bioglass and zinc oxide powder was milled together in a different weight ratio. The prepared composites were presented in **Table 6.1**.

Table 6.1 Composition of zirconia substituted bioglass and Zinc oxide composite.

Sample Code	Zinc Oxide (wt. %)	Bioactive glass (wt. %)
BZn0	0	100
BZn2	2	98
BZn4	4	96
BZn5	5	95

6.2.2. Characterization of Composites

To analyze the phases of composites, powders were subjected to X-ray diffraction with Cu-Kα radiation from 2θ range from 10°-70° and step size of 0.02°. The ATR FTIR was used to analyze the functional groups present in substituted bioglass composites. For this study, the transmittance spectrum was analyzed from wave number of 400-4000 cm^{-1} range.

6.2.3. In-vitro bioactivity in SBF

For bioactivity analysis, the apatite formation ability of all substituted bioactive glass composites was determined by immersing in the SBF for 1-28 days at 37°C. The samples in powder form were soaked in SBF and kept in an incubator at 37°C for up to 28 days. The ratio of powder to the SBF was maintained at 100:1. The SBF was unchanged during the process. After completion of immersing time, the powders reacted with SBF were filtered, dried, and further analysed for FTIR to determine the hydroxyapatite phase developed due to ion exchange between composites and SBF. Also, the pH values during SBF immersion were recorded by a pH meter after a defined duration.

6.2.4. In-vitro biocompatibility assay:

For any biomaterial, hydroxyapatite formation is not the only criteria to implant. The reaction with human osteoblast cells is also important to analyze. For this, the human osteosarcoma cell line MG63 has been purchased from the National Center of Cell Science, Pune, India, and their reactivity with the material was analyzed by MTT assay in terms of cell viability. The osteosarcoma MG 63 cells proliferation was evaluated after exposure to different Zinc bioglass composites using a light-sensitive dye (3-(4, 5dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide) (MTT) assay. The %cell viability was measured using the equations (3.5 and 3.6).

6.2.5. Statistical analysis:

The results obtained from biocompatibility are expressed as means ± SD of each composite. The Statistical Package for the Social Sciences (SPSS 16.0) software (IBM) was used for statistical analysis and analysis of variance (ANOVA) was used for statistical comparisons using $p<0.05$. The post hoc analysis using turkey's test was used to calculate the differences between composites.

6.3 Results and Discussion:

6.3.1 Characterization

The XRD pattern of substituted bioglass and zinc-containing bioglass composites were shown in **Figure 6.1**. The parent bioglass has an amorphous structure confirmed by a broad hump from 25-37°. The hump indicates the formation of silicate glass. In zinc-containing composites, the sharp diffraction peak of zinc oxide with wurtzite type structure and hexagonal phase (P63mc space groups) at 2θ= 31.8°, 34.5°, and 36.33° were present.

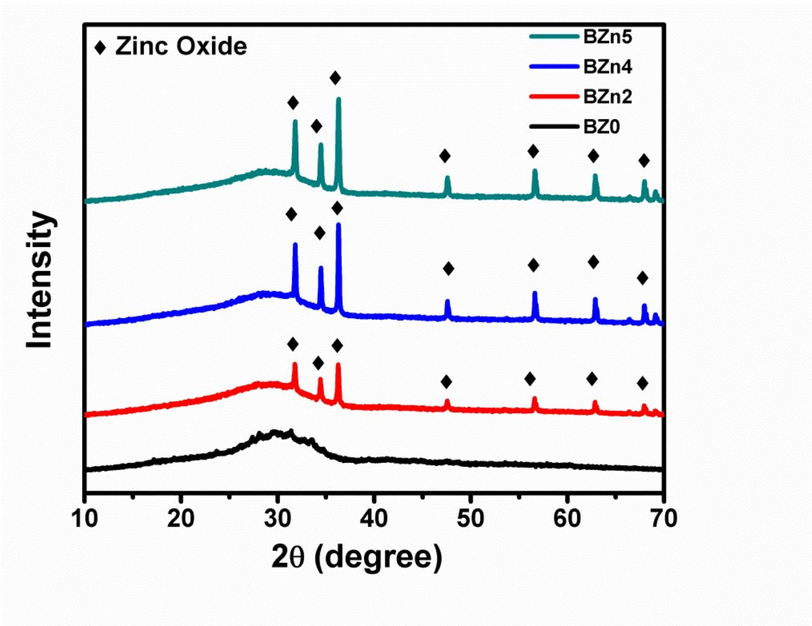

Figure 6.1 XRD pattern of substituted bioglass and zinc oxide composites.

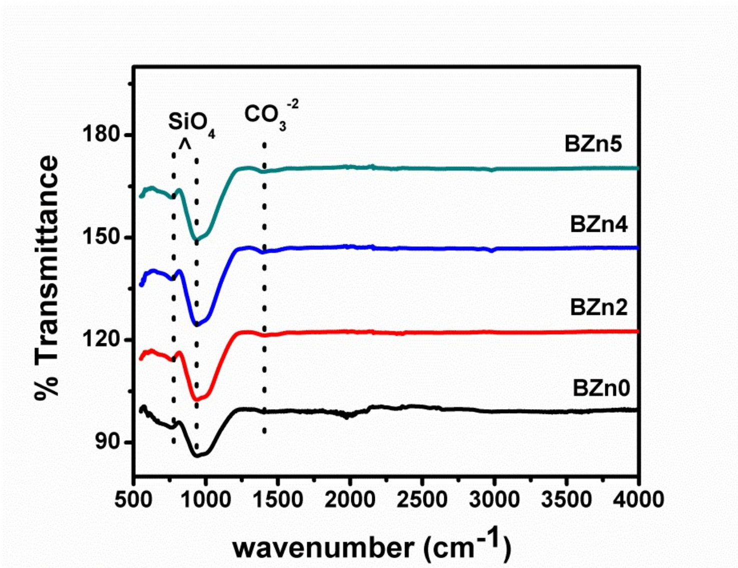

Figure 6.2 FTIR of substituted bioglass and zinc oxide composites.

FTIR spectra of the bioactive glass and composites before immersing in SBF are presented in **Figure 6.2**. The zirconia substituted bioactive glass and composites show the characteristic band of the silica network. The bands at 634, 934, and 770 cm^{-1} were contributed to the SiO_4. Transmittance bands at 1446 cm^{-1} correspond to the CO_3^{-2} group formed due to the reaction of bioglass with CO_2 present in the atmosphere during the formation process (Yadav et al., 2020).

6.3.2 In-vitro bioactivity in SBF

Figure 6.3 shows the variation of pH of the composite sample after immersing in simulated body fluid (SBF) for 28 days. It shows that for all composite samples, the pH increases to 7 days as compared to the initial pH of the SBF solution at 7.4. The increase in pH values is due to the fast release of Na^+, Ca^{+2}, and Zn^{+2}, etc. through exchange with H^+ or H_3O^+ ions into the simulated body fluid (SBF). The H^+ ions are being replaced by cations such as Na^+, Ca^{+2}, and Zn^{+2}, etc. which cause an increase in the hydroxyl concentration of the solution. This leads to an attack in the silica glass network, which results in the formation of hydroxy carbonate apatite (HCA) leading to a decrease in pH which is observed in **Figure 6.3** as composite samples immersed in simulated body fluid (SBF) for 14 to 28 days (Himanshu et al., 2016).

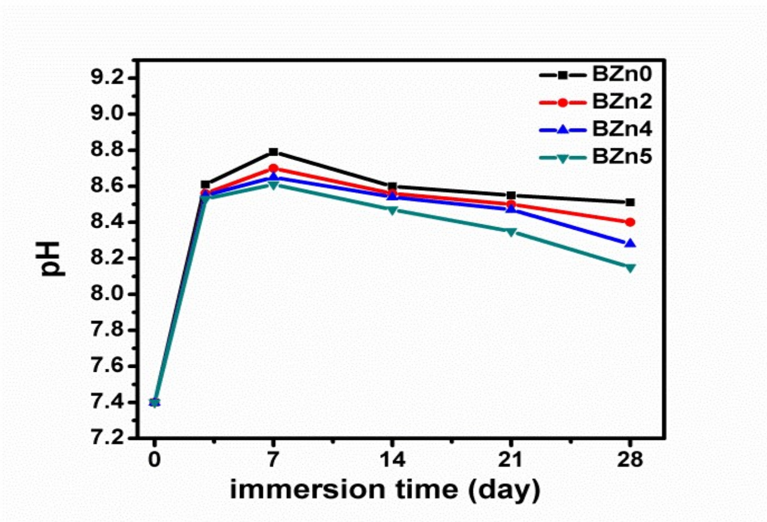

Figure 6.3 Variation of pH of the SBF solution containing composite samples with different time intervals.

Figure 6.4 shows the Transmittance spectra of composite samples after 7 days immersion in SBF. FTIR spectra show the strong peak at 1026 cm^{-1} is related to asymmetric stretching vibration mode of PO_4^{-3} group which confirms the hydroxyapatite formation.

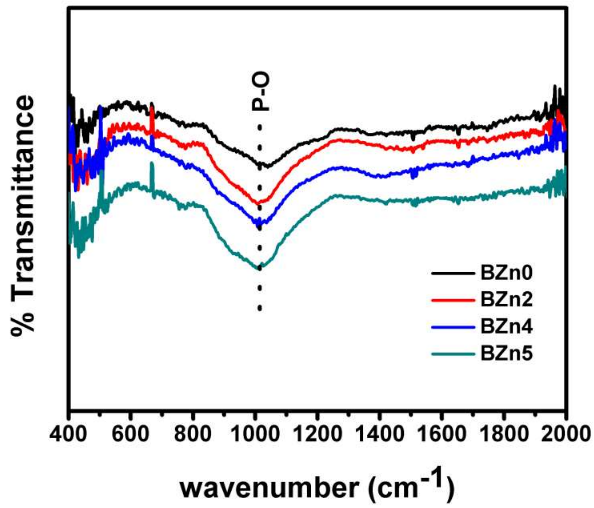

Figure 6.4 Transmittance FTIR spectra of bioglass zinc oxide composites after 7 days immersion in SBF.

6.3. 3 In-vitro biocompatibility of composites

Figure 6.5 shows the MTT result of osteosarcoma MG 63 cell line containing bioactive glass and composite for 2 and 7 days. The parent bioglass BZn0 shows no significant difference in the viability of the MG 63 cell line with the increasing culture day which indicates no proliferation in parent bioglass. However, it shows lower viability than BZn2 and BZn4 but higher viability than BZn5 composites. **Figure 6.5** also depicts that increasing the ZnO amount in composite promotes proliferation with culture time. The viability of composite BZn5 is very low as compared to other composites and considered toxic to MG 63 cells. The BZn4 composite only shows an adequate proliferation for the first 2 days.

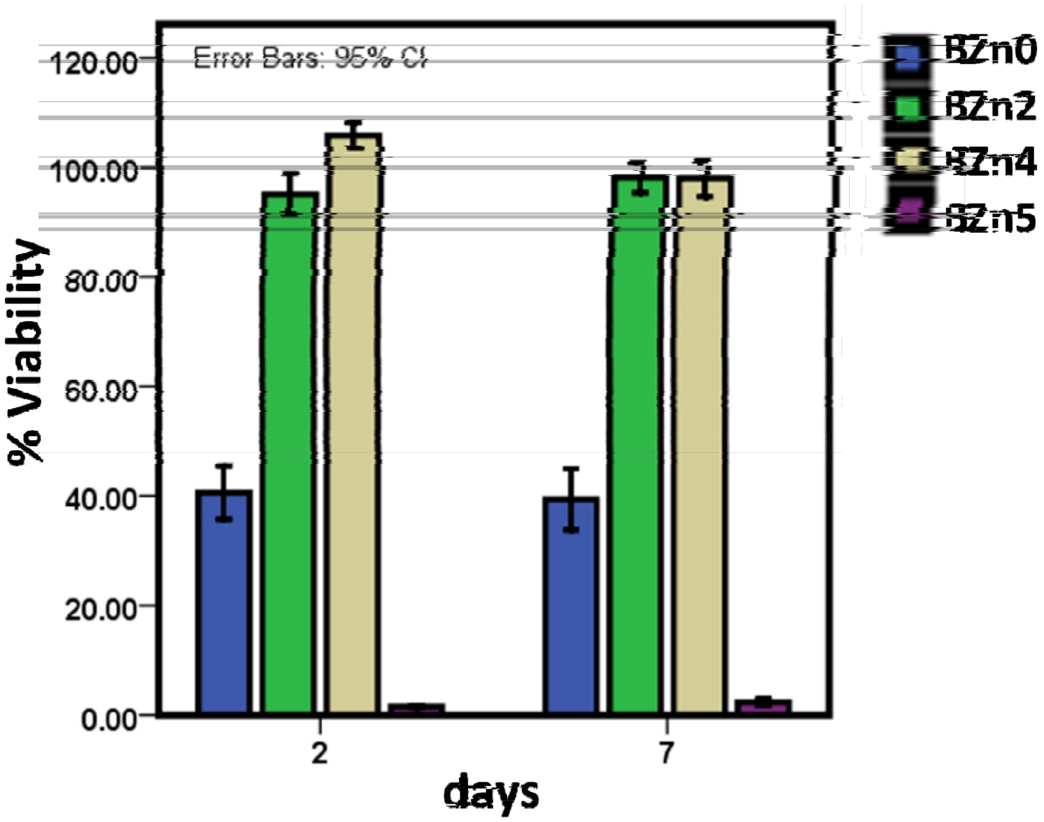

Figure 6.5 Biocompatibility of composites in terms of % cell viability on MG-63cells.

6.4 Conclusion

In this chapter, the aim was to improve the biological properties of zirconia substituted1393 bioactive glass and zinc oxide composites. The Zinc oxide was added up to 5wt% in the bioglass. The structural, functional, bioactivity, and cell compatibility on osteoblast MG 63 cells were investigated. The composites show the presence of zinc oxide phase. All composites show apatite formation in SBF. The cell compatibility shows that all composites are viable and zinc addition promotes the proliferation in bioglass composites. Composites up to 4wt% of zinc oxide show a significant difference in proliferation than zirconia substituted bioglass. The composites contain 2 wt% and 4 wt% zinc oxide show a significant difference in proliferation after 2 days but no significant difference after 7 days.

From the above results, we can conclude that zinc oxide up to 2 and 4wt% in bioglass makes good composite materials for biomedical application.