

## Chapter 7

### Conclusions and future work

*In this chapter, we provide a concise overview of the key results, which include the development and analyses of prepared bioceramics, along with the effects of surface charge on antibacterial properties. Also, the effect of surface charge and dynamic electric field stimulated cellular response as well as toxicity assessment of developed nanoparticles is presented.*

The following are the key findings of this study:

1.  $\text{Mg}_{1-x}\text{Ca}_x\text{Si}_{1-x}\text{Zr}_x\text{O}_3$  [MCSZO-X, X = 0 – 4] and hydroxyapatite (HA) were successfully synthesized by the solid-state and coprecipitation routes, respectively. The sintering temperatures are optimized to be 1380 °C, 1350 °C, 1350 °C, 1320 °C, 1320 °C for MCSZO-X, (X = 0, 1, 2, 3, 4) samples, respectively. The XRD patterns confirm the formation of orthorhombic (protoenstatite system) pure  $\text{MgSiO}_3$  [JCPDS #76-1806] phase with space group Pbcn and lattice parameters [a = 9.25 Å, b = 8.74 Å, c = 5.32 Å]. The position of peaks shifted towards lower  $2\theta$  values from 28.28° – 28.12° with increasing the concentration of Ca and Zr from 0 to 0.3 This is due to incorporation of  $\text{Ca}^{2+}$  ions (r = 1.34 Å) on smaller  $\text{Mg}^{2+}$  (r = 0.72 Å) at A-site and  $\text{Zr}^{4+}$  ions (r = 0.74 Å) at smaller  $\text{Si}^{4+}$  (r = 0.40 Å) on B-site. The grain sizes of MCSZO- X, (X = 0, 1, 2, 3, 4) samples are measured to be 1.69, 1.92, 1.98, 2, 2.01, 0.45 and 2.18  $\mu\text{m}$ , respectively.
2. The X-ray photoelectron spectroscopy (XPS) analyses and contact angle measurement enhances surface hydrophilicity as well as oxygen-deficient active sites (generation of oxygen vacancies to produce active sites for the adhesion of water, which increases the surface hydrophilicity) in  $\text{Mg}_{1-x}\text{Ca}_x\text{Si}_{1-x}\text{Zr}_x\text{O}_3$  samples without altering surface chemistry as compared to HA control, especially for negative end of electret.

3. The combined effect of electrostatic and electrodynamically stimulated *in vitro* cellular response of the prepared MCSZO-X electrets and HA bioceramics were evaluated, both quantitatively and qualitatively using human osteoblast-like MG-63 cells. The results reveal that the formation of electret and electrodynamic field stimulation accelerates cell adhesion, growth, and proliferation on negative end surfaces of MCSZO-X. Also, the mechanism of enhanced cellular functionality was revealed by the measurement of intracellular  $\text{Ca}^{2+}$ , demonstrating the activation of calcium channel pathways initiated by electrostatic-dynamic electrical stimulation.
4. The *in vitro* antibacterial response of the prepared MCSZO-X electrets and HA bioceramics were evaluated, both quantitatively and qualitatively using *E. coli* and *S. aureus* bacteria. The results reveal that the bacterial viability of *E. coli* bacteria was reduced by (29, 37, 39, 51, and 48%) and (24, 25, 30, 43 and 42 %,.) on both, negative and positive end surfaces of MCSZO-X electrets, respectively, in comparison to uncharged HA. For *S. aureus* bacteria, the bacterial viability was reduced by (21, 22, 27, 39, and 37%) and (26, 33, 35, 46, and 42%) on both, negative and positive end surfaces of MCSZO-X electrets, respectively, as compared to uncharged HA. In addition, the results obtained from different tests on enzymatic activities indicated that the production of reactive oxygen species (ROS) and the subsequent bacterial damage are most prominent on positive end of MCSZO-X electrets as compared to uncharged MCSZO-X and HA samples, respectively.
5. The surface charge of MCSZO-X electrets sample, induced via corona poling at 20 kV and 500 °C, was measured at 0.25, 0.29, 0.32, 0.17 and 0.16  $\mu\text{C}/\text{cm}^2$ , for X=0, X= 0.1, X= 0.2, X= 0.3 and X= 0.4 respectively.
6. The primary evaluation of toxicity of osteoblast-like MG-63 cells after treating with varying concentrations (0.25, 2.5, and 25 mg/ml in normal saline) of MCSZO-X (X = 0-

4) nanoparticles, demonstrate cell proliferation on MCSZO- X nanoparticles up to a concentration of 25 mg/ml. Subsequently, the *in vivo* toxicity was evaluated through the intraarticular injection of MCSZO-X nanoparticles (100 µl of 25 mg/ml each) into the knee joint of Wistar rats over a 7-day period. After the intra-articular injection, the rats did not exhibit symptoms such as, diarrhea, tremor or convulsions. Additionally, after seven days of post-injection, no notable changes were observed in behavior of rats.

7. The biochemical parameters (ALP and creatinine) revealed that MCSZO - X nanoparticles had no toxic effect on functioning of vital organs (liver and kidney). The histopathological examinations of essential organs such as the heart, liver, and kidney, indicate that there were no observable morphological alterations in the tissue structure of rats treated with nanoparticles. This finding confirms the lack of any inflammation or dispersion of nanoparticles in crucial organs like the heart, liver, and kidney.

Overall, among all the developed  $Mg_{1-x}Ca_xSi_{1-x}Zr_xO_3$  ( $x=0, 0.1, 0.2, 0.3, \text{ and } 0.4$ ) bioceramics samples, the electrodynamically stimulated negative end of higher concentration of Ca and Zr doped  $MgSiO_3$  electrets i.e.,  $Mg_{0.7}Ca_{0.3}Si_{0.7}Zr_{0.3}O_3$  demonstrate higher osteogenic response. The MCSZO-X electrets show excellent antibacterial behavior as compared to uncharged MCSZO-X samples and control (HA), while cultured with both, *S. aureus* and *E. coli* bacteria. The *in vivo* performed on the rat model reveal the confirmation of non-toxic behavior of MCSZO-X nanoparticles.

### **Scope for the future**

1. Combined action of electrostatic and electrodynamic stimulation along with compositional variation can be executed to enhance the antibacterial activity of the MCSZO-X sample.
2. The surface charge-induced bioactivity and biodegradability can be explored to improve the properties of MCSZO bioceramics.

3. It is well known that piezoelectric nature of material act as a stimulator for osteogenesis performance. Therefore, the measurement of piezoelectric properties of the MCSZO-X should be performed under the action of compressive and tensile strength.
4. The measurement of hardness, compressive strength and fracture toughness can be performed, because these are other requirements to make the orthopedic implant successful.
5. The relationship between the charge density vs voltage, temperature, and time of corona, stability in saline/bio-fluid, and its correspondence with electrical stimulation can also explored.
6. After the implantation of MCSZO-X scaffold at the defect site of animal model, the micro-CT can also be performed the evaluate the new bone formation.