ANTIBACTERIAL AND CELLULAR RESPONSE OF POLARIZED BIOCERAMIC SUBSTRATES

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By

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Chapter 7

Conclusions and Future Scope

The present chapter briefs the important outcomes including development and characterization of chosen bioceramics as well as polarization induced antibacterial and cellular response.

The key findings of the present work are as follows:

1. The model biomaterials, HA and HA- x ZnO (x = 3.0, 4.5, and 7.5 wt. %) composites were synthesized using co-precipitation and solid state mixing route, respectively. The optimized sintering temperature for HA and HA-ZnO composites is 1200 ºC for 2 h. The phase evolution and FTIR analyses confirm the formation of pure phase HA. Also, any dissociation or reaction between primary and secondary phases has not been observed. The SEM images of fractured HA and HA-x ZnO (x = 3.0, 4.5, 7.5 wt. %) composite surfaces reveal good densification of the sample at the optimal processing parameter.
2. The room temperature dielectric constant and loss values for and HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) composites are evaluated to be (17.9, 0.1137), (16.3, 0.531), (4.6, 0.0377) and (17, 0.105) respectively, measured at 10 kHz.
3. The ac conductivity values for HA and HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) composites at room temperature and frequency of 10 kHz are 1.29 × 10-8, 5.65 × 10^{-9}, 6.82 × 10^{-8} and 4.29×10^{-9} $(ohm\ cm)^{-1}$, respectively.
4. The activation energies of grains and grain boundaries for HA, and HA-3.0 wt. % ZnO composite, are calculated to be (1.36, 1.41 eV), and (1.18, 1.98 eV), respectively.
5. The quantitative and qualitative analyses for antibacterial response reveal that the addition of ZnO (up to 7.5 wt. %) as well as polarization significantly enhances

the *in-vitro* antibacterial response of the developed model biomaterials. In addition, the viability of *E. coli* and *S. aureus* bacteria significantly reduces on negatively and positively polarized HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) composites, respectively.

6. The *in-vitro* cellular response of HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) composite for SaOS2 cells are observed to improve with the addition of ZnO (up to 7.5 wt. %). The negatively polarized surfaces of HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) composites enhanced the proliferation of SaOS2 cells.
7. The NKN, BT, CT, and HA- 30 vol. % NKN/BT/CT composites were processed using solid state mixing route. The optimized sintering temperatures for NKN, BT, and CT are 1075 (2 h), 1300 (3 h) and 1450 ^{o}C (6 h), respectively. The sintering temperature for HA- 30 vol. % NKN, HA-30 vol. % BT and HA- 30 vol. % CT composites are optimized to be 1060 ^{o}C (2 h), 1250 ^{o}C (2 h) and 1350 ^{o}C (4 h), respectively.
8. The phase evolution and FTIR analyses confirm the formation of pure phase HA, NKN, BT and CT. Also, in HA- 30 vol. % NKN/BT/CT composites, distinct peaks of both primary and secondary phases are observed without any dissociation or reaction between the constituent phases. The SEM images of fractured HA and HA- 30 vol. % NKN/BT/CT composite surfaces reveal good densification of the sample at the optimal processing parameter.
9. The quantitative and qualitative analyses for antibacterial and cellular response reveal that the combined action of incorporation of piezoelectric NKN, BT and perovskite CT as well as polarization significantly enhances the *in-vitro* antibacterial response as well as proliferation of osteoblast-like SaOS2 and MG-63 cells. The enzymatic activities such as, generation of superoxide (SOD assay),

lipid peroxide (LPO assay), catalyse (H_2O_2 production) and protein concentration revealed the mechanism of polarization on antibacterial response.

10. The BBG and BBG-30 vol. % NKN/BT composites were processed using melt quenching (1300 ºC) and solid state mixing route, respectively. The optimized sintering temperature for BBG and BBG-30 vol. % NKN/BT composites is 650 ºC (30 min). The retention of pure BBG, NKN and BT in the composite has been confirmed by XRD analyses without any dissociation or reaction between the constituent phases.
11. The quantitative and qualitative analyses reveal that addition of piezoelectric NKN/ BT (30 vol. %) as secondary phase in BBG matrix increases the antibacterial as well as cellular response. In addition, the polarized surfaces demonstrate bacteria specific antibacterial response. The statistical analyses revealed that viability of *E. coli* and *S. aureus* bacteria significantly reduced on negatively and positively polarized BBG-30 vol. % NKN/BT composites, respectively.
12. The combined action of addition of piezoelectric NKN/ BT (30 vol. %) as well as surface polarization significantly enhanced the proliferation of osteoblast-like Mg-63 cells. In addition, the negatively polarized surfaces have higher cell density.

As a closure, among all the developed composite samples, HA-7.5 wt. % ZnO, HA -30 vol. % NKN and BBG-30 vol. % NKN exhibited better antibacterial as well as cellular response than the HA and BBG. The negatively polarized surfaces of HA-7.5 wt. % ZnO, HA -30 vol. % NKN and BBG-30 vol. % NKN demonstrated better antibacterial response for *E. coli* bacteria. However, positively polarized surfaces of same composition demonstrate better response for *S. aureus* bacteria.

Scope for the future work

1. Mechanical characterization of all the developed compositions can be done.
2. Polarization affects the antibacterial and cellular behavior of fabricated HA- ZnO, HA- NKN/BT/CT and BBG- NKN/BT composites. Polarization at different field strengths (kV) can be performed to observe the optimal antibacterial and cellular response.
3. Combined action of surface polarization and application of external electric field on developed HA- ZnO, HA- NKN/BT/CT and BBG- NKN/BT composites can be examined towards the antibacterial as well as cellular response.
4. Effect of surface polarization on mechanical characterization of all the developed compositions can be examined.
5. The gene expression behaviour such as ALP, Runx2 and Collagen I; and mineralisation specific gene such as osteocalcin, osteopontin, osteonectin can be performed.
6. *In- vivo* test for developed HA- xZnO (x = 3.0, 4.5, and 7.5 wt. %) HA- 30 vol. % NKN/BT/CT and BBG- 30 vol. % NKN/BT composites can be perform to verify their suitability for clinical applications.
7. Backscattered imaging and EDX surface mapping can also be performed to differentiate various phase distributions on the surface.