Chapter 1

Introduction

The chapter introduces new class of materials for orthopaedic implant applications such as electroactive and functionally graded materials (FGMs) and their advantages over conventional implant materials. In addition, a brief introduction has been provided to the electroactive property of bone as well as hydroxyapatite which is structurally, compositionally and chemically similar to the bone apatite. The need to develop electroactive biocomposites as well as FGMs is also briefly discussed. Thereafter, the objectives of the dissertation have been briefly mentioned towards the end.

1.1. Background

In the health care industry, there is an increasing demand to develop biomaterials which are biologically inspired for replacement of hard as well as soft tissues to improve the entire healing process. Biologically inspired material exhibits close biochemical, bioelectrical as well as biophysical similarities with the host tissue.[1] To enroot these properties in a single material is a challenging task in the area of biomaterials. Towards this end, various organic and inorganic synthetic implants have been developed depending on the requirement of host tissue. As far as the development of prosthetic implants for one of the major tissues i.e., bone is concerned, the number of material classes in monolithic as well as in composite forms using metals, ceramics and polymers have been suggested.[2] Due to their high mechanical reliability, metals are generally used to develop load-bearing implants.[3] Metallic implants include titanium, titanium alloys, cobalt-chromium alloys and various types of stainless steels.[4,5] On the other hand, composites of ceramics-metals, polymers-ceramics and polymers-metals are generally developed for articulating surfaces in bones such as various joints, grafts and couples.[6] Metallic implants suffer from the major drawback of 'stress shielding' effect due to their high stiffness.[7] However, according to Wolf's law, bone requires constant mechanical stress/load to develop and regrow, otherwise, it gradually loses its mass and consequently, becomes porous.[8,9] Further, such phenomenon leads to the loosening of the implant. In addition, cobalt-chromium alloys, as well as stainless steel implants, release toxic elements such as Ni, Co, and Cr due to their time-dependent corrosion in the body environment which also restricts their use as implant materials.[10,11] Therefore, titanium and its alloys are preferred in orthopaedic implants due to their excellent corrosion resistance, reliable mechanical performance, biocompatibility as well as comparatively lower stiffness than stainless steel as well as cobalt-chromium alloys. However, titanium implants also suffer from the similar drawback of stiffness incompatibility with the bone as well as poor osteointegration which leads to their failure.[4,12,13,14,15] Osteointegration refers to the structural and functional bonding between the surface of synthetic implant material and the bone tissue.[16] Titanium based alloys also cause wear debris due to their high coefficient of friction with host bone tissue which restricts their lifetime use.[17,18]

In view of the above backdrops, the recent trends towards the development of prosthetic orthopaedic implant materials are centred around (a) composites, (b) polymeric coatings on metallic implants, (c) tissue engineering and (d) functionally graded materials (FGMs).[19,20] Out of these, the concept of FGMs have recently demonstrated their potentiality as an appealing choice for orthopaedic applications. Such materials/compositions can be properly tailored to meet the various requirements such as reliable biocompatibility, strength, resistance to corrosion, appropriate elastic modulus and close chemical similarity with that of hard tissues as well as osteoconductivity and osteoinductivity for bone and dental implants.[21,22,23,24] Functionally graded materials (FGMs) refer to the class of materials where the composition, as well as the properties such as mechanical, physical and biochemical, gradually vary in space along with the thickness or the specified direction, according to the application.[25,26] On the other hand, a composite material has abrupt macroscopic boundaries/sharp interfaces between their constitutive phases. Human bone is one of the primary examples of naturally occurring functionally graded material. The simplest example can be taken in the case of long bones whose external part is the compact cortical bone layer which covers the spongy or trabecular cancellous bone.[25] Considering its cross-section, there is a gradual variation in composition i.e., pore distribution as well as mechanical properties (tensile strength and modulus of elasticity) in the direction from external compact cortical bone to the spongy cancellous bone and vice versa.[25] Similarly, there are many other types of bones having the sophisticated graded structure according to the anatomical location as well as their physiological functionality.[25]

Apart from its physical nature of having a functionally graded structure, living bone is also an electrically active tissue.[27] The electrical characteristics of bone appear in the form of piezoelectricity, pyroelectricity and ferroelectricity.[28,29,30,31,32] Piezoelectric nature of living bone is an intimidating feature by which mechanical stress is converted to a potential difference / electric charges which further facilitates the bone in its growth, proliferation of bone cells (osteocyte, osteoblast and osteoclast) and healing/reconstruction of bones in the case of fractures.[28] Therefore, materials mimicking the electrical effects in the bone can be suggested as new generation biomaterials for orthopaedic implants.[33,34] Such materials have the ability to develop charges on their surface by external stimulation such as mechanical stress or E-field which can further facilitate excellent osteointegration as well as assists in healing fractures, remodelling and growth of bone tissue.[34]

1.2. Relevance of external electric field

The application of the external electric field (E-field) on living bones to heal fractures / non-unions has generally been used for more than a decade.[35,36] Due to its piezoelectric nature, living bone develops the polarization/electric charges on the application of external electrical stimulation or mechanical stress.[27] These charges flow to the extracellular matrix (ECM) in the form of bioelectrical signals and finally reach to the bone cells (fibroblasts and osteoblasts) activating various processes of proliferation, matrix production which results in rapid healing of fractures.[37,38,39,40,41,42] From the cellular point of view, the electric field enhances the intracellular calcium concentration, DNA and protein synthesis in osteoblasts as well as fibroblasts cells.[43] Therefore, external E-field enhances bone functionality. The piezoelectric synthetic implant material with the ability to develop polarization / electric charges on the application of physiological loads or external stimulation such as E-field or mechanical stress can resemble the behaviour of bone. However, the synthetic implant materials exhibit slow osseointegration ability with the bone tissue in the absence of external/internal cues despite the ability to develop polarization / electric charges.[44] To enhance these processes, properly tuned external electric field (E-field) has been reported to be an efficient stimulating factor for improved cellular interaction with the implant material.[44,45] It is due to the characteristics of the living cell which possess inherent electrical nature and their rapid response under the external E-field. A number of studies have been conducted by considering the interaction of E-field with living cells for various applications such as electrochemotherapy, necrosis, tissue ablation, gene therapy, cellular apoptosis, etc.[46,47,48,49,50] Therefore, properly tuned external E-field can enhance the proliferation of cells on the biomaterial substrates.

1.3. Model materials

Hydroxyapatite (HA), belongs to the family of calcium phosphate, is extensively studied for the orthopaedic implants due to its ability to chemically react with the bone tissue, *in-vitro* and *in-vivo*.[51,52,53] It is a bioactive ceramic having excellent biocompatibility. Synthetic hydroxyapatite (HA) having a calcium-to-phosphate stoichiometric ratio of 1.67 is the most desirous material for the orthopaedic implants due to its chemical as well as structural similarity with the inorganic mineral phase of bone.[54] In this context, incorporating hydroxyapatite (HA) as one of the principal ingredients in the development of a biocomposite as well as FGM for the bone-implant would be an excellent choice.

HA exists in two structures i.e., in hexagonal (space group: $P6_3/m$) and monoclinic ($P2_1/b$).[55,56] The monoclinic phase possesses a non-centrosymmetric structure while hexagonal has a centrosymmetric structure. Therefore, most of the electroactive properties are associated with the monoclinic form. In addition, these electroactive properties are mainly assessed with respect to the nanocrystalline HA. HA has been reported to exhibit piezo, pyro and ferroelectric nature.[57,58,59] In bulk HA, such properties are not very significant. In this respect, HA possesses poor electrical characteristics. However, the polarization of HA via the external electric field (E-field) to generate the surface charges has been reported to provide enhanced osteobonding as well as osteoconductivity.[60,61,62] Therefore, polarized HA is suggested to have favourable biological response both, *in-vitro* and *in-vivo* to support the growth of bone cells as well as bone tissue.[60,63]

Synthetic HA possess poor mechanical properties as well. It has been reported that addition of piezoelectric secondary phase (i.e., $BaTiO_3$) in the ceramic matrix overall increases the mechanical properties of the composite system.[64] In this respect, ferroelectric $Na_{0.5}K_{0.5}NbO_3$ (NKN) is a potential alternative due to its high piezoelectric strain coefficient, d_{33} ~ 260 pC/N, high Curie temperature, T_C ~ 420°C, electromechanical coupling coefficient, k_p ~ 0.48, mechanical quality factor, Q_m ~ 280 and dielectric constant, ε ~ 657 as well as its relatively lower density, ~ 4.51 gm/cm^3 as compared with other piezoelectric biomaterials.[65,66,67,68,69] In addition, ferroelectric NKN ($Na_xK_yNbO_3$; $0 \leq x \leq 0.8$, $0.2 \leq y \leq 1$) has been patented as biocompatible orthopaedic implant material because of its excellent viability towards human monocytes.[70] $BaTiO_3$ (BT) has been demonstrated to be another piezoelectric biocompatible material, *in-vitro* as well as *in-vivo.*[44,71,72,73,74] In simulated body fluid (SBF), $BaTiO_3$ promotes apatite formation.[71,74] $BaTiO_3$ also increases the fracture toughness of HA-$BaTiO_3$

composite system due to piezoelectric energy dissipation.[75] In addition, the presence of $BaTiO_3$ as the secondary phase in HA-$BaTiO_3$ composite reveals enhanced dielectric constant as well as piezo- and pyro-electric coefficients.[64,76] The HA-$BaTiO_3$ composite system has been reported to promote osteogenesis in the dog femur bone.[72] Another electroactive non-piezoelectric perovskite biocompatible material is $CaTiO_3$ (CT) which is suggested to be a potential substrate for osteointegration and osteoconduction.[77,78]

1.4. Objectives of the dissertation

The present dissertation is divided into two parts. The first part is concerned with developing/modifying the electrical analogue of a living cell to facilitate the evaluation of the E-field parameters which can be utilized for faster osteointegration of bone cells with biomaterial substrate. In the second part, enhancement of electrical properties of HA by developing piezobiocomposite as well as FGMs has been discussed. The developed HA based piezobiocomposite and FGMs can have close resemblance with the living bone in terms of electrical, mechanical and biochemical characteristics. Surface charges developed on the biomaterial substrates are also reported to stimulate the osteointegration with the bone tissue. Therefore, the analysis of the electrical properties of polarized electroactive biomaterials has been carried out in the present dissertation. Based on these considerations, the objectives of the present dissertation are:

(i) To develop/modify the electrical equivalent of the single living cell by considering various fundamental aspects of ionic flow across the cell and nuclear membranes.

(ii) To evaluate the time constant of the modified electrical model of a single living cell.

(iii) To study the variation of the time constant with the cellular parameters such as cell size, cell and nuclear membrane capacitances and resistances and cytoplasmic and nucleoplasmic resistances and its effect on various cell fate processes.

(iv) To evaluate the E-field intensity required to electroporate the cell for different E-field pulse duration. Thereafter, to study the response of various cellular compartments to E-field pulses of various durations.

(iii) To develop the monoliths of HA and $Na_{0.5}K_{0.5}NbO_3$ (NKN) as well as composite of HA and NKN via spark plasma sintering route.

(iv) To study their electrical characteristics such as thermally stimulated depolarization current (TSDC), ac conductivity and dielectric and loss behaviour after poling at very high E-field strength (up to 100 kV/cm) over the wide range of temperature.

(v) To examine the surface characteristics of HA electrets via X-ray photoelectron spectroscopy.

(vi) To develop FGMs comprising of HA, $BaTiO_3$ and HA, $CaTiO_3$ via spark plasma sintering route.

(vii) To analyse the microstructural (SEM) properties of developed FGMs as well as to determine the integration of different layers in developed FGMs.

(viii) To study the electrical properties of FGMs such as dielectric behaviour and ac conductivity as well as impedance spectroscopy over the wide range of temperature and frequency.

1.5. Outline for the dissertation

The present dissertation has been divided into 6 chapters. Chapter 1 introduces the relevance of carrying out the present research and provide the research gap in the present domain of study. Chapter 2 reviews the bioelectrical characteristics of bone, polarization behaviour of hydroxyapatite and its relevance towards the development of electroactive prosthetic implants. In addition, the influence of E-field on various complex cellular functionalities along with the need for the development of the electrical model of the living cell and its consequences have been elaborately discussed. Also, the necessity and relevance for the development of FGM have been mentioned objectively. Chapter 3 presents the modification and analytical analyses of the electrical equivalent of the living cell. In addition, the validation of the electrical equivalent model has been carried out by evaluating the E-field parameters required for electroporation to occur and thereafter, comparing them with the experimental reports. Chapter 4 is about the study of the electrical characteristics of polarized HA, NKN and HA–NKN composite system. Also, discussion on the detailed methodology of thermally stimulated depolarization current measurements as well as various polarization and depolarization mechanisms occurring in HA is followed. Chapter 5 details the development of functionally graded materials comprising of HA, $BaTiO_3$ and HA, $CaTiO_3$. The dielectric, ac conductivity as well as impedance spectroscopic behaviour has been elaborately discussed for the developed FGMs. Chapter 6 gives a conclusion and future scope for the present dissertation. The overall structure of the present dissertation is provided in Fig. 1.1.

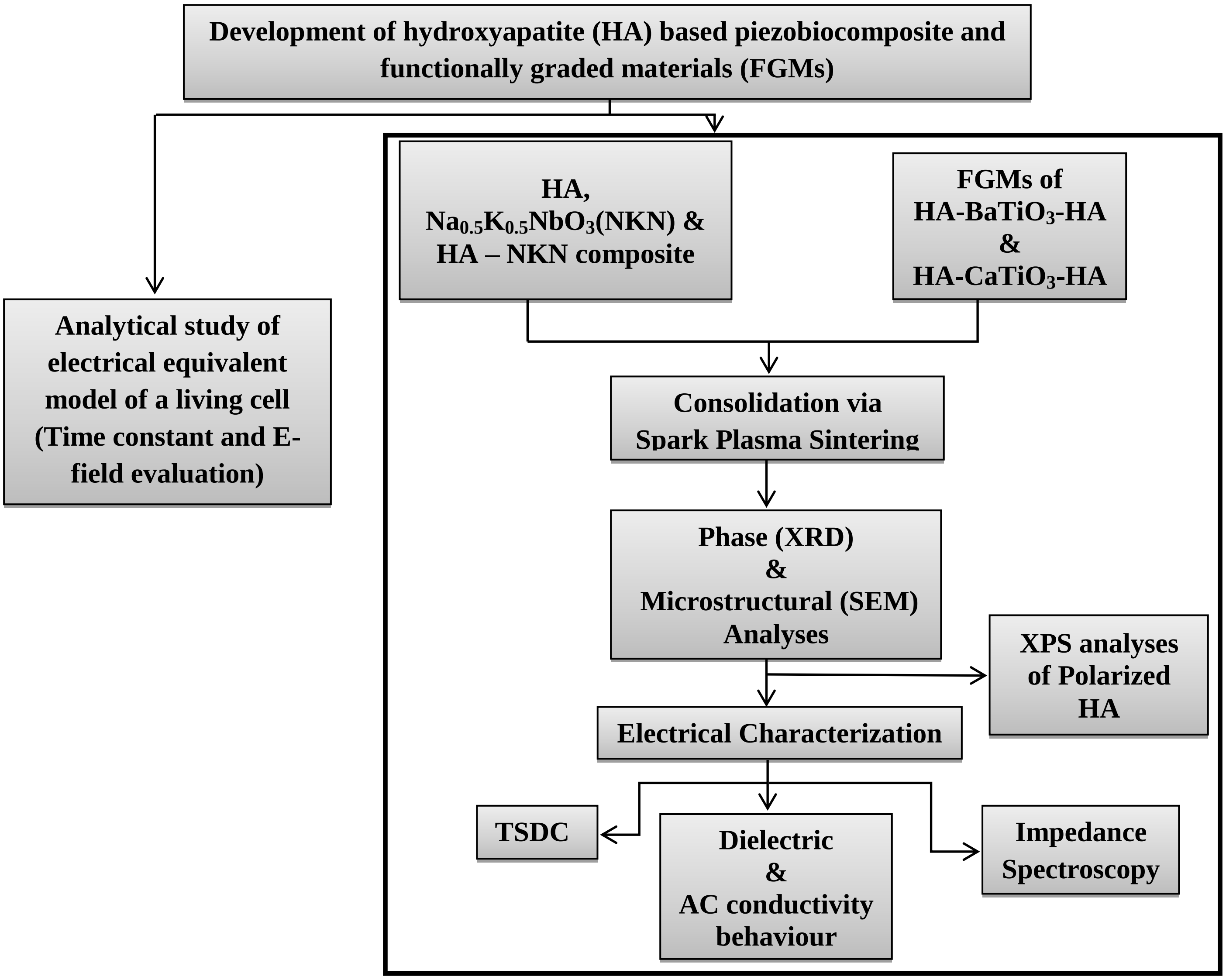

Fig. 1.1. Structure of the present dissertation

References

---

[1] W Pompe, H Worch, M Epple, W Friess, M Gelinsky, P Greil, U Hempel, D Scharnweber, K Schulte, “Functionally graded materials for biomedical applications”, *Materials Science and Engineering: A*, 362, (2003), 40-60.

[2] D. Mahmoud and M.A. Elbestawi, “Lattice Structures and Functionally Graded Materials Applications in Additive Manufacturing of Orthopedic Implants: A Review.”, *Journal of Manufacturing and Materials Processing*, 1, (2017), 13.

[3] M. Niinomi, T. Narushima, and M. Nakai, “Advances in Metallic Biomaterials, Processing and Applications”, Springer: Berlin/Heidelberg, Germany, Volume 3, 2015.

[4] A. A. Moussa and R. Yadav, “Optimization of a Functionally Graded Material Stem in the Femoral Component of a Cemented Hip Arthroplasty: Influence of Dimensionality of FGM”, *Journal of Medical Engineering*, 2017, (2017), 1-10.

[5] Q. Chen and G. A. Thouas, “Metallic implant biomaterials”, *Materials Science and Engineering: R: Reports*, 87, (2015), 1-57.

[6] A. Rajpura, D. Kendoff, and T.N. Board, “The current state of bearing surfaces in total hip replacement”, *Bone and Joint Journal*, 96-B(2), (2014) 147-156.

[7] R. Huiskes, H. Weinans and B. van Rietbergen, “The relationship between stress shielding and bone resorption around total hip stems and the effects of flexible materials”, *Clinical Orthopaedics and Related Research*, 274, (1992), 124-134.

[8] H. M. Frost, “Skeletal structural adaptations to mechanical usage (SATMU): 3. The hyaline cartilage modelling problem”, *The Anatomical Record*, 226, (1990), 423-432.

[9] H. M. Frost, “Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians”, *The Angle Orthodontist* 64(3), (1994), 175-188.

[10] Y. Okazaki and E. Gotoh, “Comparison of metal release from various metallic biomaterials in vitro”, *Biomaterials*, 26, (2005), 11-21.

---

[11] APA Wapner and L. Keith, “Implications of Metallic Corrosion in Total Knee Arthroplasty”, *Clinical Orthopaedics and Related Research*, 271, (1991), 12-20.

[12] I. D. Learmonth, C. Young, and C. Rorabeck, “The operation of the century: total hip replacement”, *The Lancet*, 370 (9597), (2007), 1508-1519.

[13] M. Thomsen and C. Lee, “In vitro rotational stability of cemented stem designs, in The Well Cemented Total Hip Arthroplasty–Theory and Practice”, S. Breusch and H. Malchau, Eds., pp. 196–205, Springer, Berlin, Germany, 1st edition, (2005).

[14] M. Jasty, W. J. Maloney, C. R. Bragdon, T. Haire, and W. H. Harris, Histomorphological studies of the long-term skeletal responses to well fixed cemented femoral components, *Journal of Bone and Joint Surgery -Series A*, 72(8), (1990), 1220-1229.

[15] M. Jasty, W. J. Maloney, C. R. Bradgon, D. O’Conner, T. Harie, and W. H. Harris, “The initiation of failure in cemented femoral components of hip arthroplasties”, *Journal of Bone and Joint Surgery*, 73B (4), (1991), 551-558.

[16] PI Branemark, USA: Quintessence Books; 2005. The Osseointegration Book – *From Calvarium to Calcaneus*; p. 24.

[17] P.G. Liang Jr., E.S. Ferguson Jr. and E.S. Hodge, Tissue reaction in rabbit muscle exposed to metallic implants, *Journal of Biomedical Materials Research*, 1, (1967), 135-149.

[18] M. Geetha, A.K. Singh, R. Asokamani, A.K. Gogia, “Ti based biomaterials, the ultimate choice for orthopaedic implants – A review”, *Progress in Materials Science*, 54 (3), (2009), 397-425.

[19] HS Hedia, “Design of functionally graded dental implant in the presence of cancellous bone”, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 75B: (2005), 74-80.

---

[20] M. Mehrali, FS Shirazi, M Mehrali, HSC Metselaar, NAB Kadri, NAA Osman, “Dental implants from functionally graded materials”, *Journal of Biomedical Materials Research Part A*, 101A, (2013), 3046-3057.

[21] B. An, R. Wang, D. Arola, D. Zhang, “The role of property gradients on the mechanical behavior of human enamel”, *Journal of Mechanical Behavior and Biomedical Materials*, 9, (2012), 63-72.

[22] W-J Li, CT Laurencin, EJ Caterson, RS Tuan and FK Ko, “Electrospun nanofibrous structure: A novel scaffold for tissue engineering”, *Journal of Biomedical Materials Research*, 60, (2002), 613-621.

[23] KF Leong, CK Chua, N Sudarmadji and , WY Yeong, “Engineering functionally graded tissue engineering scaffolds”, *Journal of Mechanical Behavior and Biomedical Materials*, 1, (2008) 140-152.

[24] E. Askari, M Mehrali, IHSC Metselaar, NA Kadri , MM Rahman, “Fabrication and mechanical properties of Al2O3/SiC/ZrO2 functionally graded material by electrophoretic deposition”, *Journal of Mechanical Behavior and Biomedical Materials*, 12, (2012), 144-150.

[25] A. Sola, D. Bellucci andV. Cannillo, “Functionally graded materials for orthopaedic applications – an update on design and manufacturing”, *Biotechnology Advances*, 34, (2016), 504-531.

[26] Dina H.A. Besisa and Emad M.M. Ewais, “Advances in Functionally Graded Ceramics – Processing, Sintering Properties and Applications”, in F. Ebrahimi (Ed.) ‘Advances in Functionally Graded Materials and Structures’, IntechOpen: Rijeka, (2016).

[27] BM Isaacson and RD Bloebaum, “Bone bioelectricity: what have we learned in the past 160 years?”, *Journal of Biomedical Materials Research Part A*, 95(4), (2010), 1270-1279.

---

[28] E. Fukada and I. Yasuda, “On the Piezoelectric Effect of Bone”, *Journal of the Physical Society of Japan*, 12, (1957), 1158-1162.

[29] C. J. Dreyer, “Properties of Stressed Bone”, *Nature*, 189, (1961), 594-595.

[30] S. B. Lang, “Pyroelectric Effect in Bone and Tendon”, *Nature*, 212**,** (1966)**,** 704-705.

[31] S. Cerquiglini, M. Cignitti, M. Marchetti and A. Salleo, “On the origin of electrical effects produced by stress in the hard tissues of living organisms”, *Life Sciences*, 6(24), (1967), 2651-2660.

[32] M.A. El Messiery, G.W. Hastings and S. Rakowski, “Ferro-electricity of dry cortical bone”, *Journal of Biomedical Engineering*, 1, (1979), 63-65.

[33] SJ Lee, JJ Yoo and A Atala, Biomaterials and tissue engineering. In: Clinical Regenerative Medicine in Urology. Singapore: Springer; 2018. p. 17-51.

[34] J. Jacob, N. More, K. Kalia and G. Kapusetti, “Piezoelectric smart biomaterials for bone and cartilage tissue engineering”, *Inflammation and Regeneration,* 38**,** (2018)**,** 2.

[35] R. K. Aaron, D. M. Ciombor and B. J. Simon, “Treatment of nonunions with electric and electromagnetic fields”, *Clinical Orthopaedics and Related Research*, 2906, (2004), 21-29, 2004.

[36] S. A. W. Pickering and B. E. Scammell, “Electromagnetic Fields for Bone Healing”, *Interantional Journal of Lower Extremity Wounds*, 1(3), (2002), 152–160.

[37] CA Bassett CA, SN Mitchelland SR Gaston, “Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields”, *Journal of Bone and Joint Surgery*, 63-A (4), (1981), 511-523.

[38] CA Bassett, AA Pilla, EI Mitchell and RE Booth, “Repair of non-unions by pulsing electromagnetic fields”, *Acta Orthopaedica Belgica*, 44, (1978), 706-724.

---

[39] CT Brighton, ZB Friedenberg, EI Mitchell, and RE Booth RE, “Treatment of nonunion with constant direct current”, *Clinical Orthopaedics and Related Research*, 124, (1977), 106-123.

[40] CT Brighton, GT Tadduni, SR Goll and SR Pollack, “Treatment of denervation/-disuse osteoporosis in the rat with a capacitively coupled electrical signal: effects of bone formation and bone resorption”, *Journal of Orthopaedic Research*, 6(5), (1988), 676-684.

[41] R Cappanna, D Donati, C Masetti, M Manfrini, A Panozzo, R Cadossi and M Campanacci, “Effect of electromagnetic fields on patients undergoing massive bone grafts following bone tumor resection”, *Clinical Orthopaedics and Related Research*, 306, (1994), 213-221.

[42] RB Heppenstall, “Constant direct-current treatment for established nonunion of the tibia”, *Clinical Orthopaedics and Related Research*, 178, (1983), 179-184.

[43] GJ Bourguignon and LYW Bourguignon, “Electric stimulation of protein and DNA synthesis in human fibroblasts”, *Journal of the Federation of American Societies for Experimental Biology*, 1(5), (1987), 398-402.

[44] A. K. Dubey and B. Basu, “Pulsed Electrical Stimulation and Surface Charge Induced Cell Growth on Multistage Spark Plasma Sintered Hydroxyapatite-Barium Titanate Piezobiocomposite”, *Journal of the American Ceramic Society*, 97, (2014), 481-489.

[45] B. Ercan and T. J. Webster, Greater Osteoblast Proliferation on Anodized Nanotubular Titanium upon Electrical Stimulation, *International Journal of Nanomedicine*, 3 [4], (2008), 477-485.

[46] D. Miklavčič, B. Mali, B. Kos, R. Heller and G. Serša, “Electrochemotherapy: from the drawing board into medical practice”, *Biomedical Engineering Online*, 13(1): (2014), 29.

[47] E. Neumann, “Membrane electroporation and direct gene transfer”, *Bioelectrochemistry and Bioenergetics*, 28, (1992), 247-267.

---

[48] M.L. Yarmush, A. Golberg, G. Serša, T. Kotnik and D. Miklavčič, “Electroporation-based technologies for medicine: principles, applications, and challenges”, *Annual Review of Biomedical Engineering*, 16, (2014), 295-320.

[49] R.V. Davalos, I.L.M. Mir and B. Rubinsky, “Tissue ablation with irreversible electroporation”, *Annals of Biomedical Engineering*, 33, (2005), 223-231.

[50] T. Kotnik, W. Frey, M. Sack, S. Haberl Meglič, M. Peterka and D. Miklavčič, “Electroporation-based applications in biotechnology”, *Trends in Biotechnology*, 33, (2015), 480-488.

[51] S. Best, A. Porter, E. Thian, and J. Huang. Bioceramics: past, present and for the future, *Journal of the European Ceramic Society*, 28(7), (2008), 1319-1327.

[52] L. L. Hench, “Bioceramics”, *Journal of the American Ceramic Society*, 81, (1998), 1705-1728.

[53] J. H. Jeong, I. J. Kwak, H. I. Kim, J. L. Ong, H. R. Rawls, and Y. J. Park, The 81st General Session of the International Association for Dental Research.Goteberg, Sweden, 2003.

[54] R Z Legeros and J P Legeros, Hydroxyapatite, Tadashi Kokubo (Ed.) in ‘Bioceramics and their Clinical Applications’, In Woodhead Publishing Series in Biomaterials, Woodhead Publishing, 2008, 367-394.

[55] M.I. Kay, R.A. Young and A.S. Posner, Crystal Structure of Hydroxyapatite, *Nature*, 204, (1964), 1050-1052.

[56] J. C. Elliott, P. E. Mackie and R. A. Young, “Monoclinic Hydroxyapatite”, *Science*, 180 (4090), (1973), 1055-1057.

[57] S. A. M. Tofail, C. Baldisserri, D. Haverty, J. B. McMonagle and J. Erhart, “Pyroelectric surface charge in hydroxyapatite ceramics”, *Journal of Applied Physics*, 106, (10), (2009), 106104.

---

[58] S. A. M. Tofail, D. Haverty, F. Cox, J. Erhart, P. Hána and V. Ryzhenko, "Direct and ultrasonic measurements of macroscopic piezoelectricity in sintered hydroxyapatite", *Journal of Applied Physics*, 105(6), (2009), 064103.

[59] S. B. Lang, "Review of Ferroelectric hydroxyapatite and its application to biomedicine", *Phase transitions*, 89(7-8), (2016), 678-694.

[60] K. Yamashita, N. Oikawa and T. Umegaki, "Acceleration and deceleration of bone-like crystal growth on ceramic hydroxyapatite by electric poling", *Chemistry of Materials*, 8(12), (1996), 2697-2700.

[61] M. Nakamura, A.Nagai, T. Hentunen, J. Salonen, Y. Sekijima, T. Okura, K. Hashimoto, Y. Toda, H. Monma, and K. Yamashita, "Surface Electric Fields Increase Osteoblast Adhesion through Improved Wettability on Hydroxyapatite Electret", *ACS Applied Materials and Interfaces*, 1(10), (2009), 2181-2189.

[62] D. Kumar, J.P. Gittings, I.G. Turner, C.R. Bowen, A. Bastida-Hidalgo, S.H. Cartmell, "Polarization of hydroxyapatite: Influence on osteoblast cell proliferation", *Acta Biomaterialia*, 6 (4), (2010), 1549-1554.

[63] Nakamura, M., Y. Sekijima, S. Nakamura, T. Kobayashi, K. Niwa, and K. Yamashita, "Role of blood coagulation components as intermediators of high osteoconductivity of electrically polarized hydroxyapatite", *Journal of Biomedical Materials Research. Part A*, 79A(3), (2006), 627-634.

[64] A. K. Dubey, A. EA, K. Balani and B. Basu, "Multifunctional Properties of Multistage Spark Plasma Sintered HA–$BaTiO_3$-Based Piezobiocomposites for Bone Replacement Applications", *Journal of the American Ceramic Society*, 96, (2013), 3753-3759.

[65] R. E. Jaeger and L. Egerton, "Hot pressing of potassium sodium Niobates", *Journal of the American Ceramic Society*, 45, (1962), 209-213.

---

[66] M. Kosec and D. Kolar, “On activated sintering and electrical properties of NaKNbO$_3$”, *Materials Research Bulletin*, 10, (1975), 335-340.

[67] R. Zuo, J. Rodel, R. Chen and L. Li, “Sintering and electrical properties of lead free Na$_{.5}$K$_{.5}$NbO$_3$ piezoelectric ceramics”, *Journal of the American Ceramic Society*, 89, (2006), 2010-2015.

[68] H. Wang, R. Zuo, J. Fu, Y. Liu, “Sol-gel derived (Li, Ta, Sb) modified sodium potassium niobate ceramics: Processing and piezoelectric properties”, *Journal of Alloys And Compounds*, 509, (2011), 936-941.

[69] D. Khare, B. Basu and A. K. Dubey, “Electrical stimulation and piezoelectric biomaterials for bone tissue engineering applications”, *Biomaterials*, 258, (2020), 120280.

[70] K. Nilsson, J. Lidman, K. Ljungstrom and C. Kjellman, Biocompatible material for implants, *U.S. Patent 6*, 526, 984 B1, 2003.

[71] Y. J. Park, Y. H. Jeong, Y. R. Lee, S. R. Noh, and H. J. Song, “Effect of Negatively Polarized Barium Titanate Thin Film, Formed on Ti, on Osteoblast Cell Activity”, *Journal of Dental Research*, 82, (2003), B212.

[72] J. Q. Feng, H. P. Yuan, and X. D. Zhang, Promotion of Osteogenesis by a Piezoelectric Biological Ceramic, *Biomaterials*, 18, (1997), 1531-1534.

[73] Y. J. Park, K. S. Hwang, J. E. Song, J. L. Ong, and H. R. Rawls, “Growth of Calcium Phosphate on Poling Treated Ferroelectric BaTiO$_3$ Ceramics”, *Biomaterials*, 23, (2002), 3859-3864.

[74] K. S. Hwang, J. E. Song, H. S. Yang, Y. J. Park, J. L. Ong, and H. R. Rawls, “Effect of Poling Conditions on Growth of Calcium Phosphate Crystal in Ferroelectric BaTiO3 Ceramics”, *Journal of Materials Science: Materials in Medicine*, 13, (2002), 133-138.

[75] X. M. Chen and B. Yang, “A New Approach for Toughening of Ceramics”, *Materials Letters*, 33, (1997), 237-240.

---

[76] A. K. Dubey, B. Basu, K. Balani, R. Guo, and A. S. Bhalla, "Dielectric and Pyroelectric Properties of HAp–BaTiO3 Composites", *Ferroelectrics*, 423 [1], (2011), 63-76.

[77] T.J. Webster, C. Ergun, R.H. Doremus, and W.A. Lanford, "Increased osteoblast adhesion on titanium-coated hydroxylapatite that forms $CaTiO_3$", *Journal of Biomedical Materials Research Part A*, 67A: (2003), 975-980.

[78] A. K. Dubey, G. Tripathi and B. Basu, "Characterization of hydroxyapatite-perovskite ($CaTiO_3$) composites: Phase evaluation and cellular response.", *Journal of Biomedical Materials Research*, 95B: (2010), 320-329.