Chapter 1

Introduction

The chapter introduces the concerns, associated with the bacterial infection in prosthetic orthopedic implants. Towards this end, number of existing solutions such as, the use of various antibacterial agents as well as application of external electric and magnetic fields, have been briefed. Further, the potentiality of one of the recent approaches i.e., surface polarization, to address the bacterial concern has been introduced. Thereafter, the objectives and structure of the thesis have been briefly mentioned towards the end.

1.1. Background

The development of suitable prosthetic antibacterial implants for human health care is one of the stimulating areas in orthopedics, as the bacterial infection at implant site has been recognized as one of the major concerns for failure of implants during/ after surgery [1]. These infections are associated with both, gram positive and gram negative bacteria [2]. It has been reported that approximately 65 % of bacterial infection in orthopedic implant is associated with gram positive cocci [*Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)*] [3]. However, gram negative bacteria [*Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aerugenosa)*] cause about 11 % of the bacterial infections in orthopedic implants [4]. Various metals (stainless steel, titanium, cobalt, and chromium based alloys etc.) polymers (carbon fibers, glass fibers, polymethyl methacrylate etc.) and ceramics (hydroxyapatite, HA), tricalcium phosphate, bioglasses etc., have been developed to replace the deceased/ defective bones [5,6]. Even with reasonable biocompatibility and bioactivity, most of the developed (metals, polymers and ceramic) implants are susceptible towards bacterial infection during/ after surgery [7]. To overcome the issue of bacterial infection during/ after surgery, various antibiotics as well as antibacterial coatings on implant materials have been used [8]. However, antibiotics have limited ability to reduce the bacterial infections and many times the bacteria develop resistance with time against antibiotics [9]. To address such an important issue of bacterial infections in orthopedic implants, various approaches such as, incorporation of antibacterial agents in existing implants as well as application of external stimuli are in continuous thrust [10,11].

Number of metallic elements (e.g., Au, Ag, Zn, etc.) as well as oxides such as CuO, ZnO, TiO_2, etc. have been suggested as antibacterial agents to reduce the bacterial infection in orthopedic implants [12]. It has been reported that addition of Ag with concentrations of 10, 50, and 100 $\mu g/ cm^3$ in HA inhibits the growth of *E. coli* bacteria by 62 %, 88 % and 100 %, respectively, after incubation of 24 h [13]. Similarly, the incorporation of 60 wt % TiO_2 in HA reduces the growth of *S. aureus* and *E. coli* bacteria by ~ 56 % and 63 %, respectively [14]. However, the excess amounts of these antibacterial elements cause adverse effects to cells and tissues [15]. In another study, it has been reported that the incorporation of ZnO (7.5- 30 wt. %) in HA matrix reduces the bacterial growth for both, *E. coli* and *S. aureus* bacteria but the addition of more than 10 wt. % ZnO in HA matrix offers detrimental effect to the cell growth [16]. Therefore, the addition of antibacterial agents raises the concern of their potential cytotoxicity [17]. To address this issue, application of external stimuli has been considered as a potential alternative [18].

The application of external stimuli such as, electric and magnetic fields have been recognized as potential means for controlling the bacterial infection in prosthetic implants [19]. The external stimuli improve the antibacterial properties of biomaterials without use of antibiotics or any other antibacterial agent [20].

The colonies of *E. coli and S. aureus* bacteria decreases by ~ (53 %, 66 %) and (59 %, 68 %) when exposed to static magnetic field of 50, and 80 mT (24 h), respectively [21]. The viability of both, *E. coli and S. aureus* bacteria, cultured on HA and HA-Fe_3O_4 composites surfaces decreases while exposed to a static magnetic field of 100 mT for 0.5 -4 h [22]. However, the application of magnetic fields is quite challenging as it depends on the number of properly tuned parameters such as field intensity, exposure duration, frequency etc., [23]. In case of high intensity of applied external magnetic field, there is a possible risk of variation in hormonal concentration and gene expression that can produce critical adverse effects [24]. Overall, the application of magnetic field can be used to inhibit the bacterial growth.

The application of electric field has been realized as a potential alternative for controlling the microbial population on biomaterial surfaces [25]. For example, the growth of *E. coli* and *S. aureus* bacteria decreases by 33 % and 31 %, respectively, after exposure to the static electric field of 4.5 kV/cm for the duration of 30 min. However, with increasing the exposure time to 2.5 h, the growth of both, *E. coli* and *S.aureus* bacteria decreased by ~ 56 and 54%, respectively, while exposed to similar electric field intensity [23]. In another report, it has been suggested that growth of *S. aureus* bacteria reduced by ~ 60 % on HA surface, after exposure to DC electric field of 1 V/ cm for 24 h. However, addition of 10 wt. % ZnO in HA as well as exposure to DC electric field of 1 V/ cm (24 h) reduces the bacterial growth by ~ 70 %, whereas pure HA exhibits almost 60 % reduction in bacterial growth [26]. Direct exposure of bacterial cells to electric field has been reported to produce some toxic substances such as, Cl_2 and H_2O_2 which can damage the other cells as well [27]. Overall, the application of external stimuli reduces the bacterial growth but high intensity electric field can cause unfavorable effect to cells and tissues [28].

Despite the significant technological advancement in this direction, bacterial infections in biomedical implants are still a serious concern. In view of above backdrop, the present work focuses to develop a new technique, i.e., polarization of biomaterial substrates, to address the issue of bacterial infections during/ after surgery. Polarization refers to the process in which a high intensity electric field is applied to develop the surface charge. Polarization also aligned all the randomly oriented domains [29].

The outer membranes of both, gram positive and gram negative bacteria possess negative charge due to the presence of peptidoglycane and lipopolysaccharides outer layers, respectively [30]. As the bacterial membrane contains charge, the electrical polarization of prosthetic implants to produce like charges anticipate to provide the antibacterial response without addition of any antibacterial agents as well as exposure to electrical or magnetic fields.

1.2. Model bioceramics

The bioceramics have demonstrated more potential over the other material classes, used for orthopedic applications. Among the existing biomedical materials such as metals, ceramics and polymers, ceramics are categorized as modern biomaterials. From material's perspective, the major component of living bone is ceramics. Therefore, the development of bioceramics for bone tissue replacement applications has been realized. As far as the material aspect of living bone is concerned, it consists of about 65 wt. % of apatite, as ceramic phase and 23 wt. % of collagen as polymeric phase [31]. Therefore, the development of various bioceramics, for human health care is one of the stimulating research areas in ceramics. Among the bioceramics, the synthetic hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] with a calcium-to-phosphate stoichiometric ratio of 1.67 is the most desirous material for the orthopedic implants due to its chemical as well as structural similarity with the inorganic mineral phase of bone. In this perspective, HA has been taken as the primary phase in the present study.

Recently, various metal oxides such as ZnO, CuO and TiO_2 have been suggested as antibacterial agents to reduce the bacterial infection [32]. Among the metal oxides, ZnO has superior antibacterial spectrum, stability and durability [33]. Moreover, Zn is the second most abundant trace elements in the human body, majority of which is present in muscles and bones [16]. The addition of ZnO as secondary phase increases the antibacterial effect for both, gram positive and gram negative bacteria [34]. For example, the addition of ZnO (25 wt. %) in HA reduces the colonies of *E. coli* and *S.aureus* bacteria by ~ 98 % and 99 %, respectively [35].

Recently, various piezobioceramics are emerged as prospective materials for bone implant, owing to the piezoelectric nature of bone [36]. The piezoelectricity of bone plays an important role in regulating the number of metabolic activities in the human body such as bone growth, controlling the bone structure, repairing the bone fractures etc. [37, 38]. The piezoelectric ceramics have ability to augment bone metabolism similar to bone without any external power source [39]. The piezoelectric $Na_{0.5}K_{0.5}NbO_3$ (NKN) and $BaTiO_3$ (BT) have been recognized as potential alternative for polarizable orthopedic implants due to their excellent viability towards human osteoblast cells [40]. In addition, piezoelectric $Na_{0.5}K_{0.5}NbO_3$ (NKN) demonstrates antibacterial behavior [41]. On the other hand, piezoelectric ceramics enhance the bioactive performance as well as cellular response [42]. The incorporation of piezoelectric material as a secondary phase in HA matrix enhances the proliferation of human osteoblast cells [43]. For example, the addition of BT (90 vol. %) as a secondary phase in HA has been reported to increase the viability of osteoblast like SaOS2 cells by ~ 97 %, after incubation of 7 days [44]. Also, HA-$BaTiO_3$ composite system has been reported to promote osteogenesis in the dog femur bone [45]. The perovskite $CaTiO_3$ (CT) has been suggested as a good substrate to promote the apatite growth [46]. It has also been reported that CT promote the osteoblast adhesion and plays an important role to enhance the osseointegration [47].

The bioglasses and glass ceramics have been widely explored for orthopedic applications due to their excellent bioactivity and biocompatibility [48]. Among the bioglasses, borate bioglass (1393B3, BBG) has demonstrated excellent biocompatibility as well as reasonably higher growth rate for the formation of apatite layer [49]. The BBG possesses inherent antibacterial property i.e., it helps to minimize the bacterial infection [50]. In this context, BBG can be suggested as a potential alternative for prosthetic implants.

In the present work, three different compositions such as, (a) HA, HA- x ZnO (x = 3.0, 4.5, and 7.5 wt. %), (b) HA- 30 vol. % NKN/BT /CT and (c) BBG, BBG- 30 vol. % NKN/ BT have been taken as model biomaterials to verify the concept of surface polarization on *in-vitro* antibacterial and cellular response.

1.3. Polarization induced cellular and antibacterial response

Recently, electrical polarization of biomaterial substrates has been recognized as potential alternative to enhance the cellular response [51].The adhesion of osteoblast-like cells on solid substrate depends on the interaction of ionic components and proteins (integrin and fibronectin proteins), present in growth media, with substrate [52]. The polarized substrates anticipate to provide the cellular response. The cations such as, Ca^{2+} are adsorbed of on negatively (N)-polarized surfaces which interacts with the adhered proteins and provide the favorable condition for cell growth and proliferation [53]. However, on positively (P) - polarized surfaces, anions such as $HPO_4^{2-}$and $HCO_3^{2-}$are attracted and cations get repelled, which is unfavorable for cell adhesion [52]. For example, the proliferation of osteoblast-like hFOB cells increased by about 200 % on negatively polarized HA compared to uncharged HA, after 7 days of incubation [54]. In another study, it has been demonstrated that the density of L929 cells increases by 40 and 62 %, on negatively polarized HA-x BT (x = 20 and 40 wt. %) composites, respectively, after incubation of 48 h [55]. Overall, it can be concluded that negatively polarized surfaces enhance the proliferation of osteoblast-like cells.

A few reports suggested that polarization also increases the antibacterial response [56,57]. The antibacterial response of *S. aureus* bacteria increases on positively polarized polymethacrylate (PMMA)-trimethylaminoethyl methacrylate chloride (TMAEMA-Cl) composite as compared to negatively charged surface, after incubation of 2 h [58]. In this perspective, the concept of surface polarization on antibacterial response has been explored in the present study.

1.4. Objectives of the thesis

The primary objective of the present thesis was to verify the concept of surface polarization for improving the antibacterial and cellular response of developed model biomaterials.

The specific objectives of the present thesis are as follows:

(a) To study the influence of incorporation of ZnO, piezoelectric NKN, BT and perovskite CT as a secondary phase in HA matrix on *in-vitro* antibacterial and cellular response.

(b) To develop the surface charge using corona poling unit at the temperature and voltage of 500ºC and 50 kV, respectively, for 30 min.

(c) To optimize the polarizing field and temperature to get the maximum antibacterial and cellular response, *in-vitro*, on polarized substrate.

(d) To observe the effect of surface polarization on *in-vitro* antibacterial response as well as adhesion and proliferation of osteoblast-like cells on selected model biomaterials.

(e) To determine the combined effect of addition of secondary phases as well as surface polarization on antibacterial as well as cellular response on selected model compositions.

(f) To measure the enzymatic activities such as, generation of superoxide (SOD assay), lipid peroxide (LPO assay), catalyse (H_2O_2 production) and protein concentration to understand the mechanism of polarization on antibacterial response.

1.5. Outlines for the thesis

The present thesis has been divided into 7 chapters. Chapter 1 briefs the relevance behind the present research work. Chapter 2 reviews various biomaterials and techniques such as, incorporation of antibacterial agents/ piezoelectric materials as secondary phases, effect of nano materials as well as application of external stimuli for improving the antibacterial response. In addition, the proliferation of osteoblast-like cells on biomaterial/ biocomposite substrates and its relevance for the development of prosthetic implant has also been explored. Chapter 3 presents the experimental methodology for the development of model biomaterials as well as characterization techniques such as X-ray diffraction (XRD) and Fourier transformation infrared spectroscopy (FTIR). This chapter also provides the details of high voltage polarizing unit (corona poling unit) for the development of surface charge on processed bioceramics. In addition, the protocols adopted for studying the antibacterial and cellular response have been detailed. Chapter 4 elaborately discusses the phase evaluation (XRD and FTIR), microstructural characterization, dielectric and electrical behavior, polarization induced antibacterial and cellular response of optimally processed HA-x ZnO (x = 0.0, 3.0, 4.5, and 7.5 wt. %) composites. In addition, to further confirm the antibacterial response, few additional measurements such as Kirby-bauer assay and superoxide dismutase (SOD) assay have also been performed. Chapter 5 presents the phase identification, microstructural characterization, as well as *in vitro* antibacterial and cellular behavior of developed NKN, BT, CT and HA- x NKN/BT/CT (x = 30 vol. %), composites. In addition, the enzymatic activities such as, super oxide assay, catalase assay, lipid peroxidation assay and protein estimation assay have been done to explore the mechanism of antibacterial response.

Chapter 6 discusses the phase evolution, polarization induced antibacterial and cellular response of the developed BBG-x NKN/BT (x = 30 vol. %) composite. Chapter 7 provides the conclusions and future scope of the thesis.

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