

Conclusions and scope for the future work

The present chapter briefs the major outcomes of the studies, performed as part of the thesis such as, development, characterization including electrical stimulation induced cellular as well as antibacterial response alongwith toxicity evaluation of prepared nanoparticulates.

The key findings of the present work are as follows:

1. Successful synthesis of sodium potassium niobate [$\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2-0.8$), NKN] piezo-bioceramics and hydroxyapatite (HA), using solid state synthesis and co-precipitation route, respectively, at the optimized processing parameters. The refined unit cell parameters (using Rietveld analyses) for sintered $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2 - 0.8$) samples confirm the formation of monoclinic structure with space group P1m1. The hardness values for $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) and HA samples are 5.8 ± 0.4 , 6.5 ± 0.3 , 6.8 ± 0.4 and 4.7 ± 0.4 GPa, respectively. The compressive strength of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) and HA samples are 121.73 ± 5.27 , 128.05 ± 4.55 , 136.47 ± 5.86 and 81.65 ± 4.44 MPa, respectively.
2. The surface charge, induced by corona poling (@ 25 kV at 500 °C) of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) samples were measured to be 0.52, 0.50 and 0.47 $\mu\text{C}/\text{cm}^2$, respectively. X-ray photoelectron spectroscopy (XPS) and contact angle measurement reveal that the surface polarization considerably increases the surface hydrophilicity (without affecting surface chemistry) of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ samples than HA control, preferably for the negatively polarized surfaces. The surface polarization remarkably promote early-stage cellular adhesion.

3. The quantitative and qualitative analyses revealed that the synergistic action of electrostatic surface polarization charge and dynamic pulsed electrical stimulation further accelerates cell proliferation and differentiation on negatively charged surfaces of Na and K rich compositions of NKN. The mechanism of augmented osteogenesis was revealed by the measurement of intracellular Calcium ions which indicates the activation of calcium channel pathways elicited by electrostatic-dynamic electrical stimulation.
4. *In vitro* bacterial culture study reveals that the viability of *S. aureus* bacteria was reduced by (41, 29, 50 %) and (28, 20, 30 %) on the positively and negatively polarized surfaces of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) samples, respectively, as compared to non-polarized HA. For *E. coli* bacteria, the negatively and positively polarized surfaces of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) samples show reduced viability of bacteria by (49, 37, 52 %) and (31, 24, 45 %), respectively, as compared to non-polarized HA. In addition to surface charge polarization, sodium ($x = 0.8$) and potassium ($x = 0.2$) rich compositions further improve the antibacterial performance of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$. The outcomes of various assays associated with enzymatic activities further elucidated that the generation of reactive oxygen species (ROS) and ROS induced bacterial damage is observed to be maximum on the positively polarized surfaces of sodium ($x = 0.8$) and potassium ($x = 0.2$) rich compositions of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$.
5. The $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) nanoparticulates were prepared using solid state synthesis route, followed by the reduction of particle sizes upto nanoscale range using high energy attrition ball milling. The high resolution scanning electron microscopy

- (HRSEM) images demonstrates the irregular shaped NKN powders of the sizes of 70 – 180 nm.
6. The initial screening of toxicity was done by the exposure of different concentration (0.25, 2.5 and 25 mg/ml in normal saline) of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ nanoparticulates in osteoblast-like MG-63 cells, which reveals the proliferation of cells on NKN nanoparticulates of concentration upto 25 mg/ml. Further, *in vivo* toxicity were assessed by the injection of NKN nanoparticulates (100 μl of 25 mg/ml for each) in the knee joint of wistar rats for 7 days. The particulates treated rats were not suffer from diarrhea, tremor, or convulsion and were not in the comatose stage, post intra-articular injection.
 7. The anti-inflammatory cytokines (IL-4 and IL-10) profile analyses of liver and spleen suggest the increased anti-inflammatory response in the treated rats as compared to non-injected (control) rats, preferably for the sodium and potassium rich NKN i.e., $\text{Na}_{0.8}\text{K}_{0.2}\text{NbO}_3$ and $\text{Na}_{0.2}\text{K}_{0.8}\text{NbO}_3$. The biochemical analyses such as alkaline phosphatase (ALP) and creatinine activities revealed normal functionality of the liver and kidney of particulate treated rats. The histopathological analyses of vital organs such as heart, liver, kidney and spleen demonstrates that the nanoparticulates treated rats did not reveal any morphological changes in the architecture of tissues which confirm the absence of any inflammation or dissemination of nanoparticulates in vital organs such as heart, liver, kidney and spleen. The knee joint of the particulates treated rats were also not showing any sign of inflammation.

Overall, among all the developed $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) piezo-bioceraics samples, the electrically stimulated negatively polarized (Na) rich NKN i.e., $\text{Na}_{0.8}\text{K}_{0.2}\text{NbO}_3$ and

potassium (K) rich NKN i.e., $\text{Na}_{0.2}\text{K}_{0.8}\text{NbO}_3$, revealed maximum osteogenic response. The polarized NKN samples demonstrated better antibacterial response than non-polarized NKN samples and HA control, which further increased on the Na and K rich compositions of NKN, for both, *S. aureus* and *E. coli* bacteria. The *in vivo* study using rat's model confirm the non-toxic nature of NKN nanoparticulates.

Future scope

1. Concerning the piezoelectric material-based therapy for bone tissue engineering, synergistic interaction among the electric field stimulation and piezoelectric properties should be explored to assess the potential to guide mesenchymal cells towards osteogenesis, contributing to bone growth.
2. Combined action of surface polarization charges and external electrical stimulation alongwith compositional modifications can be performed to improve the antibacterial response of $\text{Na}_x\text{K}_{1-x}\text{NbO}_3$ ($x = 0.2, 0.5, 0.8$) samples.
3. It is also known that the piezoelectric strain coefficients are dependent on the physical structure, e.g., fiber, film and bulk phase of the same biocompatible material can have different piezoelectricity. Towards this end, it would be important to establish the tuning between the shape of the piezoelectric material and biocompatibility.
4. The charge storage capability of sodium potassium niobate NKN piezo-bioceramics make it a suitable candidate for *in vivo* energy harvester applications such as, pacemakers etc.
5. Flexural strength and fracture toughness can be calculated for the prepared samples to establish NKN as a robust orthopedic implant.

6. FEM analyses can be performed to analyze stress distribution at different loading conditions such as tension and compression.
7. Pre-clinical studies in smaller and larger animal models are to be conducted on bulk NKN samples to further verify their potentiality for orthopedic implant applications.