# Chapter 1

# Introduction

The chapter briefly introduces the relevance of the present study in terms of development of electro-active biocomposites as well as the various techniques used to characterize the developed material system. Also, the importance of piezoelectric material as secondary phase in improving the mechanical, electrical, antibacterial and cytocompatibility of developed bioceramic composites is briefly mentioned. Towards the end, the objectives and structure of the thesis has been elaborated.

## 1.1 Natural bone

Bone, a natural nanocomposite material, is a rigid body tissue that constitutes part of the vertebral skeleton [1]. It contains almost 30-40 % of body's weight. The mineralized matrix of bone tissue consists of an organic component (mainly of collagen) and an inorganic component (hydroxyapatite) [1]. Mineral component provides the compressive strength and tensile strength is provided by the organic component [2]. The collagen also provides toughness to the bone [3]. Natural bone possesses reasonable combination of mechanical properties such as, fracture toughness (2-12 $MPa.m^{1/2}$), hardness (~1 GPa), compressive strength (~131 MPa) and bending strength (~ 160 MPa) [4– 8] and electrical properties such as, dielectric constant (~ 10) [9] and ac conductivity (~ $10^{-9}$ to $10^{-10}$ $ohm^{-1}$ $cm^{-1}$) [10]. Natural bone also possesses piezoelectric characteristic which control its metabolism [11]. Due to piezoelectric property, the natural bone polarizes in response to applied mechanical stimulation, which helps in bone growth [10]. The application of compressive stress polarizes the bone negatively and the tensile stress polarizes it positively [12]. The negative potential develops on the bone is responsible for the growth of bone – like crystals. Overall, it can be realized that in addition to reasonable mechanical strength, the natural living bone is an electrically active tissue.

## 1.2 Biomaterial

A synthetic biomaterial is the substance which has been skillfully and deliberately fabricated to communicate with the biological systems [13]. There are two classical definitions first; *Biomaterial is used to make devices to replace a part or a function of the body in a safe, reliable, economic and physiologically acceptable manner* (Hench and Erthridge, 1982) and second; *Materials of synthetic as well as of natural origin in contact with tissue, blood, biological fluids, and intended for use for prosthetic, diagnostic, therapeutic, and storage applications without adversely affecting the living organism and its components* which was (Bruck,1980).

Among the several types of biomaterials, hydroxyapatite (HA), 45S5 bioglass(45S5 BG) and 1393 bioglass (1393 BG) have generated great interest due to their excellent biocompatibility and corrosion resistance in the body fluid environment [14].

## 1.3 Hydroxyapatite (HA), 45S5 bioglass and 1393 bioglass as the matrix

In last few decades, number of attempts has been made to develop various types of biomaterials, based on the functional requirement of the host tissue. Metallic implants such as stainless steel, Ti and its alloys etc. are being used as the orthopedic implant material. Owing to wear, corrosion, mismatch in elastic modulus between implant and host, fibrous tissue encapsulation etc., the applications of such implant materials are limited, as far as the long term success of such implants are concerned. Towards this end, the bioceramics and bioglasses appear to be superior alternatives.

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] is the foremost inorganic component of bones and teeth, which exhibits hexagonal crystal structure and consists of about 65 wt. % of bone [15]. HA is structurally similar to bone and teeth minerals which can adhere or integrate with both, bone as well as soft tissues [16]. The calcium phosphate, available in form of HA, has been extensively studied for bone replacement applications. Synthetic hydroxyapatite is commonly used in tooth root replacement and healing of bones [17]. Bioglasses such as, 45S5 BG and 1393 BG also possess excellent osteoconductivity and biocompatibility and are widely used for orthopaedic applications [18 – 22]. Both, BGs form a direct bond with the bone tissue due to the formation of hydroxyapatite layer on its surface with the similar chemical composition as that of the bone which provides augmented osteogenesis by synchronizing the induction and proliferation of the cells [23– 28]. However, low mechanical properties such as, fracture toughness (0.5 – 1 $MPa.m^{1/2}$) [29], bending strength (40 – 120 MPa) [30 – 32] etc. of HA and BGs limit their application in load bearing areas [33,34].

### 1.4 Sodium potassium niobate ($Na_{0.5}K_{0.5}NbO_3$; NKN) as piezoelectric secondary phase

Recently, $Na_{0.5}K_{0.5}NbO_3$ (NKN) has been demonstrated to reveal high potentiality as the biomaterial for electroactive bone applications [35,36]. It possesses excellent dielectric and electrical properties such as, dielectric constant (~ 400), electrical conductivity ($1.2 \times 10^{-7}$ $ohm^{-1}cm^{-1}$) and reasonable mechanical properties such as, fracture toughness (1.4 ± 0.1 $MPa.m^{1/2}$), elastic modulus (140 GPa) and flexural strength (~ 90 MPa) [37 – 41]

### 1.5 Mechanical properties

It has been reported that the incorporation of piezoelectric materials such as $BaTiO_3$, $LiTaO_3$ etc., as secondary phases enhances the mechanical properties (fracture toughness, hardness, and flexural strength) of ceramic composite system [42,43]. NKN is also a promising candidate with high electromechanical coupling coefficient (~ 0.45). Apart from fundamental toughening mechanisms such as, crack deflection, crack bridging, microcrack toughening and transformation toughening, piezoelectric secondary phase provides additional toughening via energy dissipation and domain switching mechanisms.

Owing to piezoelectricity, some of the crack energy dissipates in the process of domain switching etc. which provide the additional toughening mechanism to composite system.

**1.6 Dielectric and electrical properties**

Since, natural bone is an electro-active tissue, the development of prosthetic materials for mimicking the functional performance of bone also requires to consider their electro-active response. NKN is one of the best known biocompatible piezoelectric materials with excellent electrical properties, which can be examined as the secondary phase for monolithic HA, 45S5 BG and 1393 BG compositions. The dielectric behavior of the composite samples depends on the governing polarization mechanisms. It has been reported that the conduction in HA occurs due to formation of vacancies at the hydroxyl position while migration of alkali ions are responsible for conduction in both the bioglasses.

**1.7 Antibacterial behavior**

Apart from mechanical, dielectric and electrical compatibility, bacterial infection on the implants become a serious concern which causes the implant failure or often requires revision surgery. Both, the bacterial cells, i.e., gram positive and gram negative bacteria possess the negative charge [44]. Gram negative bacterial cells have more negative charge than gram positive bacteria. Therefore, the charges on material’s surfaces can be anticipated to induce the antibacterial response. However, the nature and amount of charge needs to be analyzed to get the effective antibacterial response. NKN, being excellent piezoelectric material, can generate large amount of surface charge after electrical polarization.

**1.8 Cellular response**

HA / 45S5 BG / 1393 BG are well known biocompatible materials. Polarization induced surface charges promote the cell adhesion [38]. It has been reported that the $Ca^{2+}$ ions are attracted towards negatively charged surface and promote the attachment of cell adhesion factors (integrin and fibronectin proteins), which accelerate the cellular functionality [45]. In addition, external electrical stimulation further accelerates cell adhesion, proliferation and biomineralisation by activating voltage gated $Ca^{2+}$ channels.

**1.9 Objectives**

The objective of the present work is to synthesize the electro-active biomaterial compositions with bone mimicking mechanical, dielectric and electrical properties. In addition, the antibacterial response and biocompatibility is attempted to enhance by means of surface polarization.

The specific objectives are as follows:

(a). To synthesize HA / 45S5 BG / 1393 BG composites with varying amounts (10 – 30 wt. % for HA based composites and 10  30 vol. % for 45S5 BG / 1393 BG based composites) of piezoelectric NKN as the secondary phase and to optimize the processing parameters for these compositions to achieve maximum densification.

(b). To identify the phases, using X – ray diffraction and Fourier transform infrared spectroscopy techniques for HA – (10 – 30 wt. %) NKN, 45S5 BG – (10 – 30 vol. %) NKN and 1393 BG – (10-30 vol. %) NKN composite samples and to observe any dissociation or reaction between the matrixes and secondary phase.

(c). To observe the microstructure of the developed samples.

(d). To evaluate the mechanical properties such as, hardness, fracture toughness, flexural and compressive strength of sintered samples.

(e). To measure the dielectric and electrical properties such as, dielectric constant, loss and ac conductivity of the sintered samples.

(f). To observe the antibacterial response of developed composite samples by means of surface polarization, quantitatively and qualitatively.

(g). To investigate the influence of surface charge and external electrical field on cell adhesion and proliferation for HA / 45S5 BG / 1393 BG – NKN composites.

**1.10 structure of the thesis**

The entire thesis has been categorized into 7 chapters. Chapter 1 provides the relevance of the present study. Chapter 2 reviews the studies, associated with the development of various composite materials for bone replacement applications. This chapter also demonstrates the fundamentals of toughening as well as piezoelectric toughening mechanisms due to incorporation of secondary phase. In addition, the effect of piezoelectric secondary phase in bioceramics on mechanical, dielectric and electrical, antibacterial activity and cellular response of developed biomaterial has been reviewed. Chapter 3 covers the entire methodology, used to develop and characterize the bioceramic composites, including processing and various characterization techniques such as, mechanical, dielectric and electrical, antibacterial behavior and cellular response. Chapter 4 elaborately discusses the densification behavior, phase evolution (XRD and FTIR) and microstructural observations, mechanical, dielectric and electrical behavior, polarization induced antibacterial response and cellular response with combined action of external electric field and surface charge for the optimally processed HA – (10 – 30 wt. %) NKN composites. In addition, to verify the antibacterial behavior, this chapter also discusses the additional antibacterial characterization techniques such as disc diffusion test and ROS generation with different methods such as, catalase activity assay, SOD assay, lipid peroxidation assay and protein estimation assay. Chapter 5 covers the results and discussion such as, phase identification, mechanical, dielectric and electrical properties, antibacterial response and cellular functionality for the developed 45S5 BG – (10 – 30 vol. %) NKN composites. In addition, Kirby Bauer test has also been performed to verify the antibacterial behavior. Chapter 6 discusses the results such as, phase identification, mechanical behavior, antibacterial and cellular response for the developed 1393 BG – (10 – 30 vol. %) NKN composites. As a closure, chapter 7 provides the conclusions and future scopes of this thesis. Fig. 1.1 summarizes the overall objectives of the present work.

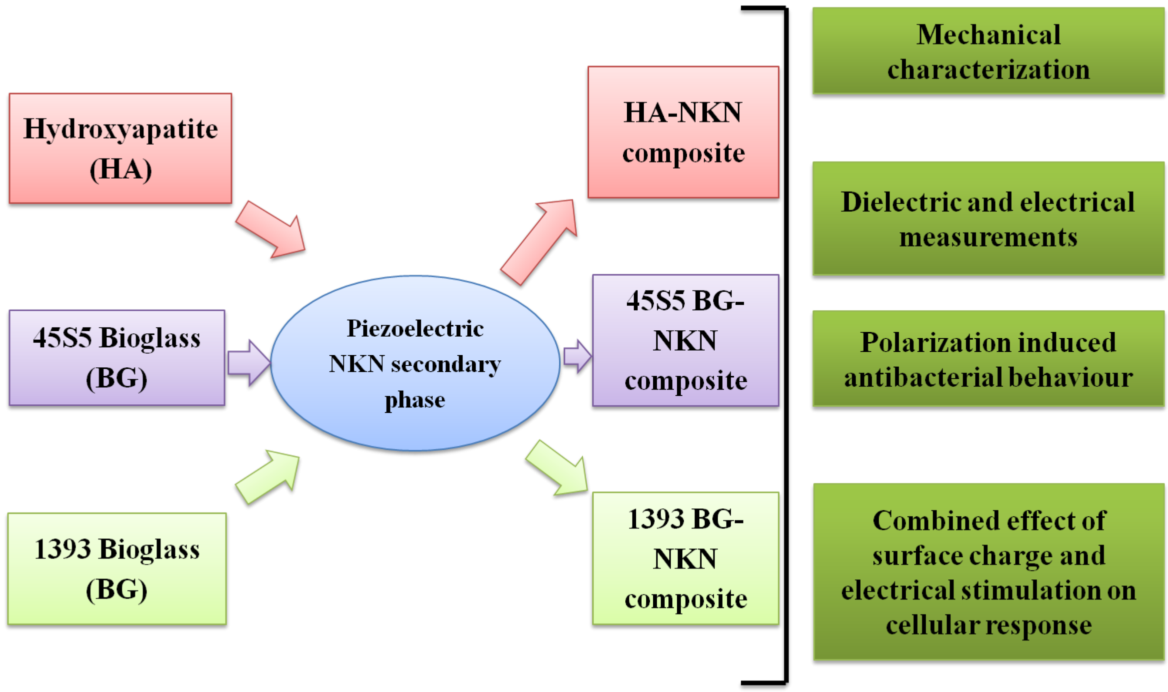


*Fig. 1.1 Schematic illustration of overall objectives of the present work*

**References**


1. A. L Boskey, “Bone composition: relationship to bone fragility and anti-osteoporotic drug effects,” *International Bone & Mineral Society*, **447** (2013).

2. D. B. Burr, “The Contribution of the Organic Matrix to Bone’s Material Properties,” *Bone* **31(1)** (2002), 8–11

3.X. Wang, R. A. Bank, J. M. TeKoppele , C. M. Agrawal, The role of collagen in determining bone mechanical properties, *Journal of Orthopaedic Research,* **19** (2001) 1021-1016.

4 P. K. Zysset, X. E. Guo, C. E. Hoffler, K. E. Moore, S. A. Goldstein, “Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nano indentation in the human femur,” *Journal of Biomechanics,* **32** (1999) 1005-1012.

5. W. T. Dempster, T. L. Richard, "Compact bone as a non-isotropic material," *American Journal of Anatomy,* **91 (3)** (1952) 331 - 362.

6. D.T. Reilly, A.H. Burstein, “The Mechanical Properties of Cortical Bone,” *Journal of Bone and Joint Surgery,* **57A [5]** (1974) 1001–1022.

7. K. Tsuda, "Studies on the bending test and impulsive bending test on human compact bone." *Journal of Kyoto Prefectural University of Medicine* **61** (1957) 1001-1025.

8. E.D. Sedlin, “A Rheological Model for Cortical Bone: A Study of the Physical Properties of Human Femoral Samples,” *Acta Orthopaedica Scandinavica* **83** (1965) 1–77.

9. M.H Shamos, S. L. Leroy, "Physical bases for bioelectric effects in mineralized tissues." *Clinical Orthopaedics and Related Research®* **35** (1964): 177-188.

10. G. B. Reinish, A. S. Nowick, “Effect of Moisture on the Electrical Properties of Bone,” *Journal of the Electrochemical Society* 123 (10) (1976) 1451-145.

11.O. Kaygilia, S.V. Dorozhkinb, T. Atesa, A.A. Al-Ghamdic, F. Yakuphanoglua, "Dielectric properties of Fe doped hydroxyapatite prepared by sol–gel method," *Ceramic International*, **40** (2014) 9395-9402.

12. A. K. Dubey, R. Kinoshita, K.Kakimoto, Piezoelectric sodium potassium niobate mediated improved polarization and in vitro bioactivity of hydroxyapatite, *RSC Advances* **5** (2015) 19638-19646.

13. R. Hussain, F. Ghafoor, M. A. Khattak, "3D Scaffolds of Borate Glass and Their Drug Delivery Applications" In *Biomedical, Therapeutic and Clinical Applications of Bioactive Glasses* (2019) 153-173 Woodhead Publishing.

14. S. Nath, B. Basu, "Designing materials for hared tissue replacements" *J Korean Ceramic Society* **45** (2008) 1–29.

15. Y.J. Song, S.-lin Wen, "Preparation and physicochemical process of nanosized hydroxyapatite powders with high purity [J]." *Journal of Inorganic Materials* **5** (2002). 985-991.

16. Dj. Veljovic, B. Jokic, R. Petrovic, E. Palcevskis, A. Dindune, I.N. Mihailescu, Dj. Janac´kovic´, "Processing of dense nanostructured HA ceramics by sintering and hot pressing," Ceramic International **35** (2009) 1407-1413.

17. L.L. Hench, "Bioceramics: from concept to clinic," *Journal of the American Ceramic Society* **74** (1991) 1487–1510.

18. L.L. Hench, "The challenge of orthopaedic materials," *Current Orthopedics*, **14** (2000) 7-15.

19. M.V. Regí, C. Ragel, A.J.Salinas, "Glasses with medical applications," *European Journal of Inorganic Chemistry,* **6** (2003) 1029-1042.

20. J. Wilson, S.B. Low, "Bioactive ceramics for periodontal treatment: Comparative studies in the patus monkey," *Journal of Applied Biomaterials*, **3** (1992) 123-129.

21. S.M. Carvalho, C.D.F Moreira, A. C. X Oliveira, A. A. R. Oliveira, E. M. F Lemos, M.M. Pereira, "Bioactive glass nanoparticles for periodontal regeneration and applications in dentistry," *Nanobiomaterials in Clinical Dentistry (Second Edition), Micro and Nano Technologies,* (2019), 351-383.

22. H. Tripathi, S.P. Singh, K.A. Sampath, M Prerna, Ashish J., "Studies on Preparation and Characterization of 45S5 Bioactive Glass Doped with (TiO2 + ZrO2) as Bioactive Ceramic Material," *Bioceramics Development and Applications* **6** (2016) 1-6.

23. L.L. Hench, R.J. Splinter, W.C. Allen, T.K. Greenlee, "Bonding mechanisms at the interface of ceramic prosthetic materials" *Journal of Biomedical Materials Research*, **5 (6)** (1971) 117-141.

24. O. Bretcanu, X. Chatzistavrou, K. Paraskevopoulos, R. Conradt, I Thompson, A R. Boccaccin, "Sintering and crystallisation of 45S5 Bioglass powder," *Journal of the European Ceramic Society*, **29(16)** (2009) 3299-3306.

25. I B Leonor, R A Sousa, A M Cunha, R L Reis, Z P Zhong, D.Greenspan, "Novel starch thermoplastic/Bioglass composites: mechanical properties, degradation behavior and in-vitro bioactivity," *Journal of Materials Science: Materials in Medicine*. **13 (10)** (2002) 939-945.

26. P Ducheyne, Q. Qiu, "Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function," *Biomaterials* **20** (1999) 2287-2303.

27. H. Oonishi, L L Hench, J Wilson, E T. Sugihara, M. Matsuura, "Quantitative comparison of bone growth behavior in granules of Bioglass, A-W glass-ceramic, and hydroxyapatite," *Journal of Biomedical Materials Research*. **51(1)** (2000) 37-46.

28. M Neo, S Kotani, T Nakamura, T Yamamuro, C Ohtsuki, T Kokubo, Y. A Bando, "Comparative study of ultrastructures of the interfaces between four kinds of surface-active ceramic and bone," *Journal of Biomedical Materials Research.*, **26** (1992) 1419-1432.

29. A. K. Dubey, E.A. Anumol, K. Balani, 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 (12)** (2013) 3753–3759.

30 H. Aoki, "Science and Medical Applications of Hydroxyapatite," Tokyo, JAAS: 1991.

31 M Jarcho, C H Bolen, M B Thomas, J Bobick, J F Kay, R H. Doremus, "Hydroxyapatite synthesis and characterizationin dense polycrystalline form," I *Material Science* **11** (1976) 2027-2035.

32 Van Dijk, H. J. A., N. Hattu, K. Prijs. "Preparation, microstructure and mechanical properties of dense polycrystalline hydroxy apatite." *Journal of materials science* **16 (6)** (1981) 1592-1598.

33. Q. Fu, "Bioactive Glass Scaffolds for Bone Tissue Engineering," *Biomedical, Therapeutic and Clinical Applications of Bioactive Glasses*, (2019) 417–442.

34. L.L. Hench, E.C.Ethridge, "Biomaterials: An Interfacial Approach." Academic Press, New York, 1982.

35. A. Jalalian, A.M. Grishin, "Biocompatible ferroelectric (Na, K)$NbO_3$ nanofibers," *Applied Physics Letters* **100** (2012) 012904.

36. A.S. Verma, D. Kumar, A.K. Dubey. Dielectric and Electrical response of Hydroxyapatite - $Na_{0.5}K_{0.5}NbO_3$bioceramic composite, *Ceramic International* **45 (3)** (2019) 3297-3305.

37. A. J. Moulson, J. M. Herbert, “Electroceramics” Second addition, Wiley, (2003).

38. A.K. Dubey, K Kakimoto, A Obata, T. Kasuga, “Enhanced polarization of hydroxyapatite using the design concept of functionally graded materials with sodium potassium niobate,” *RSC Advances*, **4** (2014) 24601-24611.

39. J. Andrejovská, J Mihalik, V Kovaľ, H.Bruncková, J Dusza, “Microstructure and fracture-mechanical properties of Pb free piezoelectric ceramics on the base $Na_{0.5}K_{0.5}NbO_3$” *Powder Metallurgy Progress* ***9*(4)** (2009) 228.

40. L. Egerton , D. M. Dillon, “Piezoelectric and Dielectric Properties of Ceramics in the System Potassium—Sodium Niobate,” *Journal of the American Ceramic Society* **42(9)** (1959) 438-442

41.A. Martin, K.Kakimoto, “Effect of domain structure on the mechanical and piezoelectric properties of lead-free alkali niobate ceramics,” *Japanese Journal of Applied Physics* **53** (2014) 09PB09.

42. X.M. Chen, B. Yang, “A new approach for toughening of ceramics,” *Materials Letters* **33** (1997) 237-240

43. Y.G. Liu, D.C. Jia, Y. Zhou, Microstructure and mechanical properties of a lithium tantalate-dispersed-alumina ceramic composite, *Ceramics International* **28** (2002) 111–114.

44. R Sonohara , N Muramatsu , H Ohshima , T. Kondo, “Difference in surface properties between Escherichia coli and Staphylococcus aureus as revealed by electrophoretic mobility measurements,” *Biophysical Chemistry* **55(3)** (1995) 273-277.

45.M Ohgaki, T Kizuki, M Katsura, K Yamashita, “Manipulation of selective cell adhesion and growth by surface charges of electrically polarized hydroxyapatite” *Journal of Biomedical Materials Research* **57** (2001) 366–373.