7 Zirconia substitute 1393 bioactive glass baghdadite composite system

7.1 Introduction

Bioactive ceramics used for bone regeneration should have properties to promote bone growth (osteoconduction), form new bone (osteogenesis), and vascularization (angiogenesis). The bioactive behavior of bioglass is characterized by forming hydroxy carbonate apatite (HCA) layer during immersion in SBF. During exposure to the biological fluid, they also promote osteoinduction. The bioactive and osteoinductive behavior makes bioactive glasses a suitable material for bone regeneration application. The substitution of metal ions or incorporation of bioceramics into glass matrix enhances the biological behavior of bioglass (Hoppe et al., 2015). Several researchers reported that bioactive glasses increase osteoblast proliferation, differentiation and improve cell adhesion. The bioactive glasses have a faster rate of apatite formation than hydroxyapatite in in-vitro and in-vivo. However poor fatigue strength, brittleness, and particle migration restrict them for load-bearing materials (Killion et al., 2013). After 45S5 bioactive glass, several modifications are done in the composition of bioglass to improve the biological and mechanical properties. 1393 bioactive glass is a modified form of 45S5 bioglass. It has a high viscosity, low crystallization behavior, and better bioactivity than 45S5.

Calcium silicate-based ceramics have better apatite formability and good mechanical strength. Due to this, these ceramics have been proposed for tissue engineering applications. But the main disadvantage is their chemical instability (Roohani-Esfahani et al., 2012). To provide stability, zirconia was incorporated in calcium silicate to enhance the biological and mechanical properties and identified as baghdadite. The baghdadite, a calcium-silica-based ceramic has wide application in bone tissue engineering due to the presence of Si, Ca, and Zr ion which provides bone formation, regeneration, and remodelling. During degradation, the released ions promote differentiation and proliferation of osteoblast cells (Yadav et al., 2021).

Baghdadite has a young modulus of 82-120 GPa, fracture toughness of about 1.3±0.1 MPa $m^{1/2}$, and bending strength of 96±16 GPa (Najafinezhad et al., 2017). As compared to wollastonite, baghdadite has higher adhesion, proliferation, and differentiation of osteoclasts, osteoblasts, and endothelial cells. But its higher degradation rate in SBF and poor mechanical properties limit it for load-bearing application (Sadeghpour et al., 2014, Schumacher et al., 2015). The composite of baghdadite with bioglass can control degradation rate and improved mechanical properties as compared to single material. A previous study by Yadav et al., 2021 showed that substituting zinc oxide in baghdadite up to 0.5% control the degradation. Further, hemolytic activity and good cell staining on MG63 cells were observed. The in-vitro study of baghdadite ceramic showed the needle-like apatite formation on the ceramic surface after 28 days of immersion in SBF. On the other hand, it is proved that baghdadite is not cytotoxic. To improve the biological and mechanical properties, composites were prepared which are expected to have better properties compared to single-phase materials. Present work aims to prepare zirconia substituted 1393 bioglass/baghdadite composites to study the effect of baghdadite on the degradation, bioactivity, and cell viability of the composites. Zirconia substituted 1393 bioglass was prepared by the sol-gel method and compared with prepared composites.

7.2 Materials and methods

7.2.1 Preparation of substituted 1393 bioglass/ baghdadite composites

Zirconia substituted 1393 bioactive glass was synthesized by the sol-gel method. To prepare bioglass, Tetraethyl Orthosilicate was mixed with an HNO_3 solution of 0.1N 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, and Potassium nitrate were added and stirred until complete sol formation. Obtained sol was aged and gel dried at 60°C for 24 hr. The complete process occurred at room temperature. The dried gel was sintered at 600°C for 4 hr to get the amorphous structure of glass.

The baghdadite ceramic of composition $Ca_3ZrSi_2O_9$ was prepared by solid-state method at 1400°C. Calcium carbonate, Zirconium oxide, and fine grain Quartz in required amounts were mixed and ball milled for the homogenous mixture.

To prepare bioactive glass/ baghdadite composites, the baghdadite ceramic was added in 0, 20, 50, and 80 wt% in bioactive glass and milled using zirconia balls. The prepared composites in their respective compositions were referred to as BG, BG20, BG 50, and BG 80 **(Table 3.5 in Chapter 3).**

7.3 Characterization of composites

The phase composition of composites was analyzed using XRD. The XRD pattern was recorded from 2θ range of 10°-70° using CuKα radiation of wavelength 1.54Å with a step size of 0.02°.

7.4 In-vitro bioactivity behaviour analysis

The In-vitro bioactivity of the bioglass/ baghdadite composites was determined by apatite mineralization ability in SBF (pH=7.4) at 37°C up to 21 days. The composites were immersed in SBF, and the specimen weight to the solution volume ratio was maintained 1:100 mg/ml. After desired time period, the composites were removed from SBF and washed with deionized water, and dried at 60°C. The phase composition after immersion in SBF was analyzed by XRD. The pH value during immersion was determined with a digital pH meter.

7.5 Density measurement

The theoretical density of zirconia substituted 1393 bioglass/baghdadite composites was determined by the rule of mixture and compared with bulk density measured by Archimedes' principle. Three repeated measurements were performed for each composition group. The relative density (ratio of theoretical density to bulk density) is also determined.

7.6 Results and discussion

7.7.1 Structural analysis of composites

X-ray diffraction analysis was used to investigate the structural properties of powders obtained by synthesized substituted 1393 bioglass, baghdadite and its composites named as BG0, BG20, BG50, and BG80. It was observed that synthesized bioglass is of amorphous nature showing a broad hump of 2θ range from 25° to 37°. Baghdadite prepared at 1400°C shows monoclinic structure and good matched with reference code 98-003-3844. All composite samples show the diffraction peaks of baghdadite only which indicates that no chemical reaction occurred between bioglass and baghdadite. Only a noticeable change in intensity was observed in diffraction pattern.

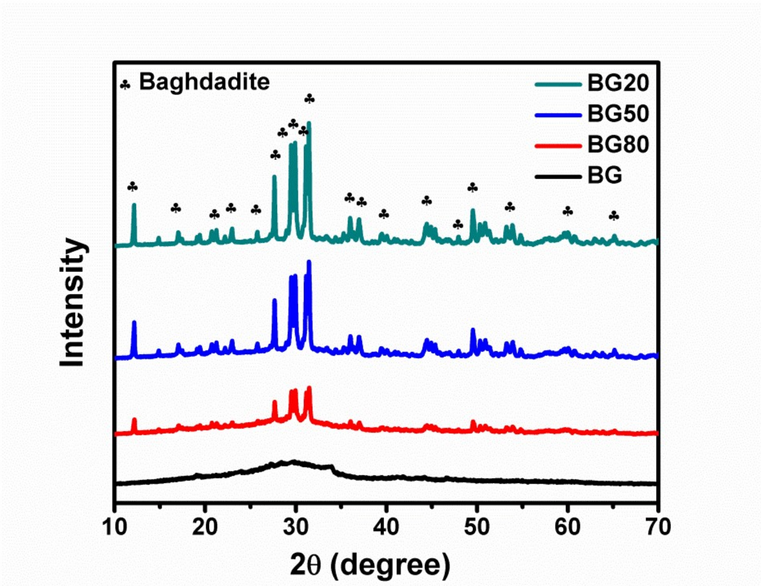

Figure 7.1 XRD pattern of composites.

7.7.2 In-vitro bioactivity analysis

Figure 7.2 shows the X-ray diffraction pattern of zirconia substituted bioglass and baghdadite composites immersed in SBF in 7 days of intervals recorded at the diffraction angle (2θ) in ranges between 10-70°. Before immersion, the samples had only peaks of baghdadite. After immersion, the composite samples exhibited additional peaks of hydroxyapatite with baghdadite; which indicates the formation of hydroxyapatite layer (HAp: $Ca_{10}(PO_4)_6(OH)_2$) on the surface of the samples. This is due to the ion leaching from sample to SBF and vice versa. After soaking for 7 days, the presence of diffraction peaks at 31.74° and 29.4° in the XRD diffractograms indicates the formation of crystalline calcium phosphate.

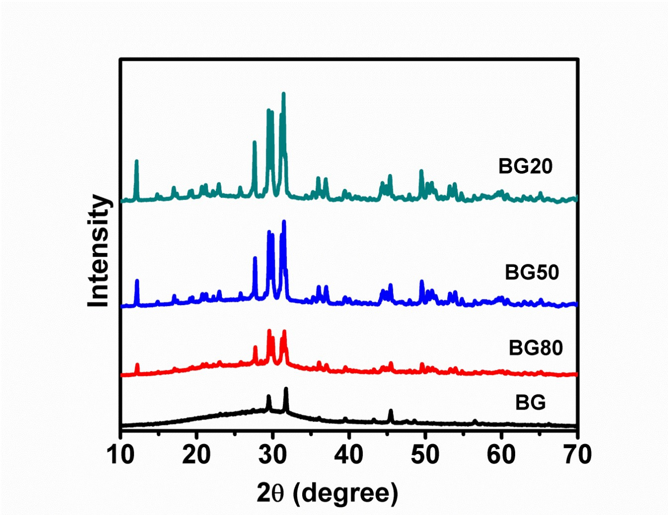

Figure 7.2 XRD pattern of composites after 7 days immersion in SBF.

7.7.3 pH analysis

The changes in pH value of composites during immersion in SBF were presented in **Figure 7.3**. The pH behaviour is observed due to the ion exchange process in SBF. The pH value suddenly increases to first 7 days which was due to fast release of alkali/alkaline ions present in composites such as Na^{+}, K^{+} , Mg^{+2}, and Ca^{+2} , Zr^{+4} in SBF. The fast releasing of these alkali/alkaline ions increases the basic nature of SBF and increases the value of pH. The exchange of alkali with H^{+} or H_3O^{+} ions of SBF solution formed negatively charged group of silanol (Si-OH) and Zr-OH. The formed Si-OH group starts apatite nucleation and this apatite nucleation accelerated with release of Ca^{2+} and Zr^{4+} ions. The migration of Ca^{+2} and PO_4^{-3} ions occurred through silica layer and form $CaO–P_2O_5$ layer. This film crystallized and form apatite layer over the composite surface. The decrease in pH value was arising due to consumption of OH^{-} ions which confirms the formation of more apatite.

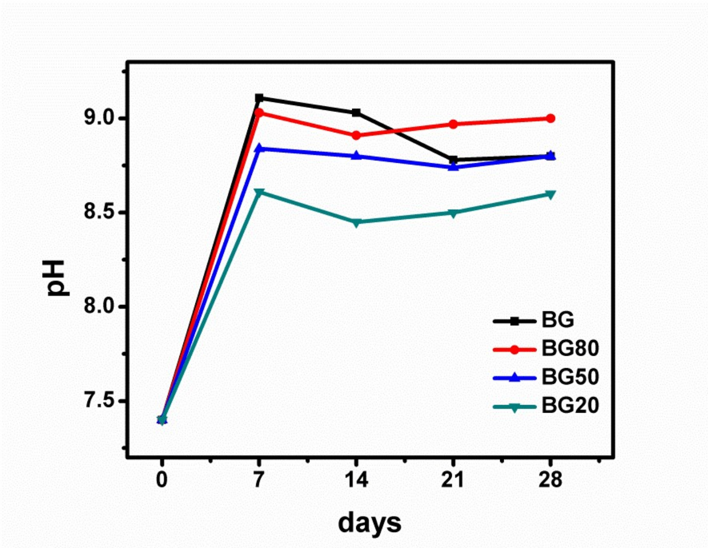

Figure 7.3 pH value of composites after immersion in SBF.

7.7.4 Density measurement

The density of composites was measured by Archimedes principle whereas the theoretical density is calculated by the rule of mixture. The theoretical density of zirconia substituted bioglass was 2.86 g/cc, whereas by Archimedes principle is 2.5 g/cc. The reinforcement of baghdadite in bioglass leads to an increase in density. This increasing trend was due to the higher density of baghdadite than bioglass. The theoretical density calculated by the rule of the mixture was 3.33g/cc, 3.145 g/cc, and 2.974g/cc for composites containing 80, 50, and 20wt% of baghdadite respectively. While the density of composites calculated by Archimedes principle was 3.12g/cc, 2.845g/cc, and 2.65g/cc for composites containing 80, 50, and 20wt% of baghdadite respectively.

7.7 Conclusion

The aim of this chapter is to study the effect of baghdadite in zirconia substituted 1393 bioactive glass on structural and biological properties. The bioglass synthesized at 600°C has an amorphous structure whereas the baghdadite prepared at 1400°C has a monoclinic structure. The composites were prepared by mixing baghdadite with bioactive glass in composition ranging from 20, 50, and 80wt%. The prepared composites show the only phase of baghdadite. The in-vitro bioactivity behavior of composites was analyzed by immersing in SBF and found that all composites have apatite formation ability after 7-day immersion in SBF. The bulk density of composites was also measured and found that baghdadite reinforcement improves the density of composites.