Chapter 7

Conclusions and Future Scope

7.1 Conclusions

Since, natural bone possesses combination of reasonable mechanical and electrical properties, the primary objectives of the present study are: (i) to develop the composite with reasonably good mechanical and electrical properties, comparable to those of the natural bone, (ii) to improve antibacterial response with the help of polarization induced surface charges and (iii) to enhance the cellular functionality by the combined action of surface polarization and electric field treatment.

The important outcomes from the present study are as follows:

(1) HA / 45S5 BG / 1393 BG – NKN composites are developed with high densification (> 95 %) using cold isostatic pressing. The optimized sintering temperatures for HA – NKN and 45S5 / 1393 BG – NKN composites are 1075°C (2 h) and 800°C (30 min), respectively. The formation of phase pure HA, 45S5 BG, 1393 BG and NKN have been confirmed by XRD and FTIR analyses. Also, any dissociation or the reaction between the primary phases has not been observed.

(2) The hardness values of pure HA, and HA – (10 - 30) NKN composites are obtained to be (4.10 ± 0.25), (5.08 ± 0.25), (5.62 ± 0.23) and (7.90 ± 1.03) GPa, respectively. The fracture toughness of HA and HA – (10 - 30) NKN composites were calculated to be 0.42 ± 0.13, 0.59 ± 0.11, 0.77 ± 0.12 and 1.03 ± 0.15 $MPa.m^{1/2}$, respectively. The compressive strength of the sintered HA and HA – (10 - 30) NKN composites are obtained to be 63.84 ± 4.9, 87.55 ± 3.4, 110.01 ± 4.49 and 135.62 ± 5.03 MPa, respectively. The flexural strength of the sintered

HA and HA – (10 - 30) NKN composite samples are obtained to be 77.21 ± 2.21, 94.53 ± 2.24, 116.5 ± 2.89 and 145.52 ± 2.21 MPa, respectively.

(3) The hardness values of pure BG, and BG – (10 - 30) NKN composites are obtained to be (0.23 ± 0.11), (3.58 ± 0.32), (4.06 ± 0.38) and (2.86 ± 0.11) GPa, respectively. The fracture toughness of BG and BG – (10 - 30) NKN composites were calculated to be 0.30 ± 0.09, 1.64 ± 0.13, 3.31 ± 0.20 and 0.95 ± 0.15 $MPa.m^{1/2}$, respectively. The compressive strength of the sintered BG and BG – (10 - 30) NKN composites are obtained to be 9.14 ± 3.84, 40.45 ± 3.51, 128.38 ± 7.06 and 135.15 ± 6.68 MPa, respectively. The flexural strength of the sintered BG and BG – (10 - 30) NKN composite samples are obtained to be 22.19 ± 0.25, 26.64 ± 0.46, 28.91 ± 0.46 and 19.92 ± 0.44 MPa, respectively.

(4) The hardness values of pure 1393 BG and 1393 BG – (10 - 30) NKN composites are obtained to be (3 ± 0.11), (3.76 ± 0.32), (4.42 ± 0.38) and (6.47 ± 0.22) GPa, respectively. The fracture toughness values for monolithic 1393 BG and 1393 BG – (10 - 30) NKN composites were evaluated to be 1.18 ± 0.09, 1.55 ± 0.13, 2.37 ± 0.20 and 3.08 ± 0.15 $MPa.m^{1/2}$, respectively. The compressive strength of the sintered monolithic 1393 BG and 1393 BG – (10 - 30) NKN composites was calculated to be 20 ± 3.25, 45 ± 4, 125 ± 3.25 and 155 ± 4 MPa, respectively. The flexural strength of the sintered 1393 BG and 1393 BG – (10 - 30) NKN composite samples are obtained to be 35 ± 3, 75 ± 4.1, 95 ± 2.95 and 120 ± 3.15 MPa, respectively.

(5) At room temperature, the dielectric constant and loss values for HA, HA – 10 NKN, HA – 20 NKN and HA – 30 NKN composites are evaluated to be (25.23, 0.33), (17.89, 0.11), (14.12, 0.10) and (17.67, 0.08), respectively, measured at 10 kHz.

(6) At room temperature, the dielectric constant and loss values for 45S5 BG, 45S5 – 10 NKN, 45S5 BG – 20 NKN and 45S5 BG – 30 NKN composites are obtained as (13, 0.09), (15, 0.15), (13, 0.16) and (19, 0.11), respectively, measured at 10 kHz.

(7) The room temperature ac conductivity for HA - 10 NKN, HA - 20 NKN and HA-30 NKN composites were measured to be 2.69×10^{-8}, 6.84×10^{-11} and 5.76×10^{-8} $(\text{ohm cm})^{-1}$ respectively, at 10 kHz of frequency. While, the ac conductivity values for 45S5 BG, 45S5 – 10 NKN, 45S5 BG – 20 NKN and 45S5 BG – 30 NKN composites are evaluated to 7.28×10^{-7}, 1.47×10^{-8} and 9.87×10^{-9} $(\text{ohm cm})^{-1}$ respectively.

(8) The activation energies for grain and grain boundary resistances for HA - 10 NKN, HA - 20 NKN and HA-30 NKN composites are evaluated to be 1.03, 1.464, 1.28 and 1.34, 1.56, 1.30 eV, respectively. For 45S5 BG, 45S5 – 10 NKN, 45S5 BG – 20 NKN and 45S5 BG – 30 NKN composites, these values are 0.59, 0.87, 0.94 and 0.76, 0.93, 1.06 eV, respectively.

(9) The addition of piezoelectric NKN secondary phase is observed to improve the antibacterial behaviour of HA / 45S5 / 1393 BG – (10 – 30) NKN composites, while cultured with *S. aureus* and *E. coli* bacterial cells. Polarization [@ 20 kV and 500°C for 30 min] induced surface charge further enhances the antibacterial response of the fabricated composites. Statistical analyses revealed that the viability of gram positive (S. aureus) and gram negative (E. coli) bacterial cells has been reduced significantly on positively and negatively charged HA / 45S5 BG / 1393 BG – (10 – 30) NKN composites samples, respectively. Various quantitative and qualitative analyses such as Nitro blue tetrazolium (NBT) assay, disc diffusion test and Kirby-Bauer test suggest that the combined effect of NKN

addition and polarization significantly enhances the superoxide production which kills the bacterial cells.

(10) The combined effect of surface charge and electrical stimulation on the cellular response of HA / 45S5 BG / 1393 BG and HA / 45S5 BG / 1393 BG – 30 NKN composites has been observed for MG63 osteoblast cells. Both, the quantitative (MTT assay) and qualitative (fluorescence imaging) results reveal the significantly enhanced cell proliferation for polarization induced negatively charged samples. In addition, application of electric field intensity and pulse duration of 1V/cm and 400 μsec, respectively, for 5 min to adhered cells, cultured on unpolarized and polarized HA / 45S5 BG / 1393 BG and HA / 45S5 BG / 1393 BG – 30 NKN, have been observed to further enhance the cell growth and proliferation.

As a closure, among all the fabricated composites, HA – 30 NKN, 45S5 BG – 20 NKN and 1393 BG – 30 NKN exhibit the better mechanical properties (such as, hardness, fracture toughness and flexural strength) than monolithic HA / 45S5 BG / 1393 BG samples, which are comparable to that of the natural bone. The polarized HA – 30 NKN, 45S5 BG – 30 NKN and 1393 BG – 30 NKN reveal the better antibacterial response and improved cellular growth and proliferation.

7.2 Scope for the future work

(1) The mechanical characterization of spark plasma sintered (SPSed) HA / BG – (10 – 30) NKN composites can be evaluated as SPSed samples exhibit almost 100 % densification.

(2) Since, the NKN secondary phase is piezoelectric, the effect of polarization on mechanical properties, mainly on fracture toughness for HA / 45S5 / 1393 BG – (10 – 30) NKN composites can be studied.

(3) Polarization induced surface charge affects the antibacterial behaviour for HA / 45S5 / 1393 BG – (10 – 30) NKN composites. Polarization at different field strengths (kV) can be performed to observe the effect of optimal surface charge on antibacterial response.

(4) The combined effect of surface charge and external electrical field enhances the cell adhesion as well as proliferation on HA / 45S5 / 1393 BG – (10 – 30) NKN composites. The cellular response can examined as a function of varying intensity of electric field to get the optimal field strength for the best cellular response.

(5) In vivo test for fabricated HA / 45S5 / 1393 BG – (10 – 30) NKN composites can provide their detailed biocompatibility behaviour.