Chapter 1

Introduction

The chapter introduces about various endogenous bioelectrical cues, present in natural bone and the relevance of surface charge and external electrical stimulation in inducing the osteogenic response. The potentiality of surface charge has also been discussed to address the concern of bacterial infection in prosthetic orthopedic implants. Further, an overview of piezoelectric bioceramics have been provided alongwith the potentiality of sodium potassium niobate (NKN) piezo-bioceramic as a prospective next-generation orthopedic implant material. Further, a short description of the biocompatibility, antibacterial and toxicity study (in vivo) on electrically / compositionally modified piezoelectric NKN ceramic are presented to reveal the potentiality of piezoelectric NKN ceramic for orthopedic applications. At the end, a brief introduction of the chapters of the thesis is presented.

1.1. Background and motivation

Endogenous piezoelectric potential of natural living bone plays a crucial role in various essential structural remodeling processes such as, growth, repair and regeneration [1, 2, 3]. Similar to bone, the electrical treatment or mechanical loading on the piezoelectric bioceramics develop polarization surface charges which promote bone regeneration by modulating the cellular activities such as, adhesion, proliferation, differentiation etc. [2, 3, 4]. Recently, polarization induced improved biocompatibility of piezoelectric bioceramics have been suggested as an appealing approach to develop new-generation electroactive prosthetic orthopedic implants [2, 3]. Apart from internal bioelectrical cues, the potentiality of external electrical stimulation has also been revealed in improving the healing capability of damaged tissues such as bone, cartilage and ligaments [5, 6, 7]. The application of direct electrical stimulation helps in regulating cellular metabolism via the activation of metabolic signaling pathways of cells [3, 6].

Apart from favorable biocompatibility, owing to the potential risk of bacterial infection during- or post-surgical operations, the development of antibacterial prosthetic orthopedic implants is in continuous thrust. The gram positive cocci (*Streptococci, Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aerugenosa, Escherichia Coli* etc.) are the most common bacteria associated with the infection of orthopedic implants which contain an electronegative outer membrane [8, 9]. Due to specific electrical surface potentials, bacteria respond against charged surface. For example, the negatively polarized surface of implant induces antibacterial response due to the electrostatic repulsion between like charges [10, 11]. Therefore, polarized ferroelectric surfaces induce antibacterial response without the addition of any antibacterial agent. In view of the above backdrop, the present work utilizes the potentiality of novel electrical stimulation techniques in improving the osteogenic as well as antibacterial response of piezoelectric sodium potassium niobate ($Na_xK_{1-x}NbO_3$, NKN).

One of the recent challenges in the design/development of prosthetic orthopedic implants is to address the concern of local/systemic toxicity of debris particles, released due to wear or degradation. Such debris particles often lead to inflammation at the implanted site or aseptic loosening of the prosthesis which results in failure of the implant during long run [12, 13]. Therefore, the present work made the first attempt to evaluate the toxicity of biocompatible NKN nanoparticles by the injection in the knee of rat's model.

1.2. The electroactive response of natural bone

Natural bone is a polymer aided ceramic hybrid composite. It carries inorganic and organic components of about 65 wt. % and 25 - 30 wt. %, respectively of the total bone mass [2, 14].

The living bone generates electrical signals by the application of mechanical stress due to the presence of piezoelectric collagen [15]. These bioelectric signals stimulate bone growth [1, 2]. The natural living bone has also been demonstrated to be a source of pyroelectricity [16] as well as ferroelectricity [17]. The electrical stimulation due to inherent bioelectricity of bone helps in bone repair [18] and the treatment of bone-related diseases, such as osteoporosis [19], bone tumor [20], etc.

In natural living bone, the hydrogen bond in collagen and HA is responsible for its polarizability [21]. The dielectric behavior of bone depends on the frequency and moisture content [22, 23]. The dielectric constant of dry human bone has been reported to be about 10 over the frequency range of 1 - 100 kHz. [21, 24]. Dielectric relaxations in mineralized and demineralized bone were observed at relaxation frequencies of about 400 MHz and 200 MHz, respectively [22]. In demineralized bone, the reduction of calcium ions gives rise to the mobility to polar side chains in collagen fiber and results in lower relaxation frequency of demineralized bone [22]. The alternating current (AC) and direct current (DC) conductivity at 1 kHz of dry human femur has been reported to be of the order of 10^{-10} $(\Omega\text{-cm})^{-1}$, at 1 kHz [23, 24]. There is an interconnection among elastic modulus, mineral density and dielectric properties of bone, which suggests that the degradation in mechanical performance of bone can be monitored by measuring their electrical conductivity [19, 25].

The natural bone is piezoelectric in nature with collagen molecules being the origin of piezoelectricity [1, 26]. The slipping of collagen fibers over each other under the application of mechanical force is specifically the cause of piezoelectricity in natural bone [27]. During physical activities like walking, stretching, climbing, etc., the collagen fibers undergo various kinds of movements like rotation, slipping, etc. and consequently, bone is subjected to stresses, like compression, tension, etc. [28]. Piezoelectric collagen leads to the development of charges in response to such kind of stress [15]. The polarity of these electrical charges depends on the direction of mechanical stress or bone deformation [29]. Compression and tension generate negative and positive charges, respectively. Halperin et al. [15] reported the value of piezoelectric strain coefficient in human tibia to vary in the range of 7.7 – 8.7 pC/N. The lack of considerable scatter in piezoelectric strain coefficients indicates that the piezoelectric property is uniform across the bone.

Natural bone shows pyroelectricity due to the presence of collagen [16]. Ramachandran et al. [30] proposed that the triple helix model of collagen, consists of three parallel helical shaped chains, where each chain contains three amino acid residues per turn. The mutual conversion between lighter and heavier residues results in the distortion in triple helix structure of collagen, which renders pyroelectricity to natural living bone [30]. The pyroelectric coefficient of a human femur has been reported to be around 0.0036 ± 0.0021 $\mu C/m^2 °K$ (in the temperature range of – 25 °C to 60 °C) [16].

The collagen fibers, present in bone tissue, change their orientation in different directions from one plate to another plate, similar to the typical ferroelectric domain alignment [17]. The presence of hysteresis loop and permanent dipoles in bone structure confirms that ferroelectricity is a fundamental characteristic of natural bone. Hasting et al. [31] further confirmed the existence of permanent dipoles as well as remnant polarization ($P_r = 0.00068$ $\mu C/cm^2$) in the bone. These dipoles can change their orientation under an external electric field, which is the reflection of reversible spontaneous polarization in natural bone.

1.3 Overview of piezoelectric bioceramics

As far as piezoelectric properties are concerned, NKN-based ceramics have been demonstrated among the best lead free candidates, because of their reasonable piezoelectricity, polarizability, dielectric strength as well as relatively lower density as compared to other piezoelectric biocompatible materials [32, 33, 34]. NKN has been patented as a potential biocompatible implant material, as it supports the growth of human monocytes [35]. $BaTiO_3$ is another piezoelectric candidate with proven biocompatibility [36, 37]. $MgSiO_3$, with an asymmetric tetragonal structure, has been reported to be a good biocompatible piezoelectric substitute for bone [38, 39]. $LiNbO_3$ is another piezoelectric material, which has been suggested as a potential alternative for bone prosthesis [40, 41]. $LiNbO_3$ possesses admirable ferroelectric, pyroelectric and piezoelectric properties [40, 41, 42]. Piezoelectric $KNbO_3$ is currently being used as a bio-probe for disease diagnosis and could be another alternative as an electroactive bone substitute [43, 44]. ZnO is another ceramic with reasonable piezoelectric, pyroelectric and dielectric potentials [45, 46]. The piezoelectric boron nitride (BN) nanotubes, in its pristine as well as composite forms, have been reported to promote the proliferation and differentiation of mesenchymal stem cells [47].

Among various piezoelectric bioceramics, NKN has recently been recognized as an emerging prospective candidate for electro-active orthopedic implant applications [3, 4, 48, 49]. The sodium and potassium ions participate in regulating the metabolism of human body such as, water-mineral balancing, maintaining nerve impulse, muscle contraction-relaxation, regulating blood pressure and cellular homeostasis [50, 51]. Recent studies reported the potentiality of niobium for hard tissue regeneration [52, 53]. The above mentioned biocompatibility of the elements, present in NKN ceramic makes it more suitable candidate than other biomaterials. It has been reported that the presence of Na and K (individual or in combination) in bacterial extracellular matrices create osmotic changes and thereby makes the environment unfavorable for the growth of bacteria and consequently, enhances the antibacterial response [54, 55]. The above mentioned cutting edge advantages of piezoelectric NKN ceramics prove its efficacy to be used as prosthetic orthopedic implant.

In the present work, three different compositions such as, $Na_xK_{1-x}NbO_3$, (a) x = 0.2, (b) x = 0.5, (c) x = 0.8 have been taken as biomaterials to analyze the combined effect of surface polarization charge, external electrical stimulation and compositional modification on the osteogenic response. Thereafter, the synergistic effect of surface polarization and variation in the contents of Na and K on the antibacterial response of NKN was investigated. Furthermore, in vivo toxicity study was performed with different compositions of NKN in rat's model. In view of utilizing the piezoelectricity induced biocompatibility and antibacterial response of sodium-potassium niobate based ceramics system for orthopedic applications, the compositions x = 0.2 ($Na_{0.2}K_{0.8}NbO_3$), x = 0.5 ($Na_{0.5}K_{0.5}NbO_3$) and x = 0.8 ($Na_{0.8}K_{0.2}NbO_3$) were selected.

1.4. Surface polarization and external electrical stimulation induced cellular response

Recently, researchers acknowledge the surface polarization as a potential technique to induce the osteogenic response of piezoelectric bioceramics [2, 3, 4, 7]. The negatively polarized surfaces attract the positively charged cations (Ca^{2+}, H^+ ions) from the biological fluid which further attract the negatively charged proteins (fibronectin and integrin) and therefore, negatively polarized surfaces act as a stimulus for cellular adhesion and proliferation [3, 7]. Moreover, external electrical stimulation activate the calcium channel pathways, which execute the gene transcription and thereby regulating the cellular metabolism [3, 49, 56]. For example, the polarized (@ 25 kV) surface of $Na_{0.5}K_{0.5}NbO_3$ has been reported to considerable improvement in the adhesion of protein as well as cells than non-polarized surface [4, 57]. The external electrical stimulation on the polarized surfaces of $Na_{0.5}K_{0.5}NbO_3$ added HA / 1393 bioglass has been reported to induce improved cellular response of piezoelectric biocomposites than their non-treated monolithic [58, 59]. The surface polarization and external electrical stimulation are considered to be new generation technique to promote the osteogenesis of piezoelectric substrates.

1.5. Surface charge induced antibacterial response

Since, bacterial cell membrane exhibits electrical charge, it interacts with polarized surfaces. The microelectric field generates on the polarized surfaces, enhances the rate of electrolysis of water and consequently, produces ROS such as H_2O_2, O_2^- etc. that damage the bacterial cell wall and results in the killing of bacteria [60, 61]. In addition, the negatively polarized surfaces have also been suggested to reduce bacterial adhesion due to electrostatic repulsion between similar charged surfaces of substrate and bacteria [10, 11]. For example, the polarized (4.5 kV/cm) surface of barium titanate – hydroxyapatite piezo-biocomposite increases the zone of inhibition (> 2 times) for *S. aureus*, *E. coli* and *P. aerugenosa* bacteria as compared to the non-polarized surface of composite [62]. In addition, the viability of *E. coli* and *S. aureus* bacterial cells are reduced by 53% and 40 %, respectively on negatively charged surfaces of HA - 7.5 % ZnO bioceramic composite [10].

1.6. Objectives

The primary objective of the present thesis is to analyze the combined effect of electrical stimulation treatments and compositional modification in inducing the cellular and antibacterial response of $Na_xK_{1-x}NbO_3$ piezo-bioceramics.

The specific objectives of the present thesis are as follows:

(a) To optimize the sintering parameters towards the development of phase pure $Na_xK_{1-x}NbO_3$ (x = 0.2 – 0.8) samples with densification, mechanical and piezoelectric properties, similar to bone.

(b) To develop the surface charge on the $Na_xK_{1-x}NbO_3$ pellets using corona poling technique at the voltage and temperature of 10 - 30 kV and 400 - 500 °C, respectively, and measurement of surface charge, stored in the polarized surfaces of $Na_xK_{1-x}NbO_3$ using thermally stimulated depolarized current (TSDC) measurement.

(c) To study the influence of polarization charge on the surface chemistry, hydrophilicity and early-stage cellular adhesion.

(d) To analyze the combined effect of electrical treatment and compositional modification on the osteogenic and antibacterial response of $Na_xK_{1-x}NbO_3$.

(e) Assessment of systemic toxicity (*in vivo*) of $Na_xK_{1-x}NbO_3$ nanoparticles in rat's model.

1.7. Outline of the thesis

The present thesis has been divided into 7 chapters. Chapter 1 briefs the relevance background and motivation behind the present research work. Chapter 2 reviews the potentiality of various piezoelectric bioceramics and biopolymers in mimicking electro-mechanical response of living bone. Towards this end, the processing-related challenges to meet the desired piezoresponsive property combination have been discussed along with the possible combinations of remedies. In addition, the advantageous effects of polarized piezoelectric substrates in augmenting the biocompatibility have been discussed objectively. Furthermore, the challenges associated with the bacterial infection were discussed alongwith their remedies such as surface polarization, electrical and magnetic stimulation. Chapter 3 demonstrates the experimental techniques for the development of dense piezo-bioceramics (with optimized parameters) alongwith the characterization techniques such as X-ray diffraction (XRD), Fourier transformation infrared spectroscopy (FTIR) and scanning electron microscopy. This chapter also elaborates the development of surface polarization charges on the piezoelectric $Na_xK_{1-x}NbO_3$ ceramic using high voltage corona poling unit. This chapter provides the details of various protocols adopted for assessment of cellular and antibacterial response of developed $Na_xK_{1-x}NbO_3$ piezo-bioceramics alongwith the evaluation of systemic toxicity of $Na_xK_{1-x}NbO_3$) in rat's model. Chapter 4 discussed the compositional modification induced structural changes in $Na_xK_{1-x}NbO_3$. Furthermore, the effect of such charges on the surface characteristics and early-stage cellular adhesion of osteoblast-like cells has been presented. In addition, the combined effect of surface polarization charge, dynamic electrical stimulation and compositional modification was analyzed towards the proliferation and differentiation of osteoblast-like cells. Chapter 5 presents the investigation of surface charge and compositional modification induced antibacterial response of $Na_xK_{1-x}NbO_3$ piezo-bioceramics. Moreover, the mechanism of surface charge induced antibacterial response of developed bioceramics was revealed by the assessment of the various enzymatic activities such as, superoxide ions, catalase activity, protein and lipid peroxide (LPO). Chapter 6 presents the development, characterization and detailed study of the assessment of systemic toxicity (*in vivo*) of $Na_xK_{1-x}NbO_3$ nanoparticles in rat's model. Chapter 7 provides the conclusions and future scope of this study.

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