8 Conclusion and Future Scope

8.1 Conclusion

This thesis deals with the preparation of some biocomposites (bioglass hydroxyapatite composite system, baghdadite zinc oxide composite, bioglass zinc oxide composite, and bioglass baghdadite composite) and their in-vitro evaluation aiming at their applicability for bone tissue regenerative application.

Herein, a summary of Chapter 1 to Chapter 7 has been delineated in brief.

In Chapters 1, 2 and 3 a comprehensive introduction, literature review, and detailed materials and methods were elaborated.

In **Chapter 4**, the zirconia substituted 1393 bioactive glass system was mixed in different proportions of hydroxyapatite and sintered at two different temperatures. The crystallization of bioglass reduces the apatite layer formation ability, therefore to improve this property; hydroxyapatite was added to the bioglass. An exothermic peak was started from 960°C that shows the crystallization temperature of composite samples. The hydroxyapatite with Akermanite was found in composites sintered at 1000°C whereas only the hydroxyapatite phase was found in composites sintered at 600°C. Bulk density (ranging from 2.5 to 2.98 g/cc) and compressive strength (ranging from 34.44 to 52.13 MPa) were improved with aid of sintering temperature and hydroxyapatite amount. In composites sintered at 600 °C; the bioglass with 80 wt% hydroxyapatite and, in composites sintered at 1000 °C, the bioglass with 50 wt% hydroxyapatite exhibit the highest proliferation of MG63 cells.

In Chapter 5 the aim was to improve the biological and antibacterial properties of baghdadite containing zinc oxide composite materials. With increasing zinc oxide content, compressive strength and density of composites have improved. All composites show the formation of the crystalline apatite layer with a controlled degradation rate in SBF. The antibacterial results suggest that the antibacterial performance of baghdadite/zinc oxide composite was dependent upon the zinc oxide content in the composite. Composite with

higher zinc oxide amount shows a higher antibacterial effect and more effective against *E*. *Coli*. The hemolysis rate was found <5% for blood compatibility of the composite. The DAPI staining on MG63 cells shows the good attachment of cells over the baghdadite surface.

In Chapter 6 the zirconia substituted bioglass was mixed with zinc oxide up to 5wt%. In composites, a peak of Zinc oxide was found which shows that no chemical reaction between bioglass and zinc oxide was taking place. 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 biocomposite materials for biomedical application.

In Chapter 7 the zirconia substituted 1393 bioactive glass was mixed with baghdadite in different amounts. The apatite formation ability, pH behavior and density were determined on the composites. In composites, the baghdadite was found as a major phase. All composites show hydroxyapatite formation after immersion in SBF. The baghdadite reinforcement improves the density of composites. The cell viability of composites was measured on MG 63 cells in terms of proliferation and found that all composites are viable and proliferate on the cells.

Salient Features
• The hydroxyapatite with Akermanite was found in composites sintered
at 1000°C whereas only the hydroxyapatite phase was found in
composites sintered at 600°C.
• Bulk density (ranging from 2.5 to 2.98 g/cc) and compressive strength
(ranging from 34.44 to 52.13 MPa) were improved with aid of sintering
temperature and hydroxyapatite amount.
• In composites sintered at 600 °C; the bioglass with 80 wt%
hydroxyapatite and, in composites sintered at 1000 °C, the bioglass with
50 wt% hydroxyapatite exhibit the highest proliferation of MG63 cells.
A reduction in synthesis temperature was found due to incorporation of
ZnO.
• Increasing zinc oxide content, compressive strength and density of
composites have improved.
• All composites show the formation of the crystalline apatite layer with a
controlled degradation rate in SBF.
• Composite with higher zinc oxide amount shows a higher antibacterial
effect and more effective against E. Coli.
• The hemolysis rate was found <5 % for blood compatibility of the
composite.
• The DAPI staining on MG63 cells shows the good attachment of cells
over the baghdadite surface.

• The Zinc oxide was added up to 5wt% in the bioglass.
• The composites show the presence of zinc oxide phase.
• The cell compatibility shows that all composites are viable and zinc
addition promotes the proliferation in bioglass composites.
• 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.
The prepared composites show the only phase of baghdadite.
• All composites have apatite formation ability after 7-day immersion in
SBF.
• The baghdadite reinforcement improves the density of composites.

8.2 Future perspectives

"If we knew what it was we were doing, it would not be called research, would it?"

- Albert Einstein

A good research work should ask more questions to ponder and investigate further potentialities. Similarly, based on the techniques and results discussed in this work, few ideas for future perspectives are suggested here.

1. Developing superior mechanical properties

In these investigations we observed that the Zirconia substituted 1393 bioglass and calcium zirconium silicate ceramics are excellent biomaterials. They can be converted to hydroxyapatite (the mineralogical component of bone and teeth) and tailored to the natural bones. However, due to the brittleness, we cannot achieve the desired mechanical strengths just by using them only. From the material science perspective, the main objective is to engineer mechanically competent systems. The composites developed in this work could be improved, in terms of mechanical strength, by forming metal-matrix composites (khodaei et al., 2019, Kowalski et al., 2017). This will enable the composites to compete in load-bearing applications. Furthermore, the bioactivity of the construct will not be inhibited.

2. Investigation of ALP, and alizarin red stain activity of composites

ALPs are plasma membrane-bound glycoproteins which are widely distributed in the nature, including plants, algae, bacteria, fungi and animals. Robison et al, in 1923, made two important discoveries which reports the presence of phosphatase in bone, especially growing bone and the role of phosphatase in bone formation. He also pointed out the absence of calcification in tissues not containing ALP. He believed

ALP to be the cause of bone formation as the main function of ALP is to release phosphate from phosphate esters. Alizarin-red staining is commonly used to detect the formation of calcium nodules (mineralization) formed by osteoblasts in the late stage of differentiation – in osteoblast cultures.

3. Additional in-vivo studies of these derived composites for easy clinical translation for attaining FDA approval and can be marketed.