

Chapter 1

Introduction

This chapter provides a concise overview of the significance of the current study on silicate-based bioceramics. It also introduces the importance of silicate-based bioceramics for the application of bone tissue engineering along with the role of various ions such as, Mg, Ca, and Si in bone metabolism. Further, surface charge and electrodynamical stimulation have also been proposed as potential solutions to enhance the osteogenic response. Additionally, surface charge-induced antibacterial responses have been discussed, which is a major problem in prosthetic orthopedic implants.

1. Introduction

1.1. Bone

Bone is living tissue, which make the skeleton of our body [1]. The capacity of bone for regeneration enables it to initiate self-repair provided the injury remains below the "critical size defect (CSD)." Notably, the freshly regenerated bone closely resembles healthy bone tissue, making it difficult to differentiate between the two [2, 3]. The point at which bone fractures become non-regenerative is termed the CSD. In these scenarios, comprehensive research has been documented on bone restoration techniques, encompassing the utilization of autografts (healthy tissue taken from patient own body) and allografts (tissue is taken from a healthy person) involving both autologous and allogeneic grafting procedures [4, 5].

Autograft contains indispensable components which is required to achieve osteoconduction, osteogenesis and osteoinduction without risk of immunogenic reaction [6]. Nonetheless, autograft transplantations are costly procedures, entailing significant harm to the donor site, potential health issues, impairment, scarring, as well as surgical hazards: hemorrhaging, inflammation, infection, and persistent pain [6].

In contrast to autografts, allografts exhibit reduced revascularization and integration. Furthermore, they display distinct patterns of remodeling, susceptibility to immune response, and the potential transmission of viral diseases [7]. The bone grafting is the surgical process which replaces the damaged bone with the synthetic substitute [8]. The ideal characteristics for biomaterials to be used in the development of bone graft substitutes encompass four key attributes: chemical compatibility, structural durability, resorbability, and the capability for low-temperature processing [9]. In general, biomaterials are classed into four groups polymer, metal, ceramics and composite [10]. Among them, ceramic based biomaterials are most widely used for filling material and implant in bone tissue engineering application because of excellent bioactivity, and like apatite phase of bone and good osseointegration [11-13].

Recently, biodegradable implants attracted significant attention for variety of clinical applications, including bone regeneration, replacement, and repair [4, 14]. Such perspective implants minimize the possibilities of revision surgery for their replacement and other related concerns [15, 16]. Additionally, the transfer of load from the implant to host tissue occurs successfully if the pace of degradation of the implant coincides well with the rate of neo-bone growth. Bone contains Mg^{2+} , Ca^{2+} , and Si^{4+} ions, which are essential for the regulation of several metabolic processes, including promotion of osteogenesis and prevention of osteoporosis [17-19].

Silicate-based bioceramics come in a variety of chemical compositions with attractive physical, chemical qualities and biocompatibility properties for bone graft applications. Some of the silicate- based bioceramics show the uncontrolled degradation rate, which restricts their widespread applications, such as wollastonite, bredigite and tricalcium silicate [20].The mismatch between the degradation rate of such bioceramic implants and the rate of formation of new bone remain as a major challenge [21].The dissolution properties of these bioceramics

can be tailored with the substitution of various ions like, Ca^{2+} , Zn^{2+} , Sr^{2+} , and Mg^{2+} [14]. Also, the issue of local and systemic toxicity caused by the release of debris particles resulting from wear and degradation is currently one of the main challenges in the designing and developing of prosthetic orthopedic implants. These particles frequently induce inflammation at the site of implantation or cause aseptic loosening of the prosthetic device [22, 23]. Therefore, Thus, the current study aims to assess the toxicity of biocompatible Ca and Zr doped MgSiO_3 nanoparticles through injection into a rat model's knee.

1.2. Significance of Mg, Ca and Si ions in bone metabolism

Silicate-based perovskites are recognized as a new family of biomaterials for hard tissue regeneration [20, 24-26]. In bone and a few connective tissues (such as, skin, hair, and nails), Si content has been reported to be about 100 and 600 ppm, respectively [18, 27, 28]. In human body, the concentration of Si in serum, muscle and lung tissue are 0.6, 41 and 57 ppm, respectively [29-31]. It has been demonstrated that Si ions enhance the proliferation and differentiation of mesenchymal stem cells (MSCs), bone mineralization, synthesis of type I collagen and prevent the osteoporosis [32-36].

Si (0.8 wt. %) substituted hydroxyapatite (HA) stimulates the formation of type I collagen and increases the proliferation of human osteoblast cells (HOBs) [37]. In a similar study, it has been reported that the addition of Si (5 mol. %) into HA increases the proliferation rate of osteoblast cells, where the dissolution of Si doped HA (0.5% Si-HA) was observed to be higher as compared to pure HA [38]. Bavya et al. [39] demonstrated that the Mg^{2+} and Si^{2+} ions, dissolved or degraded from the MgSiO_3 -based implanted materials, enhance the bone regeneration. Overall, Si ion plays a crucial role in bone formation and maintaining its health [35, 40, 41]. In general, Si ions (metasilicate) spread throughout the connective tissues [42]. It has also been reported that the calcification process initiates by the association of Si and Ca ions, i.e., Si is also important in maintaining the bone density [43-45].

Ca is the most abundant (98% of the total body Ca) element, present in the living bone [19, 46]. For the nucleation and growth of HA, Ca plays the crucial role which also influence the metabolic activities of osteoblastic cells [17, 47]. However, it has been reported that higher concentration of Ca^{2+} ions (> 10 mmol.) causes the toxic concern to cells. Whereas, the medium concentration (6 to 8 mmol) of Ca^{2+} is favorable for mineralization of extracellular matrix (ECM). Also, low concentration (2 to 4 mmol) of Ca^{2+} is preferable for differentiation and proliferation of osteoblast cells [46, 48-50]. Generally, 10-15 mg of Ca ions are found per 100 mg of cells or body fluid [42]. It has been reported that a proper balance between the intake and excretion of calcium ions in body is necessary to maintain the calcium homeostatis and bone strength. In human body, Ca homeostasis is maintained by a rapid exchange of Ca ions, present in blood and bone [51].

Mg, the second most abundant element, present in cellular matrix (e.g., 28-35 gm in mature human body, around 50 % in bone, 30-40 % in soft tissues and 1 % in serum) plays a key role in regulating its metabolism [19, 52-54]. Also, the Mg ions augment the proliferation and differentiation of osteoblast cells and mesenchymal stem cells (MSCs). The deficiency of Mg ions in the body enhances the risk of osteoporosis and hamper the growth of osteoblast cells [19, 54, 55]. In presence of Mg ions, the expression of differentiation markers, such as BMP2, OPN Runx2 and COL1 in MSCs and osteoblasts cells were observed to increase. It has also been reported that Mg ions promote the new bone formation by regulating osteogenic gene expression and the activation of the MAPK-ERK pathway [50, 56, 57]. Further, the incorporation/substitution of different ions such as Sr^{2+} , Zn^{2+} , and Zr^{4+} influence the dissolution rate and biocompatibility of silicate- based bioceramics [39, 58, 59]. The response of different ionic dissolution products of Mg and Ca-based silicate bioceramics have been illustrated in Figure. 1.1.

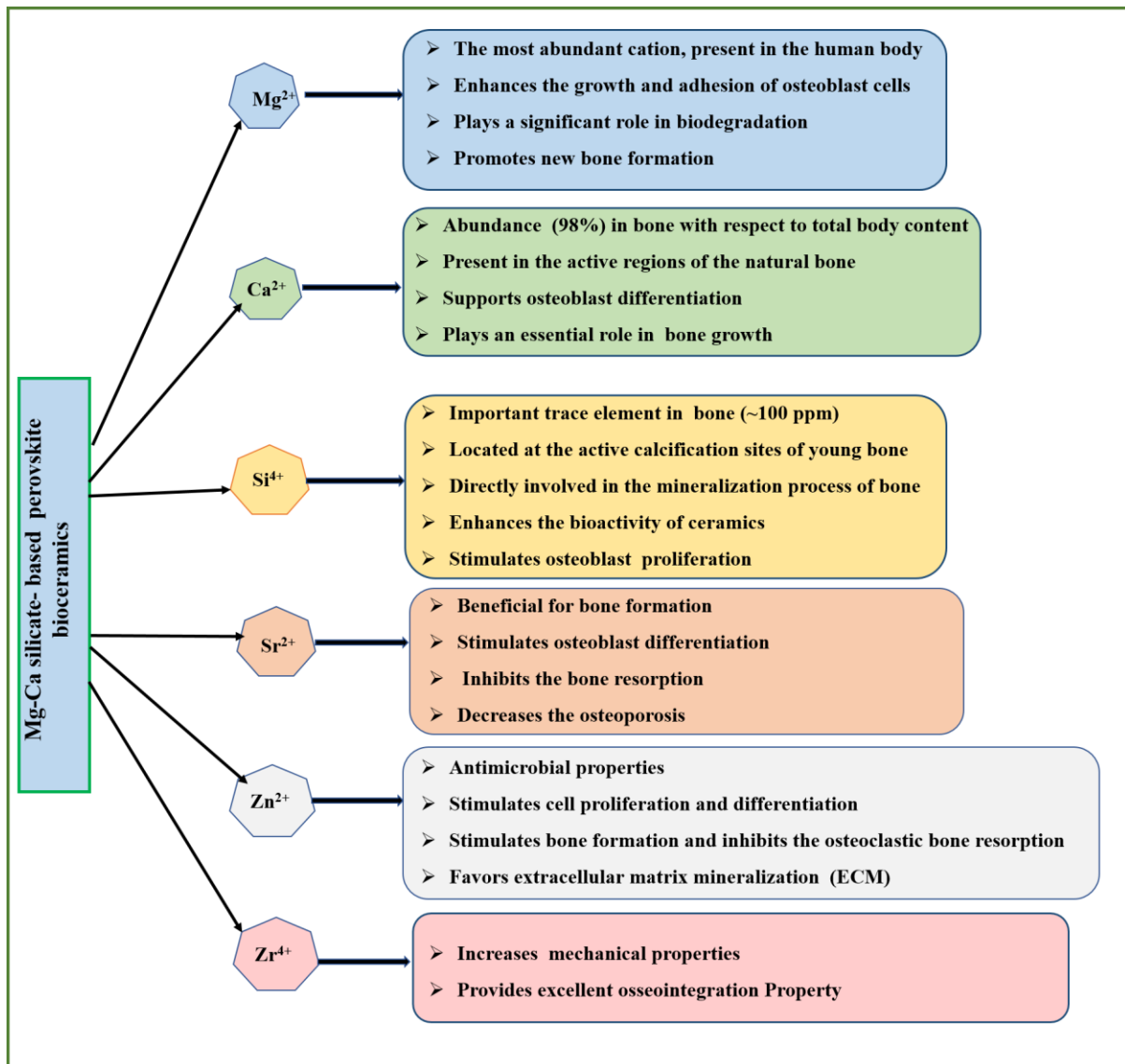


Figure 1.1. Biological response of Mg^{2+} , Zn^{2+} , Ca^{2+} , Sr^{2+} , Si^{4+} and Zr^{4+} ions in Mg/Ca silicate-based perovskite bioceramics.

1.3. Bioceramics

In orthopedic applications, bioceramics show greater promise than other material classes. Among the various types of biomedical materials such as ceramics, metals, and polymers, in which the ceramics are modern biomaterials. Living bone is mostly composed of ceramics, as seen from the standpoint of materials. Therefore, bioceramics have been successfully developed for orthopedic implant applications. Bone is composed of 60% inorganic component (hydroxyapatite, $(Ca_{10}(PO_4)_6(OH)_2)$), 10% water and 30% organic component

(collagen, proteins) [60]. The durability requirement of bone is compressive strength (~131 MPa), bending strength (~160 MPa), fracture toughness (2-12 MPa.m^{1/2}) and hardness (~1 GPa) [61-65]. Bone resorption is a critical process in the maintenance and remodeling of bone tissue. It involves the following key characteristics: like osteoclast Activity (osteoclasts play a central role by secreting acids and enzymes that dissolve bone minerals), Regulation by Hormones (hormones like parathyroid hormone (PTH) and calcitonin help regulate the balance between bone resorption and formation. PTH increases resorption to raise blood calcium levels, while calcitonin inhibits it) and Excessive bone resorption can lead to osteoporosis and bone weakening, while inadequate resorption affects bone remodeling and healing [66, 67].

Additionally, piezoelectric properties in natural bone regulate its metabolism [68, 69]. Because of its piezoelectric characteristic, natural bone undergoes polarization when exposed to mechanical stimulation, contributing to the facilitation of bone growth [70, 71]. The compressive load generates negative charge which promotes the osteoblastic activities. On the other hand, tensile load generates positive charge where osteoclastic activities dominate [72, 73].

Recently, polarization induced improved biocompatibility of piezoelectric bioceramics has been recognized as an interesting strategy for the development of prospective electroactive prosthetic orthopaedic implants [74-79]. Piezoelectric materials such as Na_{0.5}K_{0.5}NbO₃ (NKN) and BaTiO₃ (BT) have gained attention as promising alternatives for polarizable orthopedic implants due to their remarkable biocompatibility with human osteoblast cells [80-82]. MgSiO₃, a piezoelectric material [24, 26], has been reported as a good biocompatible substitute for bone through various *in vitro* and *in vivo* studies. MgSiO₃ is biodegradable, where degraded Mg²⁺ and Si⁴⁺ ions stimulate osteogenic performance through the differentiation of mesenchymal cells into osteoblasts cells [83-86].

Apart from MgSiO_3 , Ca- based silicate bioceramic also was used for bone tissue engineering application due to their excellent bioresorbability and osteoconductivity as compared to Ca phosphate implant. The CaSiO_3 ceramics are well known for their excellent bioactivity [87, 88].

Wu et al. [89] demonstrated that doping of Sr (0-10 mol.%) into the CaSiO_3 results in decreased dissolution rate of CaSiO_3 . Also, Sr doped CaSiO_3 stimulates the proliferation of human bone derived cells (HBDCs), even at lower extract concentration range (12.5 to 50 mg/ml) as compared to CaSiO_3 (100 to 200 mg/ml). In another study, Lin et al. [90] demonstrated that Sr doped CaSiO_3 , implanted in ovariectomized (OVX) rat calvarial defect model, enhances the osteoporotic bone regeneration as compared to CaSiO_3 . The new bone area of Sr incorporated macroporous CaSiO_3 was 11.7 % higher than the pure CaSiO_3 (8.07 %) after 4 weeks of implantation. However, Sr doped porous CaSiO_3 scaffold, helps to promote osteoporotic bone defects repair, attributed due to the Sr and Si ions.

1.4. Effect of surface charge and electrodynamic stimulation on cell viability

Recently, it has been recognized that surface polarization is as a promising method for triggering the osteogenic response [74, 75, 79, 91]. The negative charged surface operates as a stimulus for the adhesion and proliferation of cells, because they attract cation like Ca^{2+} , H^+ etc. from culture media which further attract fibronectin and integrin proteins (negatively charged) [Figure. 1.2] [79, 92]. It has been reported that the configuration of voltage - gated Ca^{2+} channels and endoplasmic reticulum's receptor channel changes, when the cells are activated by the electric field [75, 77, 93]. The production of transforming growth factor and bone morphogenic protein-2, which aid in controlling cellular metabolism, is triggered by enhanced amount of intracellular Ca^{2+} ions [77, 92]. Srirussamee et al. [94] reported that the physical and chemical properties of the extracellular microenvironment are influenced by the application of direct electrical stimulation, which affect the metabolic activities of MSCs

cells. It has been reported that, negative charged surface of HA shows the increased in cell proliferation while cultured with human fetal osteoblastic- like cells (hFOB) cells, after 7 days of incubation [95]. Another study also reported that, electrically stimulated charged surfaces of $\text{Na}_{0.5}\text{K}_{0.5}\text{NbO}_3$ and the addition of $\text{Na}_{0.5}\text{K}_{0.5}\text{NbO}_3$ into HA and 1393 bioglass enhanced in cellular response as compared to uncharged surfaces of respective samples [80, 96, 97].

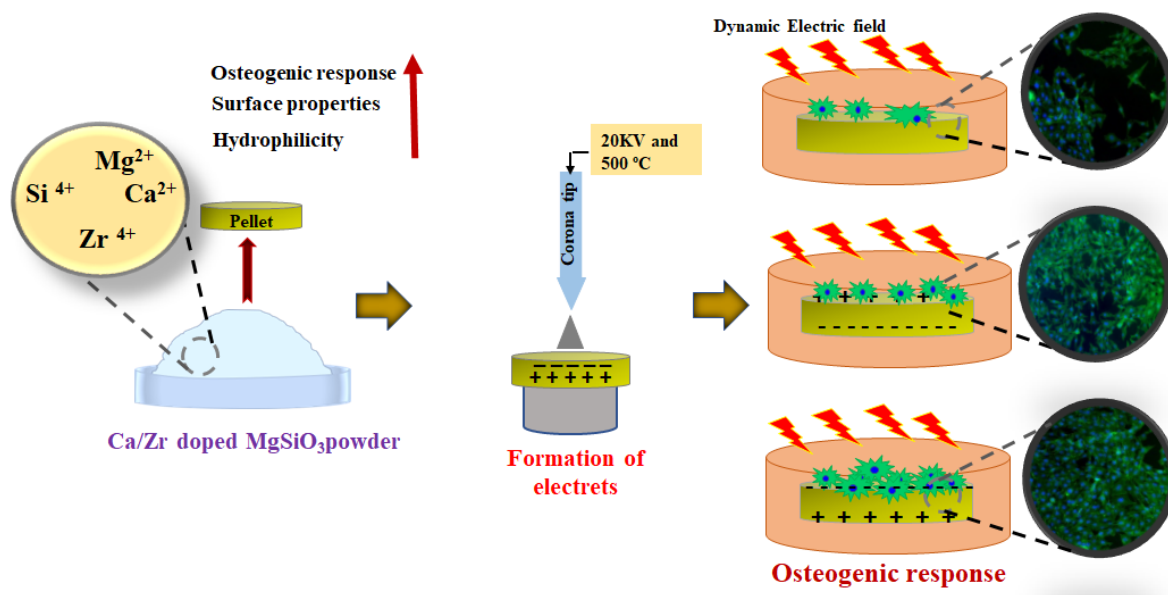


Figure 1.2. Schematic representation of electrostatic and electrodynamic stimulated cellular response.

1.5. Surface charge induced antibacterial response

Because bacterial cell membranes carry an electric charge, they have the ability to interact with surfaces that are polarized. Bacteria respond to external electric or magnetic fields due to their surface potentials, which triggers the generation of reactive oxygen species (ROS) [98]. The production of ROS damages the bacterial cell wall [99, 100]. In addition, bacteria communicate with electret surfaces through their electrical potential and micro electric field of charged surface of bacterial membrane [101-103]. It has been observed that the population of both, gram positive (GP, *S. aureus*) and gram negative (GN, *E. coli*) bacteria are reduced on the positively and negatively charged surfaces, respectively. Also, the charged (polarized

at 4.5 kV/cm) surfaces of BaTiO₃-HA bioceramics demonstrate the antibacterial response for the *P. aeruginosa*, *S. aureus* and *E. coli*, bacteria in comparison to their respective uncharged surfaces [103]. It has been suggested that the population of both, GP (*S. aureus*) and GN (*E. coli*) bacterial cells decreased by 53 % and 40 %, respectively, on negative surfaces of 7.5 wt% ZnO-HA bioceramics composite [104].

The wear resistance of implants and the biological response, generated by debris particles are also the key factors in determining the long-term success of implants [23]. The debris particles, released due to degradation, friction, and wear, have the ability to trigger harmful biological reactions at the implantation sites, resulting in periprosthetic osteolysis, inflammation, and aseptic loosening [22, 23]. Specifically, inflammation activates osteoclast cells, leading to the improper balance between osteoclasts and osteoblasts. This imbalance initiates osteolysis, ultimately causing aseptic loosening of the prosthetic implant [22, 23, 105]. Also, the properties of debris particles, such as their composition, morphology, volume, and size, play a crucial role in their biological response and profoundly affect the fate of peri-implant cells [106].

Numerous of *in vitro* and *in vivo* studies, have demonstrated that nanoparticles consistently raise concerns because of their specific features, such as surface area, morphology, size and concentration [107, 108]. The wear particles smaller than 2 μm can be easily enter to other organs, which then penetrate inside the cell through plasma membrane and induce cell toxicity [109, 110]. Wang et al. [110] revealed that intra-articular injection (with concentrations of 2 and 20 mg/ml) of TiO₂ nanoparticles (38 to 54 nm) into the knees of rats, allowed the migration of TiO₂ nanoparticles into crucial organs such as the heart and liver, accompanied by pathological damage to these organs. Additionally, biochemical assessments, including alkaline phosphatase (ALP) and creatinine, demonstrated impairments in the renal and hepatic systems of the rats treated with TiO₂ particulates [110].

Mabrouk et al. [111] reported that the performance of liver does not affect after implantation of pure and BaO (3 and 5 %) doped MgSiO_3 nanopowder in tibia fractured rats. Number of studies have reported that the *in vitro* biological response of Mg-Ca silicate based bioceramics shows excellent osteogenic activity and antibacterial efficiency which confirm the potentiality of these material for bone implant application [14, 39, 55, 112-116].

In this research, Ca and Zr substituted MgSiO_3 perovskite bioceramic was electrically charged using corona poling unit and their cellular and antibacterial response examined. The surface charge induced Ca and Zr substituted MgSiO_3 silicates perovskite are expected to show an excellent cellular response and inhibit the bacterial growth. Therefore, the present thesis focuses on the surface charge induced biocompatibility as well as antibacterial response. In this perspective, the potentiality of silicate perovskites bioceramics can be realized as a new generation prosthetic implant material. However, the nature and amount of surface charge and consequently, the polarization parameters need to be optimized which can accelerate the osteogenesis with the desired antibacterial response. Further, the *in vivo* toxicity of MCSZO-X ($X = 0 - 4$) nanoparticles in a rat model was also performed.

1.6. Objectives

The main objective of the present thesis is to evaluate the synergistic effect of surface charge and dynamic pulsed electric field stimulation as well as a compositional variation on *in vitro* cellular response of $\text{Mg}_{1-x}\text{Ca}_x\text{Si}_{1-x}\text{Zr}_x\text{O}_3$ ($x = 0 - 0.4$); MCSZO-X [where, $X = (0 - 4)$, corresponding to $x = 0 - 0.4$] bioceramics. In addition, the effect of surface charge on antibacterial response of MCSZO-X ($X = 0 - 4$) is to be explored.

The following are the precise goals of this thesis

- (a) To synthesize the MCSZO-X ($X = 0 - 4$) bioceramics using solid-state synthesis route and optimize the processing parameters (calcination and sintering temperatures) to obtain the pure phase $Mg_{1-x}Ca_xSi_{1-x}Zr_xO_3$ ($x = 0 - 0.4$); MCSZO-X ($X = 0 - 4$) samples.
- (b) To observe the phase formation and microstructure of the prepared MCSZO-X ($X = 0 - 4$) samples, using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and Scanning electron microscopy (SEM).
- (c) To develop the surface charge on the surface of MCSZO-X ($X = 0 - 4$) using a corona poling unit at voltage and temperature of 20kV and 500°C, respectively, for 30 min.
- (d) To study the effect of co-doping of Ca and Zr doping in $MgSiO_3$ and surface charge on *in-vitro* cellular and antibacterial response.
- (e) To study the synergistic effect of electrodynamical stimulation and surface charge on *in-vitro* cellular response of MCSZO-X ($X = 0 - 4$) bioceramics.
- (f) To study the influence of release ions on the surface chemistry and hydrophilicity of MCSZO-X ($X = 0 - 4$) bioceramics.
- (g) To study the *in vivo* toxicity of MCSZO-X ($X = 0 - 4$) bioceramics in a rat model.

1.7. Outline of the thesis

This present thesis is designed into seven chapters, each addressing a specific aspect of the research topic. Chapter 1 presents a brief description of the significance of the current investigation. Chapter 2 critically reviews the potentiality of Mg-Ca silicate-based crystalline bioceramics such as, $MgSiO_3$, Mg_2SiO_4 , $CaSiO_3$, Ca_2SiO_4 , Ca_3SiO_5 , $CaMgSi_2O_6$, $Ca_2MgSi_2O_7$, $Ca_7MgSi_4O_{16}$, $CaMgSiO_4$ and $Ca_3MgSi_2O_8$ as new generation orthopaedic prosthetic implants. Mg^{2+} , Ca^{2+} and Si^{4+} ions are abundant in bone and play a crucial role in various bone metabolic activities such as enhancing osteogenesis, inhibiting osteoporosis etc.

The release rate of Mg^{2+} , Ca^{2+} and Si^{4+} ions from these bioceramics depends on the crystal structure that consequently, influences their bioactivity and biocompatibility. In addition, the release rate of these ions can vary by tailoring the processing parameters/routes and compositional modifications and subsequently, bioactivity, cellular response as well as bone regeneration ability can improve. Towards this end, the present thesis thoroughly reviews and analyse the influence of crystal structure, processing parameters/routes and compositional alteration on *in vitro/in vivo* biocompatibility and degradation behavior of the above ceramics. Further, a correlation between structure, processing and properties has been established. Chapter 3 provides the synthesis and experimental methodology for the development of bioceramics as well as different characterization techniques like, XRD, FTIR and SEM. Also, the influence of electrical treatment on the osteogenic response (viability and differentiation) of prepared bioceramics samples has analyzed using osteoblast-like MG-63 cells. This chapter also provide the detailed procedure for development of $Mg_{1-x}Ca_xSi_{1-x}Zr_xO_3$, [(x = 0, 0.1, 0.2, 0.3 and 0.4); MCSZO-X (X = 0 – 4)] electret samples using high voltage corona (20 kV) poling unit at the temperature of 500 °C. The influence of formation of electrets on surface hydrophilicity, surface chemistry and leaching behavior has also examined using X-ray photoelectron spectroscopy (XPS), contact angle and inductive coupled plasma (ICP) measurements, respectively. This chapter provides a detailed explanation of the specific protocols used to assess the cellular and antibacterial responses of the MCSZO-X bioceramic, as well as to evaluates the toxicity of MCSZO-X (X = 0 – 4) nanoparticles in a rat model. Chapter 4 discusses the effect of co-substitution of Ca and Zr in $MgSiO_3$ [$Mg_{1-x}Ca_xSi_{1-x}Zr_xO_3$, (x = 0, 0.1, 0.2, 0.3 and 0.4); MCSZO-X (X = 0 – 4)] and influence of electrets formation of prepared MCSZO-X ceramics on their surface chemistry, hydrophilicity and leaching behavior which has been assessed by XPS, contact angle measurement and ICP, respectively. Chapter 5 explores the effect of surface charge and co-

substitution of Ca and Zr in MgSiO_3 [$\text{Mg}_{1-x}\text{Ca}_x\text{Si}_{1-x}\text{Zr}_x\text{O}_3$, ($x = 0, 0.1, 0.2, 0.3$ and 0.4); MCSZO-X ($X = 0 - 4$)] on their antibacterial response. Also, the effect of co-doping of various concentrations of Ca and Zr in MgSiO_3 on their crystallite size and lattice strain has been explained by X-ray peak profile analyses. In addition, the charge stored on the surface of electret MCSZO-X samples has also been measured using thermally stimulated depolarized current (TSDC). The antibacterial activity of MCSZO-X ceramics is measured by means of ROS, the levels of superoxide dismutase, catalase, and level of protein and lipid peroxide. Chapter 6 elaborately discusses the development and examination of the *in vivo* toxicity assessment of MCSZO-X ($X = 0 - 4$) powders in a rat model. Chapter 7 presents the key findings and potential future directions of this investigation.

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