

PIEZOELECTRIC BASED HYDROXYAPATITE NANOCOMPOSITE COATINGS AS POTENTIAL BIOIMPLANTS

Submitted in partial fulfilment of the requirements for the award of
Master of Technology Degree in
Medical Instrumentation

by

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DEPARTMENT OF BIOMEDICAL ENGINEERING

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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This is to certify that this Project Report is the bonafide work of **NANDHINI.A** (Reg. No.40440004) who carried out the project entitled "**Piezoelectric based Hydroxyapatite nanocomposite coatings as potential bioimplants**" under our supervision from **September 2021 to March 2022**.

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ABSTRACT

The piezoelectric material promotes the growth of bone. However, the material should be subjected to stress before it can produce an electric charge that promotes repair of bones and reconstruction of it conducive to healing of fracture. In the present study, Barium Strontium Titanate-Hydroxyapatite nanocomposite coatings (BST-HAp nanocomposite coatings) would be developed onto medical grade titanium alloy substrate by simple dip coating method. X-Ray Diffraction and scanning electron microscope (SEM) analysis would be done to determine crystal structure and surface morphology of the coatings. Tribology test has to be performed by using linear reciprocating test to examine the wear resistance of the coated sample. Piezoelectric effect would be studied from the BST-HAp nanocomposite coatings. Further, electrochemical corrosion behavior was carried out.

Keywords: *Piezoelectricity; HAp; Bioimplants; osteointegration; Corrosion behavior*

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LIST OF ABBREVIATIONS

ABP	ARTIFICIAL BLOOD PLASMA
AO	ACRIDINE ORANGE
ARS	ALIZARIN RED S
BST	BARIUM STRONTIUM TITANATE
CPD	CONTACT POTENTIAL DIFFERENCE
EDX	ENERGY DISPERSIVE X RAY
EIS	ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY
FESEM	FIELD EMISSION SCANNING ELECTRON MICROSCOPY
HA	HYDROXYAPATITE
JCPDS	JOINT COMMITTEE ON POWDER DIFFRACTION STANDARDS
OCP	OPEN CIRCUIT POTENTIAL
PBS	PHOSPHATE BUFFERED SALINE
PDP	POTENTIO DYNAMIC POLARIZATION
PRP	PLATELETS RICH PLASMA
SBF	SIMULATED BODY FLUID
SDS	SODIUM DODECYL SULFATE
XRD	X RAY DIFFRACTION

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CHAPTER – 1

INTRODUCTION

1.1 BIOMATERIALS

Biomaterials are the synthetic or natural materials intended to function appropriately in a bio-environment. This materials used for making devices that can interact with biological systems to coexist for long service with minimal failure (Praveen Kumar *et al.*, 2022). Meaning of bio implant is a biomaterial which is surgically implanted in a person's body to replace damaged tissue. The usage of implants and medical equipment's has become a popular practice and it is necessary to save the lives of thousands of people every day. For example, about one million hip replacements and more than 250,000 knee replacement surgeries are reported throughout the world every year (Rezaie *et al.*, 2015). The engineering objective is to provide minimal physiological stress to the remaining bone system so that the integrity and functionality of the bone and prosthetic materials are maintained over a long service life (Gurbhinder Singh *et al.*, 2010). Such engineered Nanomaterials have extended its potential therapeutic and preventive applications in biomedical and clinical field (Muthukrishnan *et al.*, 2019). This strategy can provide biomedical engineers with appropriate constructs in term of mechanical stability similar to the natural bone structure.

Biomaterials can generally be classified as biological and synthetic materials. Examples of biological biomaterials are collagen and chitin. Synthetic biomaterials consist of four main types: ceramics, metals, polymers, and composites. Examples of medical ceramics include phosphate ceramics, ceramics, and glass-ceramics. Ceramics and glass-ceramics are widely used in the fields of dentistry and bone replacement. (Ismail Hamisah *et al.*, 2021). Biomaterials, and specially bioceramics, have been developed and modified from being inert to bioactive (Krishnan *et al.*, 2013)

1.2 PIEZOELECTRIC MATERIAL

It has been also reported that natural bone is a piezoelectric material (Tang *et al.*, 2017). Human bone has been found to be a piezoelectric material that can supply the

necessary bioelectricity inside the body (Gittings *et al.*, 2009). It has been affirmed that whenever human bone comes under mechanical stress electric signal is produced that subsequently promotes bone regeneration and growth. Hence, piezoelectric materials as bone substitutes may be preferred over other biomaterials (Yasuda *et al.*, 1954). Piezoelectric material are the material that can produce electric energy upon application of mechanical stress and promotes the growth of bone. In general, piezoelectric materials can generate charges on two opposing surfaces in response to a mechanical strain, such as stretching, compression, or bending (Qianqian *et al.*, 2021). So, piezoelectric materials can provide the obligatory electrical energy deprived of using an external electrical stimulus inside the body. Actually, it has been asserted that when the bone is mechanically stressed, electrical signals are produced which in turn promote bone growth and repairing (Ribeiro *et al.*, 2015). As an alternative approach, piezoelectric materials have the ability to deliver electrical signals to bone cells and can enhance the cell proliferation in physiological bio-electrically stimulated environment to accelerate bone repair (Zhang *et al.*, 2016). Piezoelectricity is the name given to a very interesting phenomenon which simply consists in the generation of electricity as a result of a mechanical pressure applied to a solid. The meaning of the prefix word “piezo” derives from the Greek word “piezein” which means “to press”, so, literally, piezoelectricity means “pressure electricity”. A more sophisticated definition of piezoelectricity was given by Cady (Cady *et al.*, 1946) as being an electric polarization produced by mechanical strain in some crystals belonging to certain classes of materials. The phenomenon of piezoelectricity, which is a special property, characteristic only to a group of materials, was discovered by the Curie brothers Jacques and Pierre in 1880, during their investigation on the effect of pressure on the generation of electrical charge by some natural crystals such as quartz or tourmaline. The piezoelectricity generated in bones found to be applicable in sensing the biologic forces, which promotes the bone regeneration at the repair site (Ribeiro *et al.*, 2015).

1.3 BARIUM STRONTIUM TITANATE

Barium Strontium Titanate (BST) is a ferroelectric material with a perovskite structure. Perovskite materials, such as BST, have been attracted an increased

interest due to their inherent high dielectric constant (Nagaraj *et al.*, 1999, Kuroiwa *et al.*, 1994, Serraiocco *et al.*, 2002). BST is a promising material, due to its dielectric properties, Among other nano materials $\text{Ba}_{1-x}\text{Sr}_x\text{TiO}_3$ has attracted attention due to i) high chemical stability ii) high permittivity iii) high tunability iv) low dielectric losses at room temperature. That can be used as microspheres or scaffolds or bone filler materials. Conversely, BST has been extensively used as a piezoelectric material and biocompatible properties that is additionally supposed to be an intelligent material for bone regeneration. This property has previously been replicated by other piezoelectric materials such as Barium titanate composites also involving hydroxyapatite (Baxter *et al.*, 2009).

1.4 HYDROXYAPATITE

Hydroxyapatite [HAp , $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_6$] because of its chemical resemblances, structure of the crystal and good biocompatibility has been widely used as implantation material for coating in the usage of porous, granulated or compact forms (Vikas Rattan *et al.*, 2018). As a bone curative materials due to its resemblance to the inorganic constituents of humanoid bone and its biological performance (Tang *et al.*, 2017). HAp bioceramics belongs to a class of phosphate- based biomaterials known to be surface active or bioactive containing calcium orthophosphate with water, and can have structural functions as joint or bone/body rebuilds, tissue replacements. It can also be fabricated as coatings to improve the bioactivity, biocompatibility, compressive strength and corrosion resistance of metal implants (Blackwood *et al.*, 2003). Calcium phosphates have been considered as a good option for implant coatings that may promote accelerated bone healing around implants (Praveen Kumar *et al.*, 2022). In recent year hydroxyapatite (HAp) has become the first choice for coating of bio implants due to its Ca/P ratio, which is almost similar to the human bone. It has been proven by many researchers that HAp coating allows a controlled and rapid osseointegration between living bone and the surface of an implant (Ben-Nissan *et al.*, 2015). Natural bone is a nanocomposite consisting of a collagen and non-collagenous proteins -based soft hydrogel and it is also a chemical and crystallographic template of hard inorganic ceramic phase of HAp. In this study, we

have conventionally integrated HAp with naturally synthesized pure and biocompatible HAp goat femur bone (HAp) by calcination method. HAp can be coated on metallic implants using various coating techniques (Awasthi *et al.*, 2020). The metallic implant with HAp coating supports new bone development due to strong interface between the coating and host tissue (Song *et al.*, 2008). The biomaterials can be fabricated using several coating methods like physical vapor deposition, chemical vapor deposition, thermal spray, spin coating, dip coating, electrochemically and electrophoretic deposition methods (Sathishkumar *et al.*, 2016). Dip coating possesses several characteristics including ease in processing, uniform coating layers, lower operating temperatures, cost-effective and process complex assemblies (Dorozhkin *et al.*, 2015).

1.5 BIOGLASS CERAMICS

Silicate glasses (bioactive glasses) as materials for bone bonding were presented for the first time by Hench in the late 1960s (Hench *et al.*, 1973). The first composition that was confirmed to establish strong bond to bone was labeled as 45S5 due to its silica content (45% wt.) (Jones *et al.*, 2013). As bioactive glasses have the ability to bond with living tissues forming an apatite layer, they represent an attractive group of materials for the development of coatings on metallic implants for dental and orthopedic applications (Hench Larry *et al.*, 2015). Silica based nanomaterials and their synthesis processes are biocompatible, cost effective, easily to handle as well as scalable for industrial purposes, they are good candidates for developing functional coatings (Ducheyne *et al.*, 2015). Depending on size and dose, they are also catalogued as hydrophilic and non-toxic (Kim *et al.*, 2015). A further feature is that the degradation product (silicic acid) beneficially supports the formation of connective tissues (Wang *et al.*, 2017). In the typical nanoparticle shape (spherical), silica is investigated as a promising carrier system for drug delivery (Zhang *et al.*, 2013).

1.6 TITANIUM ALLOY

Titanium (Ti) and its alloys (Ti-6Al-4V, Ti-13Nb-13Zr and Ti-5Al-6Nb) have received considerable attention due to the fascinating mechanical properties such as

fatigue resistance, more strength and superior biocompatibility. Moreover, in various fields such as osteosynthesis, oral implantology and joint prosthetics Ti alloys have exhibited their potential as an implant material (Praveen Kumar *et al.*, 2022). Titanium alloys are light, strong and highly resistant to fatigue and corrosion, six times stronger than compact bone and offer more opportunities for designs with thinner sections (Praveen Kumar *et al.*, 2022). Due to the excellent property of biocompatibility, Titanium and its alloys are highly used in the biomedical field such as in orthopaedic implants. Ti alloys have gained great importance over other alloys in the medical and dental fields (Grover *et al.*, 2020). As due to overwhelming characteristics of titanium alloys like its excellent mechanical property, resistant to corrosion and wear make it biocompatible (Pompe *et al.*, 2003). Nevertheless, there are a number of complications in developing such crystalline uniform coating on Ti metal surface with proper hardness and adhesion (Das *et al.*, 2016). After implantation, the surrounding body cells/tissues (osteogenesis) should adhere and proliferate onto the implanted surface which determines the success rate of implant materials (Kaliaraj *et al.*, 2019). In order to improve the materials performance and durability, surface tailoring play a vital role to enhance the mechanical strength, biological and corrosion behavior of the implant materials. In particular, ceramics materials have taken considerable attention in biomedical coatings due to their aesthetic, good mechanical strength, corrosion resistance and better biocompatibility properties (Gobi Saravanan *et al.*, 2019).

1.7 OUTLINE OF THE PROJECT

In this study, HAp was used as a basic bioactive material and BST as a piezoelectric material in the composite samples. So, in this dip coating technique, the metallic substrate is immersed in the prepared HAp-BST solution at a constant speed. After a fixed time interval of dipping the metallic substrate, pull out of the coating solution. The coating thickness is a function of speed, concentration of sol-gel, number of dips, and time of dipping. This method has good control over HAp film thickness (Motealleh *et al.*, 2016). These implants are placed to hold the bones in place and help to promote osseointegration with bone tissue. Implants, used for tissue support, must be biocompatible and give clues to host cells to start a quick natural cell healing

process (Zhang *et al.*, 2013). Metallic substrate coated with ceramic coating has advantages of HAp-BST materials. Where metallic substrate give mechanical stability, there porous ceramic coating will provide excellent bio compatibility to bio implant (Gurbhinder Singh *et al.*, 2010). Orthopedic implant devices are intended to restore the function of load-bearing joints which are subjected to high level of mechanical stresses, wear, and fatigue in the course of normal activity (Sameer *et al.*, 2009). As such, biomaterial implants employed for load-bearing applications must have many reliable properties, including appropriate mechanical properties, excellent corrosion resistance, high wear resistance, excellent biocompatibility, osseointegration, and non-cytotoxicity to avoid the revision surgery (Kurtz *et al.*, 2007). After implantation, the surrounding body cells/tissues (osteogenesis) should adhere and proliferate onto the implanted surface which determines the success rate of implant materials. Hence, it is necessary to evaluate the cytocompatibility behavior of surface modified Bioimplants (Subramanian *et al.*, 2011). The metal implants (any non-degradable metallic material like titanium and its alloys and 316 L stainless steel) with calcium phosphate coating allow the required porosity for bone in-growth and can overcome the poor mechanical strength, weak adhesion and brittleness of HAP (Sridhar *et al.*, 1999).

In this research were using Barium Strontium Titanate (BST) for piezoelectric effect and Hydroxyapatite (HAp) for bone repair and regeneration. So with the combination of BST-HAp materials were used as the nanocomposite coating for potential Bioimplants. So, the present study is to investigate microstructure, strength and corrosion analysis of dip coating of BST-HA, 70 wt% of HA + 30 wt% of BST, 60 wt% of HA + 40 wt% of BST, and 50 wt% of HA + 50 wt% of BST coatings. Mechanical, hardness and corrosive resistant analysis of the BST-HA coatings on the Titanium alloy substrate with the varying percentages of the Hydroxyapatite and Barium Strontium Titanate as the piezoelectric material has been carried out and surface characterization has been done using XRD and surface morphology characterization technique using SEM with elemental mapping using EDX studies. We further examined the mechanical, electrical and biological properties of the HAp-BST

composites. The surface hardness, wettability and in vitro biological analysis were also performed. Then Biomineralization study was also performed to evaluate the bioactivity of the coated and uncoated samples. Finally, Bacterial adhesion behavior study using Gram Negative Bacteria (*Pseudomonas aeruginosa*) is one of the important microorganism causes associated implant infections which is frequently isolated from patients with burns, cystic fibrosis and clinical surroundings (Erdem *et al.*, 1999). The selected implants must possess different crucial characteristics such as considerable biocompatibility, high mechanical strength, corrosion, wear resistance, hydrophilicity and osseointegration. Coatings on biomedical implants are considered as a promising technique to enhance the implant-tissue interactions and promote their biocompatibility and bio functionality without altering the material's properties (Priyadarshini *et al.*, 2019). The success of bioceramic implant will be exemplified by their ability to produce bone growth and osteointegration at the implant and tissue interface (Paital *et al.*, 2009).

CHAPTER – 2

LITERATURE REVIEW

Subhasmita Swain *et al* studied that to mimic the electrical properties of natural bone, controlled strontium substitution of both hydroxyapatite and ferroelectric barium titanate were achieved by mixing in the ratio 30:70 by weight. The composites were characterized by X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy to investigate the phase composition and microstructure of the composites. Unpolarized and polarized strontium hydroxyapatite (SrHA)-barium strontium titanate (BST) composites with controlled degree of Sr substitution were examined, including 5SrHA-5BST (5% Sr substitution in both components) and 10SrHA-10BST composites. The 10SrHA-10BST composite showed a higher osteoblast activity, as observed from the cell viability studies performed using CCK-8 assay. The polarized composites showed promise against *Staphylococcus aureus* bacteria by minimizing the adhesion and growth of bacteria, as compared with their unpolarized counterparts. The polarized 10SrHA-10BST was found to be superior to all other composites. As a result, the approach of polarization of SrHA-BST composites has been found to be an effective bone substitute material in controlled enhancement of osteoblast growth with simultaneous reduction of bacterial infection (Subhasmita Swain *et al.*, 2021).

Gobi Saravanan Kaliaraj *et al* evaluated Surface tailoring of bioimplants is a prerequisite for reducing inflammation and improving durability. In this work, novel calcia stabilized zirconia (CaSZ) and silver-CaSZ nanocomposite coatings were developed on medical grade stainless steel (316L SS). We analyzed their microstructure, mechanical, biological and corrosion resistance performance. Ag-CaSZ nanocomposite coating effectively arrested *Pseudomonas aeruginosa* bacterial growth without compromising its biocompatibility. Hemolytic behavior was studied and the results confirmed that the samples expressed hemocompatible nature. Further, CaSZ and Ag-CaSZ nanocomposite coatings enhanced osteoblast-like cells (MG-63) adhesion with improved calcite precipitation (biomineralization) using Alizarin red-

based assay (ARS). Finally, electrochemical bio-corrosion was performed with Artificial Blood Plasma (ABP) solution. The chemical inertness of ceramics and the formation of the passive layer could enhance corrosion resistance. (Gobi Saravanan Kaliaraj *et al.*, 2021).

Qianqian Xu *et al* observed that piezoelectric materials, with their unique ability for mechanical-electrical energy conversion, have been widely applied in important fields such as sensing, energy harvesting, wastewater treatment, and catalysis. In recent years, advances in material synthesis and engineering have provided new opportunities for the development of bio-piezoelectric materials with excellent biocompatibility and piezoelectric performance. Bio-piezoelectric materials have attracted interdisciplinary research interest due to recent insights on the impact of piezoelectricity on biological systems and their versatile biomedical applications. This review therefore introduces the development of bio-piezoelectric platforms from a broad perspective and highlights their design and engineering strategies. State-of-the-art biomedical applications in both biosensing and disease treatment will be systematically outlined. The relationships between the properties, structure, and biomedical performance of the biopiezoelectric materials are examined to provide a deep understanding of the working mechanisms in a physiological environment. Finally, the development trends and challenges are discussed, with the aim to provide new insights for the design and construction of future bio-piezoelectric materials (Qianqian Xu *et al.*, 2021).

G.S. Kaliaraj *et al* stated that surface engineered materials are presently used as dental, orthopedic and cardiovascular applications made of titanium, cobalt-chromium, stainless steel alloys, etc. In this perspective, different phases of zirconia (ZrO_2 ; henceforth ZrO_2 allotropes) coatings (m- ZrO_2 , t- ZrO_2 and c- ZrO_2) were developed by electron beam physical vapor deposition (EBPVD) technique. ZrO_2 allotropes obtained a smooth surface with a drastic reduction of human pathogenic bacterial adhesion. t- ZrO_2 coating evolved higher surface hardness followed by c- ZrO_2 and m- ZrO_2 coatings. ZrO_2 allotropes showed higher blood plasma protein adherence

using a whole blood test. Whilst, the role of proteins in the degradation of ZrO_2 allotrope coatings was examined in detail by electrochemical impedance spectroscopy (EIS) in the presence and absence of bovine serum albumin (BSA) in artificial blood plasma (ABP) solution. (G.S. Kaliaraj *et al.*, 2020).

Gobi Saravanan Kaliaraj *et al* studied that the effect of piezoelectricity from piezoelectric materials promotes the growth of bone. However, the material should be subjected to stress before it can produce an electric charge that promotes repair of bones and reconstruction of it conducive to healing of fracture. This mainly includes the coatings, hardness, tribometer and biological tests on the composite material. Initially the widely used stainless steel metal of 316L grade is selected and then it was cut into small samples of specific dimensions 10 mm 10 mm 4.5 mm and 20 mm 10 mm 4.5 mm. Then BaTiO_3 (Barium titanate) doped hydroxyapatite (HA) and SrTiO_3 (Strontium titanate) doped HA powders were coated on the stainless steel samples by Plasma Spray Technique. X Ray Diffraction was done to characterise the material. Vickers hardness test was performed to check the hardness of various coated samples. Then tribology was performed using linear reciprocating test to examine the wear resistance of the coated sample. The effect of piezoelectricity of BaTiO_3 and SrTiO_3 promoted the growth of osteoblasts and interaction with HA, which was better than the piezoelectric effect of HA alone. The result show that BaTiO_3 doped hydroxyapatite and SrTiO_3 doped hydroxyapatite coatings could enhance the bio mineralization (calcium precipitation) process (Senthilkumar *et al.*, 2020).

Shikha Awasthi *et al* stated that the production of hydroxyapatite (HAP) composite coatings has continuously been experimented for bone tissue applications during the last decades due to its significant bioactivity and osteoconductivity. This report unravels the recent progress of experimental and theoretical perspectives of the HAP coatings, which may seal the existing gap between theory and practice. The experimental studies are mainly dealing with the electrochemical (EC) and electrophoretic (EP) depositions for the synthesis of nano-HAP in the form of coatings. Additionally, the biocompatible coating method for the fabrication of the HAP

composite coatings, plasma spraying (PS) technique and its mechanism are adorned in the review. The adhesion strength, mechanical, tribological and electrochemical phenomenon of the HAP composite coatings are critically analyzed. The ameliorated bactericidal activity is also discussed to recognize the possibility of substituted HAP coatings from a clinical perspective. In addition, the computational studies, grafted with the HAP system are explored in this report with the deployment of first-principle density functional theory method, ab initio modeling approach and molecular dynamics simulations. Thus, the utmost significance of the review is to reveal a collective discussion on structural features, interfacial mechanics and binding aspects by experimental and theoretical investigations for the HAP-based biomaterial, which can provide clear insights for future research related to the orthopedic applications. (Shikha Awasthi *et al.*, 2020).

Bilal Beig *et al* evaluated biomaterials are in use for the replacement and reconstruction of several tissues and organs as treatment and enhancement. Metallic, organic, and composites are some of the common materials currently in practice. Metallic materials contribute a big share of their mechanical strength and resistance to corrosion properties, while organic polymeric materials stand high due to their biocompatibility, biodegradability, and natural availability. To enhance the biocompatibility of these metals and alloys, coatings are frequently applied. Organic polymeric materials and ceramics are extensively utilized for this purpose due to their outstanding characteristics of biocompatibility and biodegradability. Hydroxyapatite (HAp) is the material from the ceramic class which is an ultimate candidate for coating on these metals for biomedical applications. HAp possesses similar chemical and structural characteristics to normal human bone. Due to the bioactivity and biocompatibility of HAp, it is used for bone implants for regenerating bone tissues. This review covers an extensive study of the development of HAp coatings specifically for the orthopaedic applications that include different coating techniques and the process parameters of these coating techniques. Additionally, the future direction and challenges have been also discussed briefly in this review, including the coating of

HAp in combination with other calcium magnesium phosphates that occur naturally in human bone. (Bilal Beig *et al.*, 2020).

Tara Tariverdian *et al* observed that to promote bone healing, new generations of biomaterials are under development. These biomaterials should demonstrate proper biological and mechanical properties preferably similar to the natural bone tissue. In this research, 3D-printed barium strontium titanate (BST)/ β -tricalcium phosphate (β -TCP) composite scaffolds have been synthesized as an alternative strategy for bone regeneration to not only induce appropriate bioactive characteristics but also piezoelectric behavior. The physical, chemical and biological performance of the scaffolds have been examined in terms of mechanical, dielectric properties, apatite-forming ability, Alizarin Red Staining (ARS), Alkaline Phosphatase activity (ALP), and cytotoxicity. The samples composed of 60% BST and 40% β -TCP showed the highest compressive strength, bending module, elastic modulus and the Young's modulus. The dielectric constant increased with further addition of the BST phase in the constructs. Scanning Electron Microscope (SEM) and energy dispersive X-ray (EDX) analyses showed that 60% BST/40% β -TCP sample had the highest amount of bone-like apatite formation after 28 days in simulated body fluid (SBF). Moreover, the results of ARS proved that 60% BST/40% β TCP composite could present higher quantities of mineral deposition. The ALP activity of osteosarcoma cells on 60% BST/40% β -TCP sample showed higher activities compare with the other composites. None of the samples demonstrated any sign of toxicity using MTT test. It can be suggested that BST/ β -TCP composite scaffolds can be potentially used as the next generation of bone tissue engineering scaffold materials. (Tara Tariverdian *et al.*, 2019).

Biranche Tandon *et al* stated that the process of bone repair and regeneration requires multiple physiological cues including biochemical, electrical and mechanical - that act together to ensure functional recovery. Myriad materials have been explored as bioactive scaffolds to deliver these cues locally to the damage site, amongst these piezoelectric materials have demonstrated significant potential for tissue engineering

and regeneration, especially for bone repair. Piezoelectric materials have been widely explored for power generation and harvesting, structural health monitoring, and use in biomedical devices. They have the ability to deform with physiological movements and consequently deliver electrical stimulation to cells or damaged tissue without the need of an external power source. Bone itself is piezoelectric and the charges/potentials it generates in response to mechanical activity are capable of enhancing bone growth. Piezoelectric materials are capable of stimulating the physiological electrical microenvironment, and can play a vital role to stimulate regeneration and repair. This review gives an overview of the association of piezoelectric effect with bone repair, and focuses on state-of-the-art piezoelectric materials (polymers, ceramics and their composites), the fabrication routes to produce piezoelectric scaffolds, and their application in bone repair. Important characteristics of these materials from the perspective of bone tissue engineering are highlighted. Promising upcoming strategies and new piezoelectric materials for this application are highlighted. (Biranche Tandon *et al.*, 2018).

Namdev More *et al* studied that bone and cartilage are major weight-bearing connective tissues in human and possesses utmost vulnerability for degeneration. The potential causes are mechanical trauma, cancer and disease condition like osteoarthritis and osteoporosis, etc. The regeneration/ repair is a challenging, since their complex structures and activities. Current treatment options comprise of auto graft, allograft, artificial bone substituent, autologous chondrocyte implantation, mosaicplasty, marrow stimulation and tissue engineering. Were incompetent to overcome the problem like abandoned growth factor degradation, indistinct growth factor dose and lack of integrity and mechanical properties in regenerated tissues. Present, paper focuses on the novel hypothesis for regeneration of bone and cartilage by using piezoelectric smart property of scaffold material. (Namdev More *et al.*, 2017).

CHAPTER – 3

AIM AND SCOPE

AIM & OBJECTIVES:

- ❖ To deposit HAp-BST nanocomposites by dip-coating method on medical-grade Titanium alloy.
- ❖ To characterize HAp-BST nanocomposite by XRD, FESEM, Contact angle measurement.
- ❖ To examine the mechanical property by wear resistance analysis of HAp-BST nanocomposite.
- ❖ To determine piezoelectric effect
- ❖ To examine the electrochemical behaviour by corrosion resistance study of HAp-BST nanocomposite.
- ❖ To evaluate biocompatibility behaviour of HAp-BST nanocomposite by Protein adsorption, Platelet adhesion and cytotoxic analysis.
- ❖ To investigate bioactivity behaviour through Biomineralization studies

SCOPE:

The scope of the project is enormous, when the biomaterial is synthesized or engineered into nanomaterial. The engineered Nanomaterials have extended its potential therapeutic and preventive applications in biomedical and clinical field.

CHAPTER- 4

MATERIALS AND METHODS

4.1 NATURAL SYNTHESIS OF HAP

Goat femur bone were collected and used as a sole source of HAp. Initially, the bones were boiled in distilled water for 1hour for de-fattening and easier removal of adhering impurities. Afterwards, the bones were washed and cleaned well with water to remove all attached meat, tendons, bone marrows, and other soft tissues. The bones were then immersed in 99% ethanol for two hours and washed with distilled water for 3 times. The bones were dried at 37°C to evaporate the absorbed water. After that, the dried femur bones were calcinated at 700°C for 5 hours using muffle furnace. Calcinated bone were crushed into powder using mortar pestle and then milled into smaller particle sizes using rotary ball mill (SPEX 8000M-230 dual mixer mill, 230V/50Hz) and further characterization of sample was carried out.



Fig 4.1: Processed Calcinated bone from goat



Fig 4.2: Calcinated bone into powder form by mortar pestle



Fig 4.3: Ball milling is used for preparation of nanomaterial

4.2 SUBSTRATE PREPARATION

Medical grade titanium alloy (TiAl4V6) with dimensions of 3x1cm² was used as substrate materials. Before deposition, all specimens were polished using different grades of silicon carbide (SiC) sheets (600, 800, 1000, 2000 and 5000 grits) to obtain uniform roughness. After polishing, they were ultrasonically cleaned with acetone followed by distilled water for 15 min to remove surface residues. Finally, the cleaned samples were sterilized by autoclave (under steam pressure for 15 min at 121 C°) and dried. (Kaliaraj *et al.*, 2018). The *figure 4.4* shows that the smooth morphology after the substrate preparation.



Fig 4.4: Image of an uncoated Medical grade titanium alloy (TiAl4V6)

4.3 PREPARATION OF 5 WT% POTASSIUM SILICATE SOL FOR COATING PURPOSE

For the binding of BST with HAp, Potassium silicate binder was used. 5g of Potassium silicate was dissolved in 10ml of distilled water with continuous mixing until it gets dissolved completely.

4.4 DEPOSITION OF TITANIUM BY DIP COATING

Different concentration of Hydroxyapatite and Barium Strontium Titanate: 0.5 wt% HAp- 0.5 wt% BST, 0.6 wt% HAp- 0.4 wt% BST and 0.7 wt% HAp- 0.3 wt% BST respectively to make nanocomposites in presence of potassium silicate binder which was further coated onto medical grade Ti6Al4V substrate using dip coating method.

After coating, the samples have been dried in air for a day and were calcinated in a furnace at 150° C for 3 hours.



a) 0.5g HAp-0.5g BST

b) 0.6g HAp-0.4g BST

c) 0.7g HAp-0.3g BST

Fig 4.5: Comparative study of HAp-BST different concentration in 50wt% of K_2SiO_3 in 10 ml of deionized water

4.5 CHARACTERISATION

4.5.1 XRD Studies

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

XRD is one of the techniques for investigation of crystalline compounds. XRD analysis of coated samples of HAp-BST was carried out by Rigaku Mini Flex 660

(9kW) with an X-ray source of Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over the range of 2θ angle 20 to 80°.

4.5.2 FESEM Analysis

A field-emission cathode in the electron gun of a scanning electron microscope provides narrower probing beams at low as well as high electron energy, resulting in both improved spatial resolution and minimized sample charging and damage. Under vacuum, electrons generated by a Field Emission Source are accelerated in a field gradient. The beam passes through Electromagnetic Lenses, focusing onto the specimen. As result of this bombardment different types of electrons are emitted from the specimen. A detector catches the secondary electrons and an image of the sample surface is constructed by comparing the intensity of these secondary electrons to the scanning primary electron beam. Finally the image is displayed on a monitor.

FESEM with EDX studies. The morphology and chemical composition of HAp-BST coating were investigated by using FESEM (Carl Zeiss, SUPRA® 55 with GEMINI®) equipped with EDX to obtain a clear photomicrograph of coated samples.

4.5.3 Contact Angle Measurement

The surface wettability of coated and uncoated substrates was determined by contact angle measurement using the sessile drop method by a JJC-1 contact angle goniometer. 18.2 M Ω ultrapure water (liquid surface tension (γ_1) = 72.8 mJ m⁻²) was used as the wetting liquid (Lugscheider *et al.*, 2003) Contact angle was measured, after 1 min of water addition (3 μ L) on the surface of the specimen to provide the configuration between the measurements. (Kaliaraj *et al.*, 2014)



a) 0.5g HAp- 0.5g BST



b) 0.6g HAp- 0.4g BST



c) 0.7g HAp- 0.3g BST

Fig 4.6: Measurement of Contact angle of coated samples

4.6 MECHANICAL STUDIES

4.6.1 Tribology

The wear-resistant behavior of the coating was tested by a linear reciprocating nano tribometer (CSM Instruments, Switzerland). A steel ball (100Cr6) with a diameter of 2 mm was used as a sliding counter body. For analysis, applied load and sliding speed was constantly maintained at 2N and 3 cm/s, respectively. For each experiment, the test was carried out at three different locations at room temperature. The average friction coefficient value was determined. Error bars represent standard deviations. (Zhu et al., 2017)

4.7 ELECTROCHEMICAL STUDIES

4.7.1 Corrosion Analysis

The electrochemical behavior of the test samples was determined in artificial blood plasma solution (ABP) (composition (g/l): NaCl-6.8; CaCl₂-0.200; KCl-0.4; MgSO₄-0.1; NaHCO₃-2.2; Na₂HPO₄-0.126; NaH₂PO₄-0.026) at room temperature. All electrochemical measurements were performed in a standard three-cell electrode setting with an exposed area of 1m. Open circuit potential (OCP) was measured for about 60 min, before corrosion experiments to attain a stable potential for the working electrode. Electrochemical impedance spectroscopy (EIS) was performed in the frequency range from 100 kHz to 10 MHz with an AC amplitude of 10 mV. After EIS analysis, potentiodynamic polarization (PDP) was studied at a potential of ± 500 mV vs SCE with a scan rate of 10 mV/s. The corrosion potential (E_{corr}) and corrosion current density (I_{corr}) were determined by the Tafel plot. (Kaliaraj et al., 2018)

4.8 PIEZOELECTRIC EFFECT

The variation of the surface barrier upon illumination (surface photo-voltage) or upon bending (piezoelectric effect) was measured as the change in the contact potential difference (CPD) with respect to a gold reference electrode. The use of a small vibrating-boom electrode and single harmonic detection of the signal permitted off-null linear CPD measurements with an accuracy of the order of 1 mV. Details of

this technique and of the technique for applying stress on the coating through mechanical bending have been reported.

4.9 BIOLOGICAL CHARACTERISATION

4.9.1 Protein Adsorption Studies

For the protein adsorption study, the coated and uncoated titanium alloy surfaces were equilibrated in isotonic Tris buffer for about 20 h at 37 °C. Then, the samples were kept in a screw cap bottle containing 5 ml of plasma and incubated for 3 h. After incubation, the surface was gently washed by isotonic Tris-buffer followed by incubation with 2% sodium dodecyl sulfate (SDS) solution for 24 h in order to elute adsorbed proteins. Finally, the protein eluates were stored at – 70 °C and the concentration of adsorbed proteins were determined by Lowry's method (Lowry *et al.*, 1951) Triplicate experiments were done for data accuracy.

4.9.2 Platelets Adhesion Studies

Thrombogenicity of the coated material surface was studied by platelet adhesion analysis. The Human blood was collected from healthy volunteers by venipuncture into EDTA containing sterile tubes. The blood was centrifuged at 3000 rpm for 3 min to collect platelet-rich plasma (PRP). PRP was centrifuged at 4000 rpm for 15 min to separate platelets. The separated platelets were further washed, suspended and diluted with 1X PBS buffer. Coated samples surface was hydrated in Millipore water for 24 h followed by PBS soaking for 1 h. The platelet adhesion study was performed in a well plate and the two set of coated and uncoated TiAl4V6 samples were incubated in platelet solution at 37 °C for 1 h with slight orbital shaking to avoid platelet sedimentation. After incubation, one set of samples were gently washed with PBS and stained by 0.1% AO for Epifluorescence micrograph (Nikon Eclipse E600).

4.9.3 Bacterial Adhesion Studies

A pure *Pseudomonas aeruginosa* bacterial culture was obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH, Chandigarh). The bacteria were sub cultured in nutrient broth and

incubated at 37 °C for 12 hrs as recommended by MTCC. The bacterial culture was transferred into 20 ml conical flask containing nutrient broth and incubated for 3h in a shaking incubator. The final bacterial concentration was adjusted to an optical density of 0.1 to 0.3 (at 660 nm) in which, the approximate bacterial concentration was expected to be between 1×10^5 and 3×10^5 CFU ml⁻¹ (colony forming unit per milliliter).

The test samples (50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST) were sterilized in an autoclave (120 °C, 15 lbs pressure for 15 minutes) to avoid cross contamination. The diluted bacterial culture was inoculated into nutrient broth containing the test samples and incubated for 24 hrs at 37 °C in a shaking incubator. After incubation, the samples were washed with distilled water and the sample surface was swabbed with sterile swab to remove adhered bacteria and collected into phosphate buffered saline (PBS) solution (composition (g/l): NaCl-8.0; Na₂HPO₄.12H₂O-2.08; KCl-0.20; KH₂PO₄-0.20) for serial dilution. Finally, 0.1 ml of serially diluted samples were poured onto an agar plate and incubated for 24 hrs. The bacterial colony was expressed as colony forming unit per square centimeter (CFU/cm²). (Pradhaban *et al.*, 2014) (Song *et al.*, 2008). For this, the incubated samples were contacted with 20 µl of acridine orange (AO), incubated for 10 min, and examined by epifluorescence microscopy (Nikon Eclipse, E600).

4.9.4 Cytotoxic Studies

Human osteoblast (MG-63) like cells were procured from National Centre for Cell Sciences (NCCS), Pune, India. These cells were used to carry out cytotoxic behavior against test samples. Cells were cultured following the standard protocols suggested by the supplier. The cells were grown until confluency before the addition of mineralizing medium (α-MEM containing: 20% FCS (fetal calf serum), 100 µg/ml– 1 streptomycins, 100 µg/ml– 1 penicillin 50 mM L-ascorbate, 10– 8 M dexamethasone, and 2 mM glutamine along with osteogenic supplements). All chemicals were procured from Sigma-Aldrich. Cells were incubated at 37 °C in presence of 5% CO₂ under standard humidified conditions. Test samples (HAp-BST coated, and uncoated titanium alloy) were kept in 6 well plates with osteoblast-like cells with a density of 104 cells/well. Incubation was done for 2 weeks at 37 °C under humidified conditions. After

incubation, loosely bound cells were removed by repeated washing with distilled water before adding 1 ml of 40 ml of ARS in each sample with gentle shaking for 5 min. Then, the attached cells were observed by Epifluorescence microscopy. Further, a quantitative analysis of cell attachment at different days was done by a WST assay (Invitrogen). The assay was conducted with 10 μ l of WST-1 reagent by replacing the medium and incubation in 5% CO₂ at 37 °C for 3 h. Finally, the measurements were made by a plate reader (Biotek) at 440 nm. (Sathishkumar et al., 2016)

4.10 BIOACTIVITY STUDIES

4.10.1 Biomineralization

To evaluate the in vitro bioactivity, the coatings were immersed in a 10-mL simulated body fluid (SBF). The evaluation of biomineralization ability was carried out in Kokubo's simulated body fluid (SBF), the composition of which is presented in *Table- 4.1*. The solution was kept at 37 °C, and the pH was regulated to be in the range of 7.3–7.4. The biomineralization test was carried out by soaking the coated samples mounted vertically in a special platinum holder in 45 ml of SBF in polyethene containers maintained at 37 °C for various periods (4, 8, 14 days). After that, the samples were gently rinsed with deionized water to remove SBF solutions followed by drying at room temperature. (Prem Ananth et al., 2015)

Table 4.1: Study of Ion concentrations (mM/dm³) of SBF and Human blood plasma

Ionic concentration (mM/dm³)	Simulated Body Fluid	Blood plasma
Na ⁺	142	1425.0
K ⁺	5.0	5.0
Mg ⁺	1.5	1.5
Ca ⁺	2.5	2.5
Cl ⁻	147.8	103.0
HCO ₃ ⁻	4.2	27.0
HPo ₄ ⁻	1.0	1.0
So ₄ ⁻	0.5	0.5

CHAPTER- 5

RESULT AND DISCUSSION

5.1 CHARACTERISATION

5.1.1 XRD Studies

By comparing XRD patterns of 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST coatings on TiAl4V6, it is observed that there are slight changes in the intensity of the peaks. *Figure.5.1* shows the majority of the diffraction peaks at 2theta are assigned to corresponding hkl value planes of HAp-BST coatings on Titanium alloy. The standard JCPDS database for XRD pattern was used for phase identification for a large variety of crystalline phases in HAp-BST coating. The HAp peaks were indexed according to JCPDS the standard pattern (structure card no. 74-0566) for the hexagonal of HAp and BST peaks were indexed according to the standard pattern (structure JCPDS card no. 89-8211).

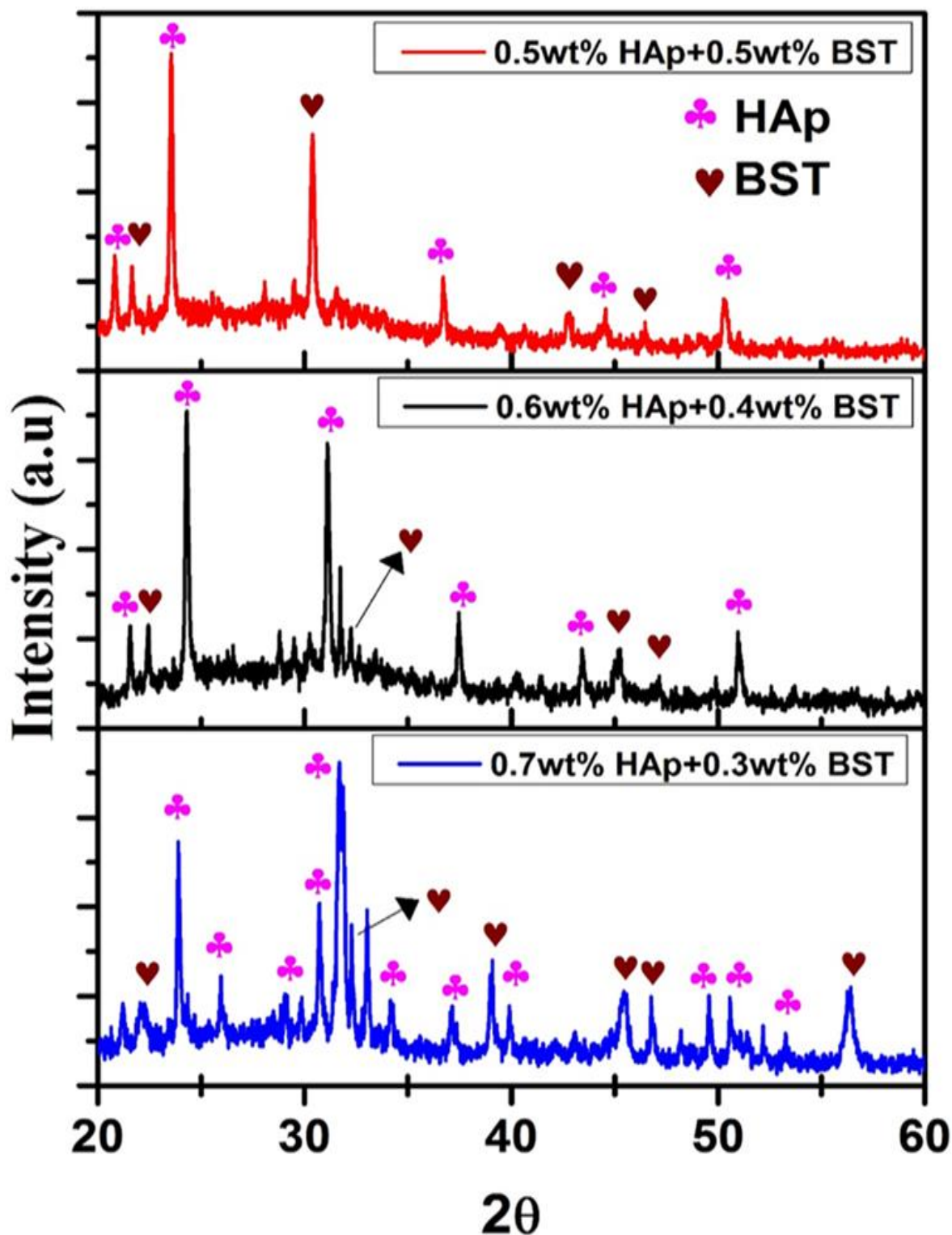


Fig 5.1 Study of XRD patterns of coated HAp-BST

(Coated samples (a) coated of 50wt% HAp- 50wt% BST (b) coated of 60wt% HAp- 40wt% BST (c) coated of 70wt% HAp- 30wt% BST)

5.1.2 FESEM Analysis

The Surface morphologies of 50wt% HAp- 50wt% BST, by Field Emission Scanning Electron Microscope (FESEM) is given in *Fig. 5.2*, whereas the Surface morphologies of 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST is given in *Fig. 5.4 and Fig. 5.6* respectively. The FESEM images of the sample 50wt% HAp- 50wt% BST films has shown smooth structure, but the sample 60wt% HAp- 40wt% BST shown smooth and hairy and 70wt% HAp- 30wt% BST shows smooth and Rod agglomerated morphologies of coated samples. The elemental analysis pattern of the coated samples of 50wt% HAp- 50wt% BST, by EDAX has shown in *Fig 5.3*, which confirms the presence of O (47.490), P (2.507), K (23.833), Ca (11.284), Ti (0.308), Sr (11.746), and Ba (2.832). On the other hand the elemental pattern of 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST has given in *Fig. 5.5 and Fig. 5.7* respectively. The EDAX pattern of the sample 60wt% HAp- 40wt% BST confirms the presence of O (44.236), K (26.814), Ca (0.976), Ti (0.231), Sr (27.290), and Ba (0.453) (*Fig. 5.5*) and the sample 70wt% HAp- 30wt% BST confirms the presence of O (44.366), P (0.000), K (29.498), Ca (1.553), Ti (0.025), Sr (23.995), and Ba (0.563) (*Fig. 5.7*).

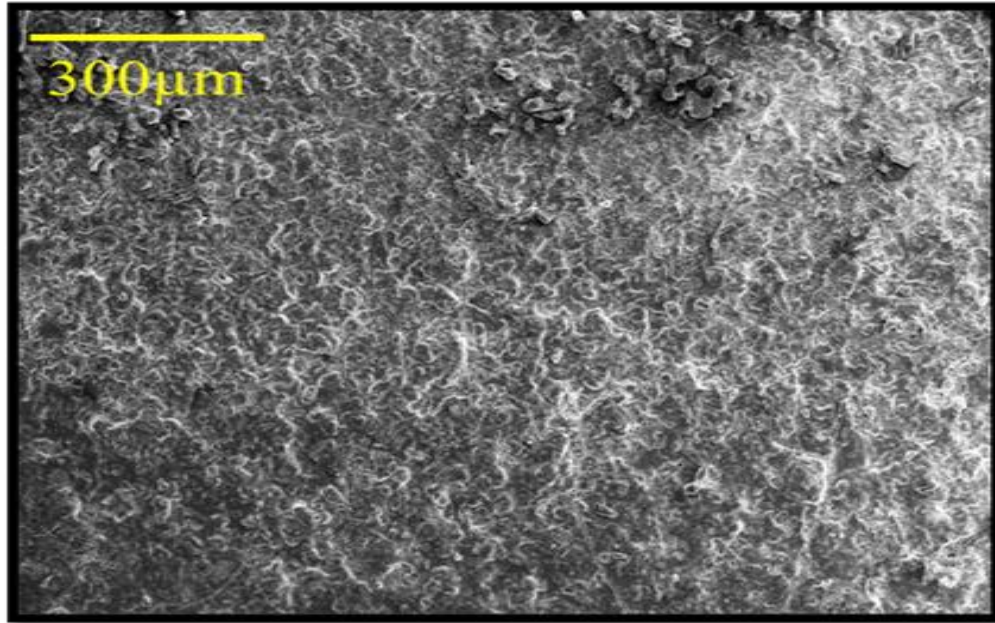


Fig. 5.2. The FESEM images of films shows the Surface morphology of smooth structure of coated samples of 50wt% HAp- 50wt% BST

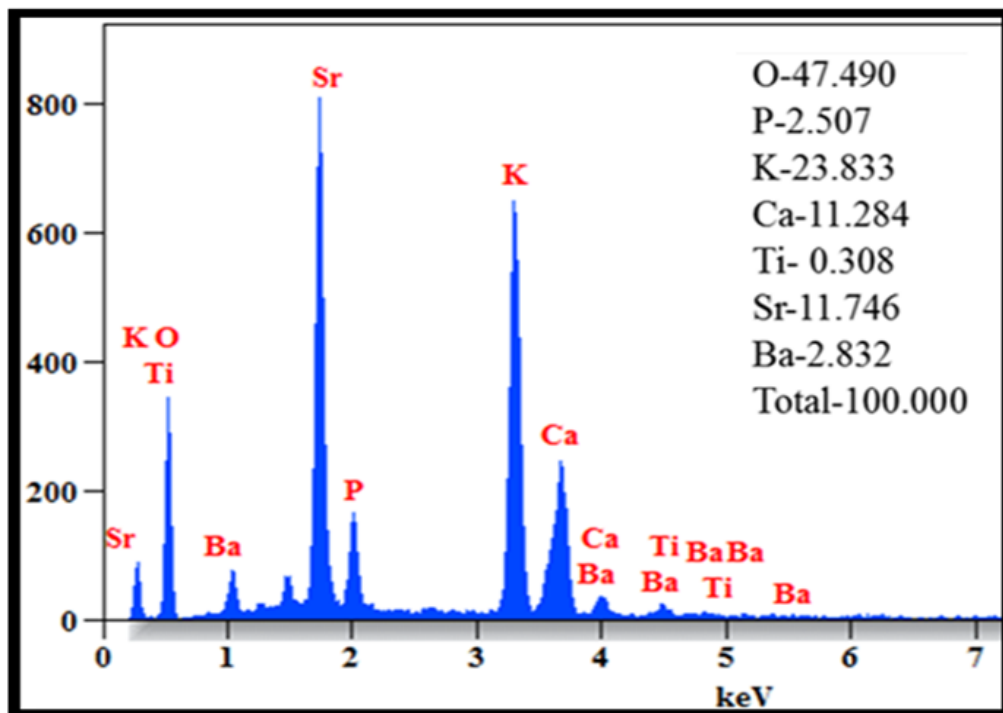


Fig. 5.3. The EDAX elemental analysis pattern of the coated samples of 50wt% HAp- 50wt% BST

[EDAX confirms the presence of O (47.490), P (2.507), K (23.833), Ca (11.284), Ti (0.308), Sr (11.746), and Ba (2.832)]

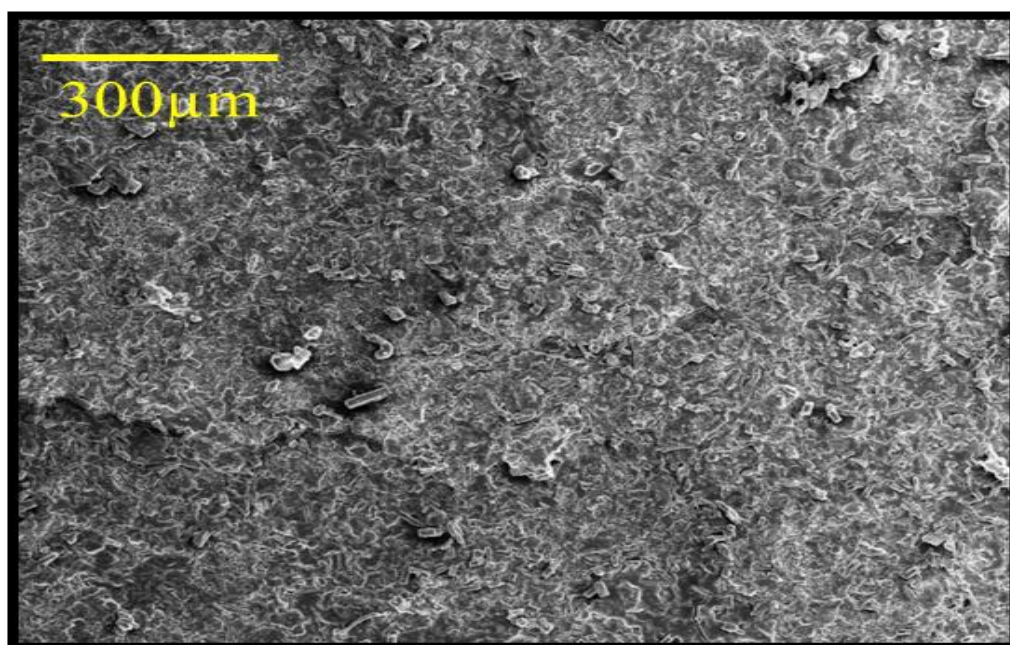


Fig. 5.4. The FESEM images of films shows the Surface morphology of smooth and hairy structure of coated samples of 60wt% HAp- 40wt% BST

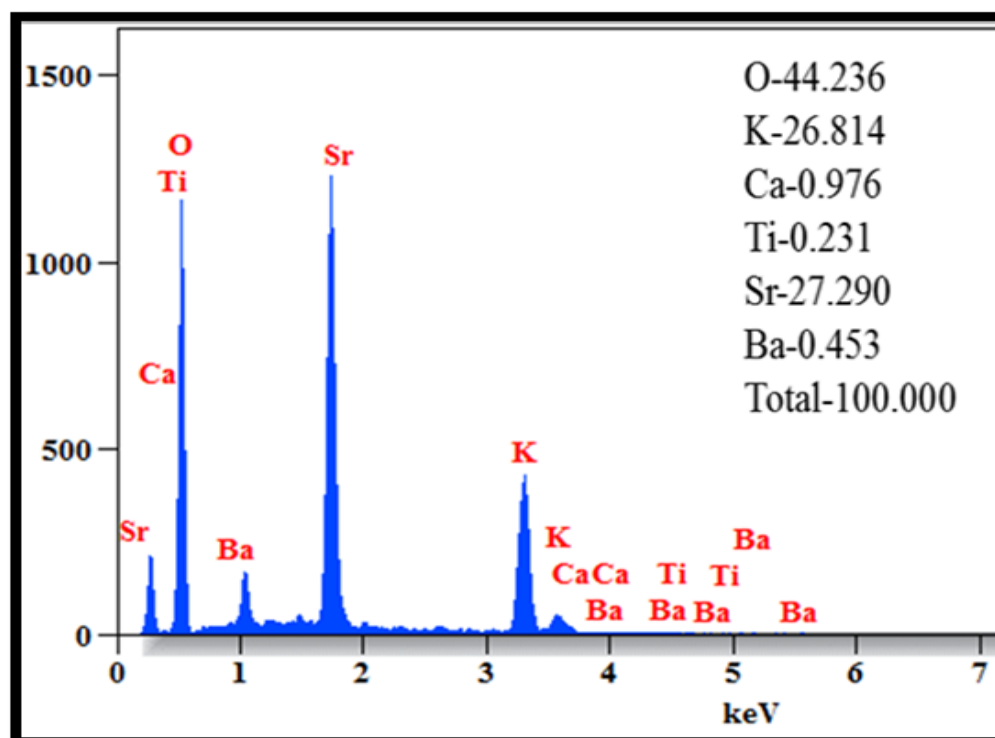


Fig. 5.5. The EDAX pattern of the coated samples of 60wt% HAp- 40wt% BST

[EDAX confirms the presence of O (44.236), K (26.814), Ca (0.976), Ti (0.231), Sr (27.290), and Ba (0.453)]

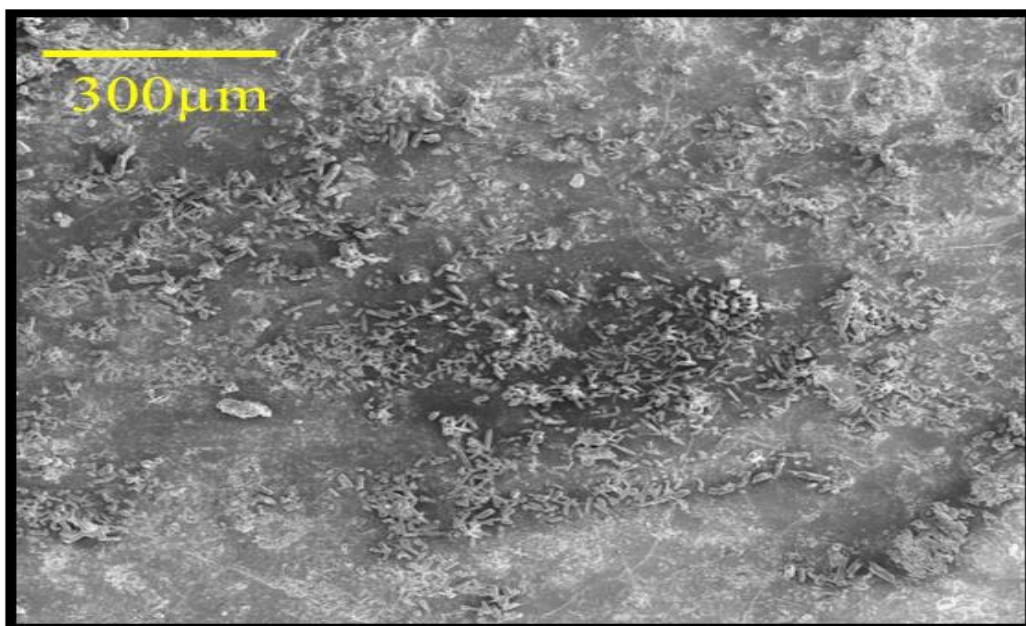


Fig. 5.6. The FESEM images of films shows the Surface morphology of smooth and rod structure of coated samples of 70wt% HAp- 30wt% BST

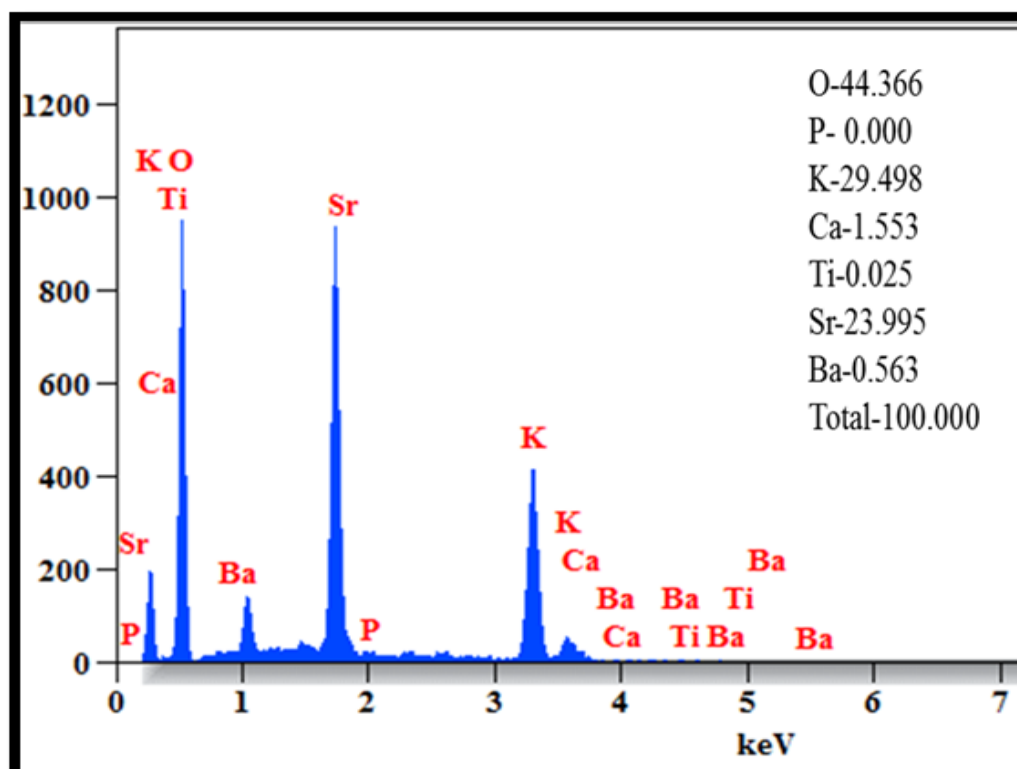


Fig. 5.7. The EDAX pattern of the coated samples of 70wt% HAp- 30wt% BST

[EDAX confirms the presence of O (44.366), P (0.000), K (29.498), Ca (1.553), Ti (0.025), Sr (23.995), and Ba (0.563)].

5.1.3 Contact Angle Measurement

The contact angle values of uncoated and coated substrates are shown in *Figure 5.8*. The contact angle of the uncoated alloy is found to be 85° , whereas, for 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST coatings, the values are 55° , 45° , 31° respectively. Low contact angle values suggest that the surface energy of coated surfaces is low, thereby resulting in the super hydrophilic behavior of the coated sample surface than uncoated sample showing least hydrophilic 85° .

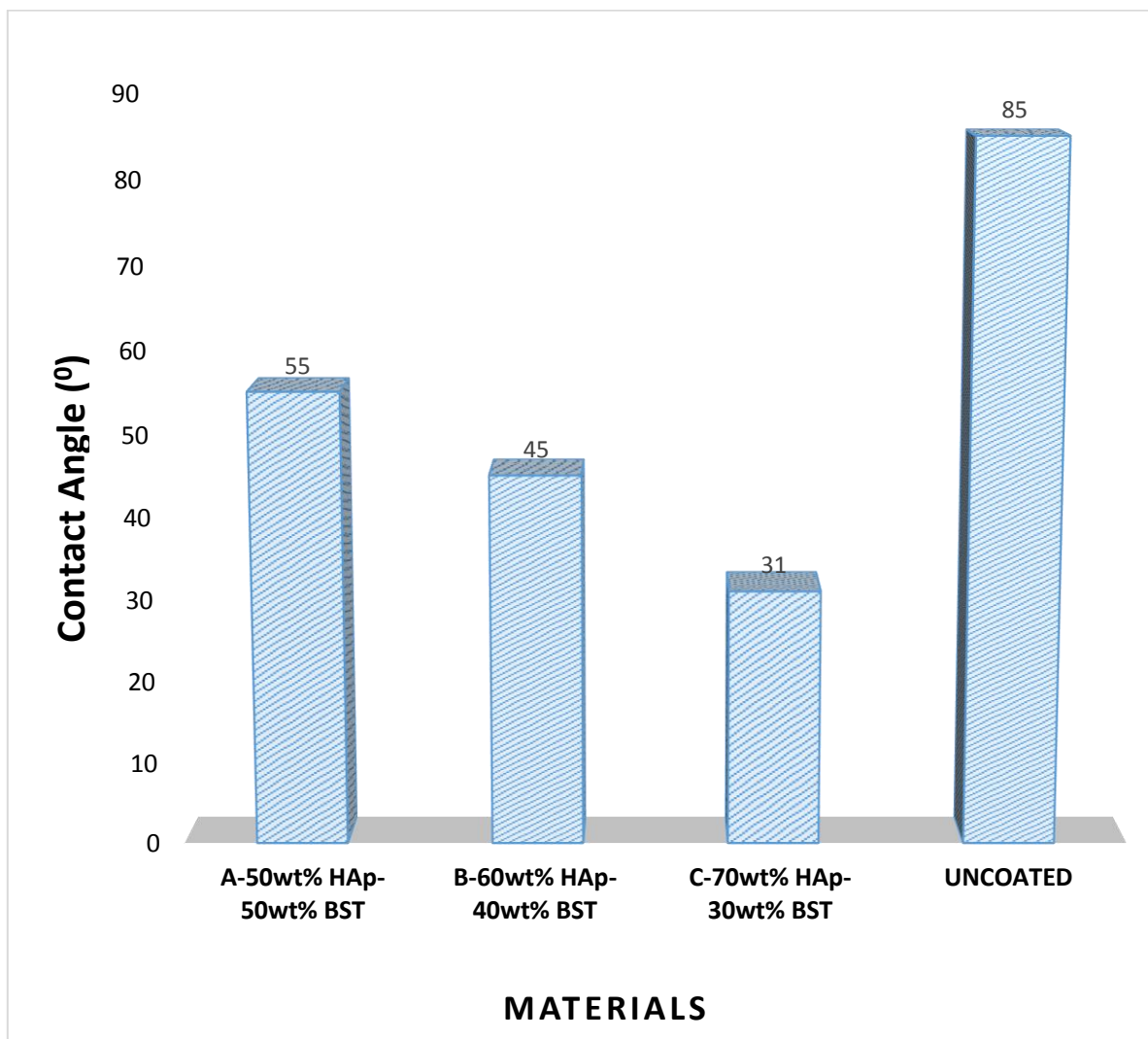


Fig 5.8: Study of Contact angle values of coated HAp-BST

(Coated samples (a) coated of 50wt% HAp- 50wt% BST (b) coated of 60wt% HAp- 40wt% BST (c) coated of 70wt% HAp- 30wt% BST (d) Uncoated)

5.2 MECHANICAL STUDIES

5.2.1 Tribology Properties

The friction coefficient of HAp-BST coatings on Titanium alloy are shown in *Figure. 5.9*. The steady friction state was observed throughout the sliding distance in coatings. This indicates strong adhesion between the coating and the substrate. Also, there is no fluctuation noticed in the friction coefficient value during steel ball-coating sliding interaction, which confirms that the coating did not undergo any wear or stress. HAp-BST coating showed a steady wear rate owing to its surface oxide which alters adhesion and abrasion wear to stable oxidation wear. (Zhu *et al.*, 2017) (Tronci *et al.*, 2015).

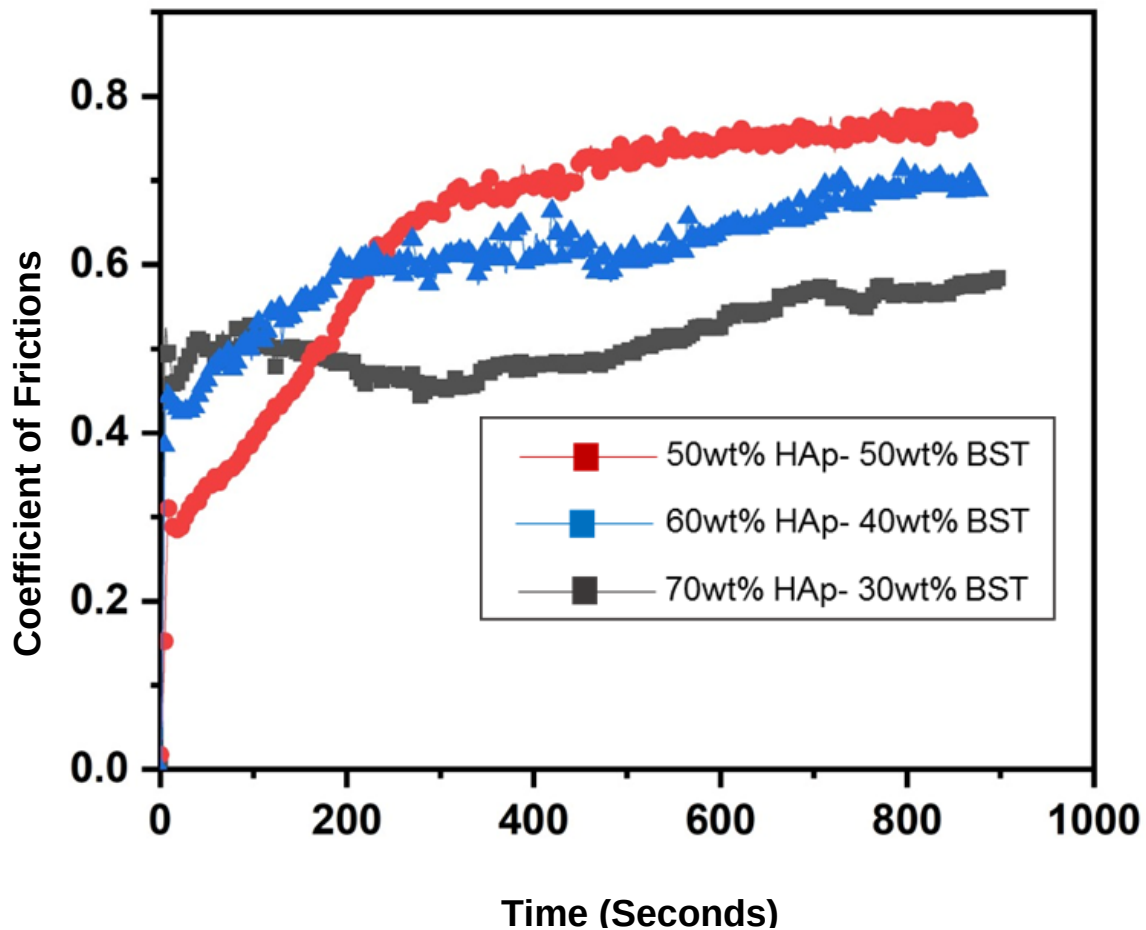


Fig 5.9: Analysis of Wear coefficient values obtained for coated sample
(Coated samples (a) coated of 50wt% HAp- 50wt% BST (b) coated of 60wt% HAp- 40wt% BST (c) coated of 70wt% HAp- 30wt% BST)

5.3 ELECTROCHEMICAL CORROSION ANALYSIS

5.3.1 Corrosion Analysis

The potentiodynamic polarization curves of uncoated TiAl6V4 and 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST -coated sample are presented in *Figure 5.10*. It can be seen from potentiodynamic polarization curves, the coated sample showed higher corrosion potential (E_{corr}) and lower corrosion current density (I_{corr}) than the uncoated sample, demonstrating that the HAp-BST significantly increases the corrosion resistance of the TiAl6V4 substrate are given in *Table 5.1*. From the polarization test results, the protective efficiency, P_i (%) of the film can be calculated by P_i (%) = $[1 - (I_{corr})]$. (Gobi Saravanan Kaliraj et al., 2015).

Table 5.1: Study on Analysis of Corrosion values obtained from different coated samples at different proportion of HAp-BST.

S.No	Sample Name	E_{corr} (V)	I_{corr} (μ A)
1	50wt% HAp- 50wt% BST	-0.370	0.289
2	60wt% HAp- 40wt% BST	-0.210	0.112
3	70wt% HAp- 30wt% BST	-0.244	0.078
4	Uncoated sample	-0.397	7.348

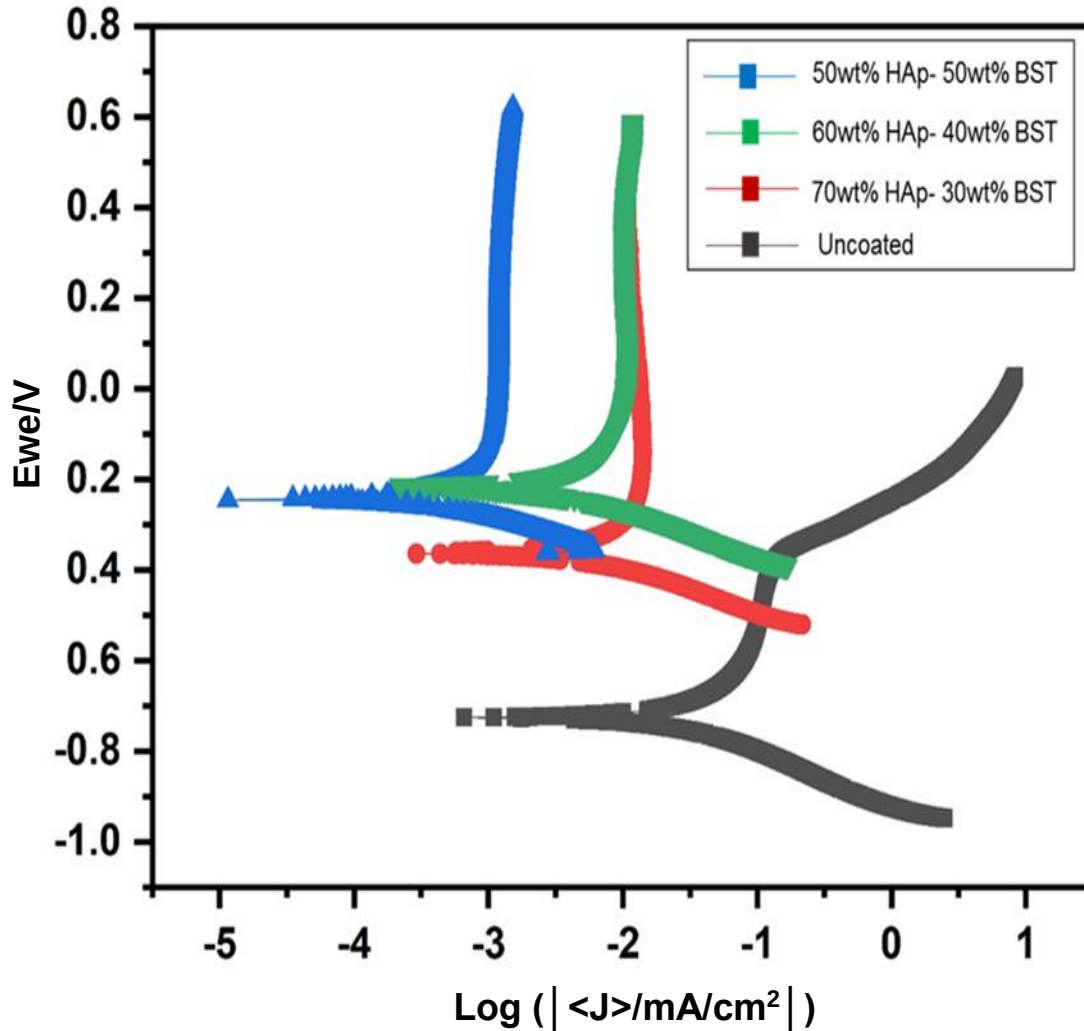


Fig 5.10: Comparative Analysis of Corrosion values obtained from various materials

(Coated samples (a) coated of 50wt% HAp- 50wt% BST (b) coated of 60wt% HAp- 40wt% BST (c) coated of 70wt% HAp- 30wt% BST)

5.4 PIEZOELECTRIC EFFECT

The piezoelectric values of HAp-BST coated substrates are shown in *Figure.5.11*. It is seen that the piezoelectric effect is increased with increasing concentration of BST (50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST and 70wt% HAp- 30wt% BST) were respectively 12, 16, 21, indicating that 50wt% HAp- 50wt% BST nanocomposites exhibited better piezoelectric response (surface barrier) which is suitable for biological responses. The d_{33} value is given in the *figure 5.11*.

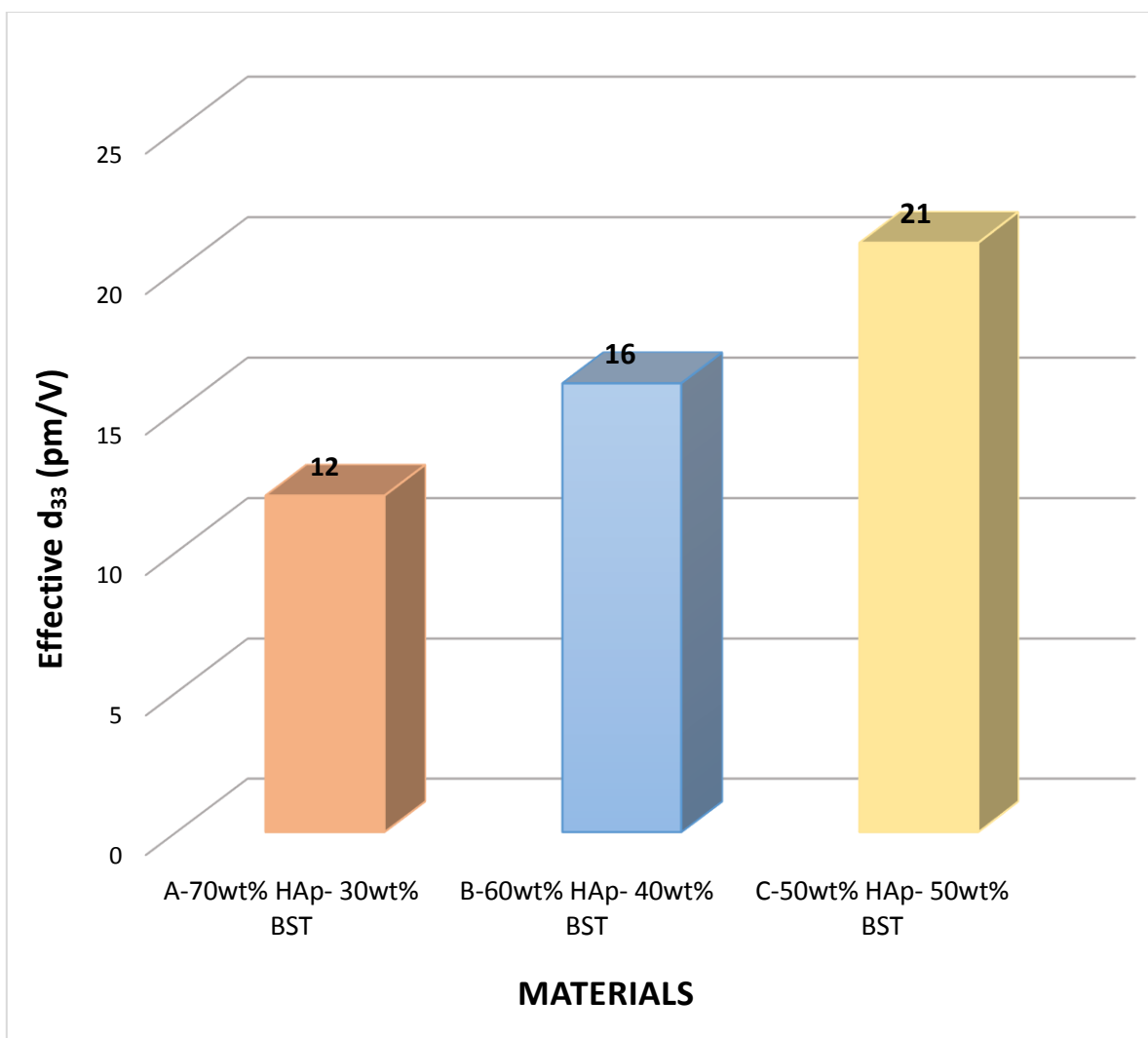


Fig 5.11: Comparative Analysis of Piezoelectric values obtained from various materials

(Coated samples (a) coated of 70wt% HAp- 30wt% BST (b) coated of 60wt% HAp- 40wt% BST (c) coated of 50wt% HAp- 50wt% BST)

5.5 BIOLOGICAL STUDIES

5.5.1 Protein Adsorption Studies

Figure 5.12 shows the time-dependent profile of the adsorbed amount of proteins. The quantity of proteins adsorbed on coated substrate prepared from 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST, 70wt% HAp- 30wt% BST were respectively 0.3mg/ml, 0.6mg/ml and 0.9mg/ml. The adsorption progressed gradually

and a higher amount of protein adsorbed on coated samples 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST, 70wt% HAp- 30wt% BST compared to uncoated samples.

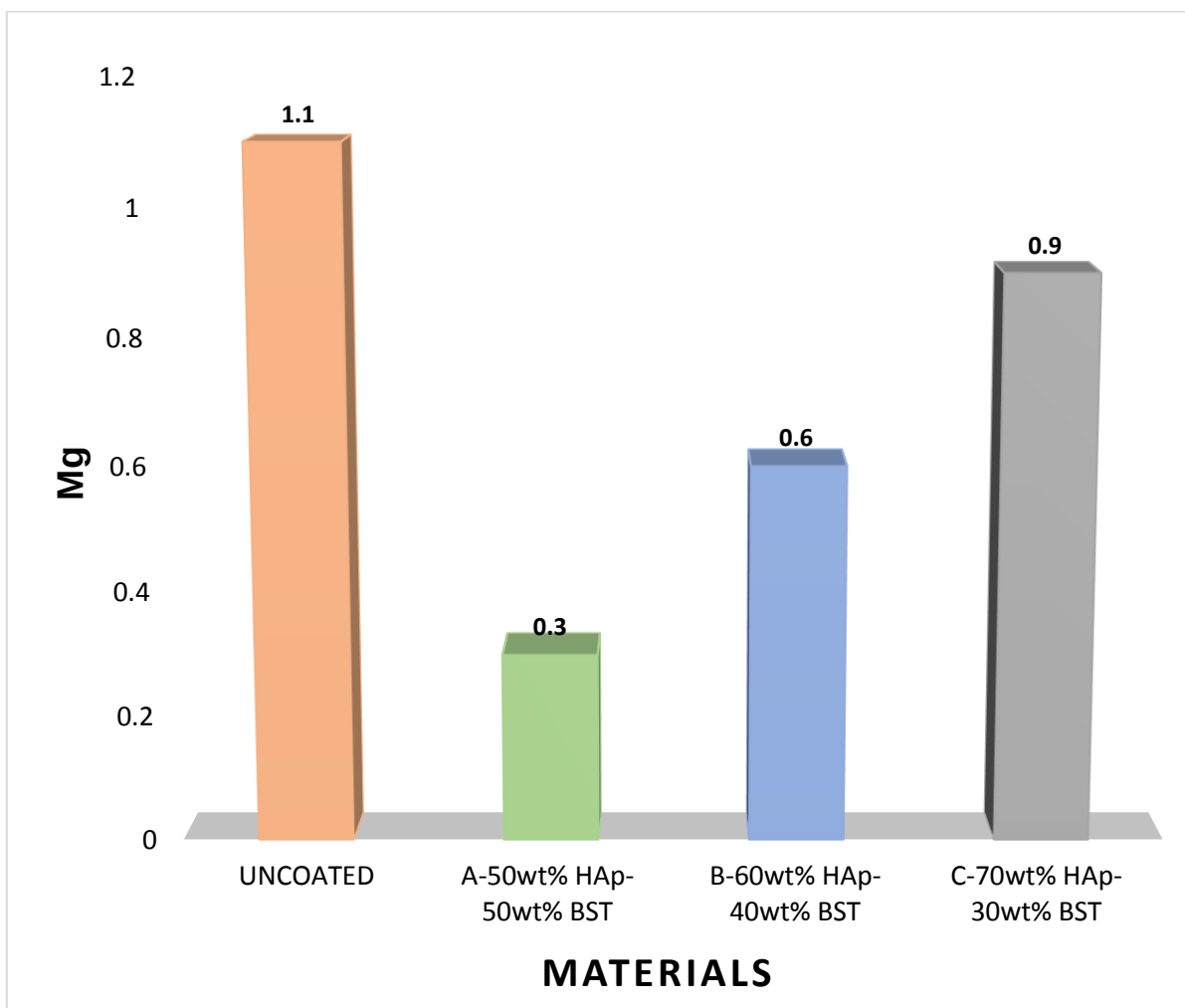
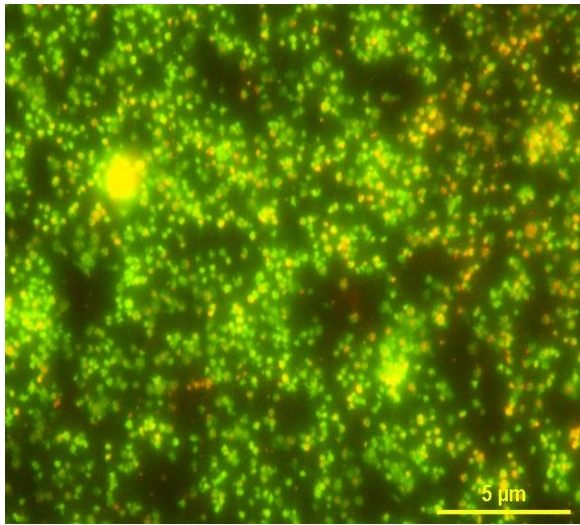


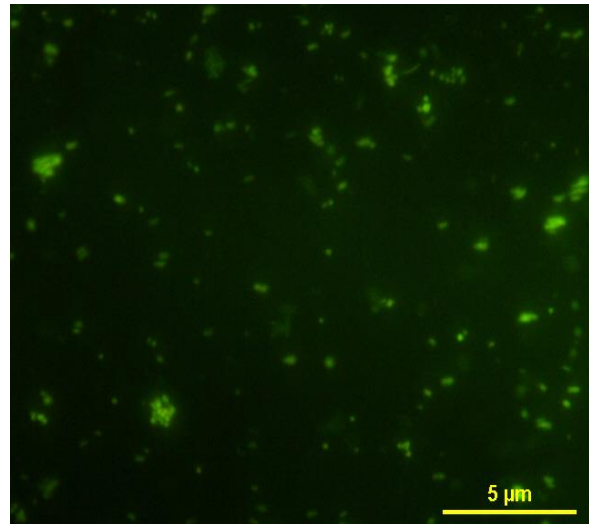
Fig 5.12: Comparison of Protein adsorbed on various Sample Materials (Coated samples (a) Uncoated (b) coated of 50wt% HAp- 50wt% BST (c) coated of 60wt% HAp- 40wt% BST (d) coated of 70wt% HAp- 30wt% BST)

5.5.2 Platelets Adhesion Studies

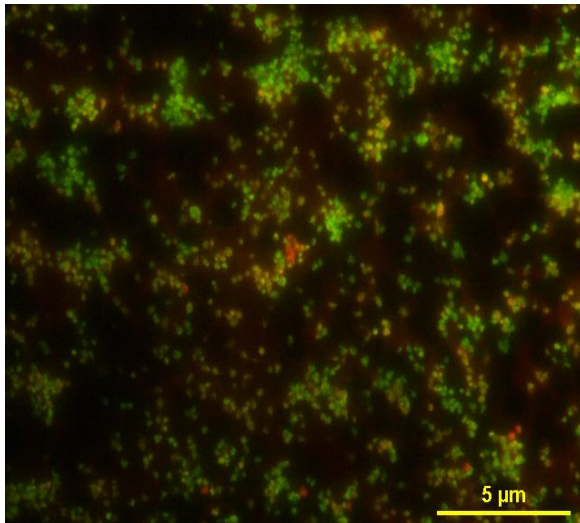
The samples were stained with acridine orange to examine platelets adhesion and activation under epifluorescence. The platelets adhesion and activation behaviour on HAp-BST coating is shown in *Figure 5.13* which shows the HAp-BST coating showed no activation of platelets and least adhesion of platelets compared to uncoated substrate which proved its hemocompatible behaviour.



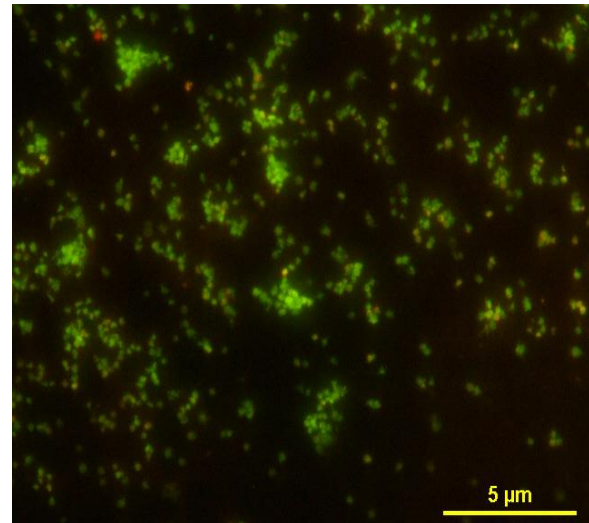
(a) Uncoated



(b) coated of 50wt% HAp- 50wt% BST



c) coated of 60wt% HAp- 40wt% BST



(d) coated of 70wt% HAp- 30wt% BST

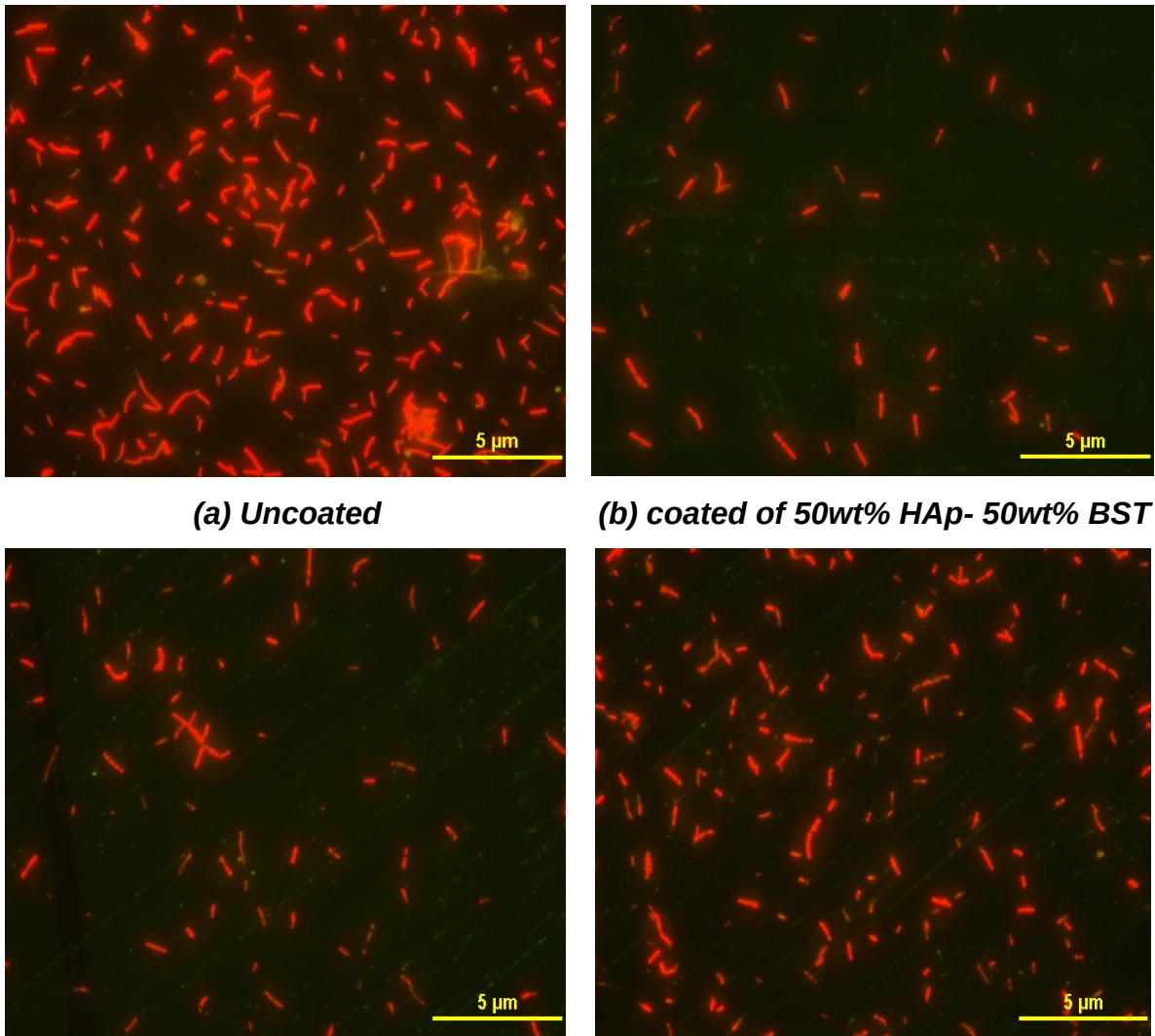
Fig 5.13 Study of Epifluorescence images of platelet adhesion on coated HAp-BST

(coated samples (a) Uncoated (b) coated of 50wt% HAp- 50wt% BST (c) coated of 60wt% HAp- 40wt% BST (d) coated of 70wt% HAp- 30wt% BST)

5.5.3 Bacterial Adhesion

Antibacterial activity of HAp-BST and uncoated Ti6Al4V were evaluated by epifluorescence microscopy analysis. Figure 5.14 shows the bacterial adhesion behaviour against *Pseudomonas aeruginosa*. The uncoated Ti6Al4V has more

bacterial adhesion comparing coated Ti6Al4V with reduced bacterial adhesion nature were observed. Drastic reduction in bacterial viability in the coated films, against, *P. aeruginosa*. The antibacterial activity decreases with increasing silica concentrations.



(a) Uncoated

(b) coated of 50wt% HAp- 50wt% BST

c) coated of 60wt% HAp- 40wt% BST

(d) coated of 70wt% HAp- 30wt% BST

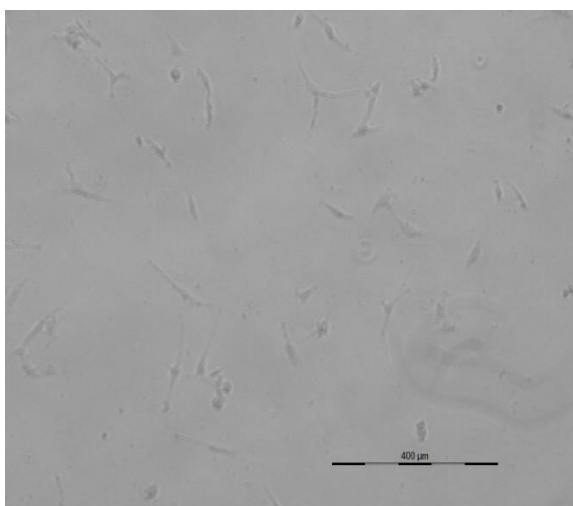
Fig 5.14: Study of Epifluorescence images of bacterial invasion (*Pseudomonas aeruginosa*) on coated HAp-BST

(coated samples (a) Uncoated (b) coated of 50wt% HAp- 50wt% BST (c) coated of 60wt% HAp- 40wt% BST (d) coated of 70wt% HAp- 30wt% BST)

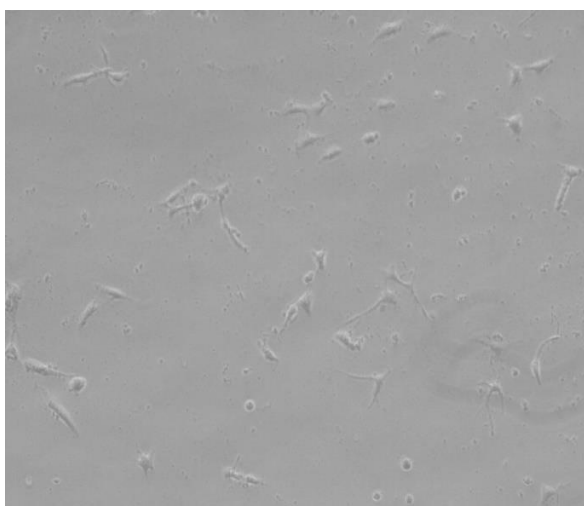
5.5.4 Cytotoxic Studies

The Mg-63 Osteoblast cells displayed good adhesion and well-spread morphology on the sample shown in *Figure 5.15*. Moreover, a layer formed when the

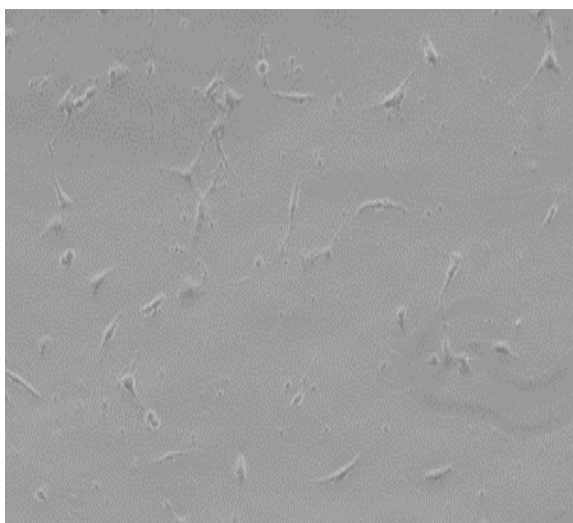
cells were spindle-shaped. Compared with the initial cell density, a significant increase was observed in the number of cells after seven days of culture on the surface of the samples. This indicates that the samples are viable for use with osteoblastic cells, and thus have comparable bioactivities in vitro. The cell viability of samples was tested with Mg63 cells using MTT assay and the results are shown that the coated samples exhibit higher cell viability than uncoated titanium alloy.



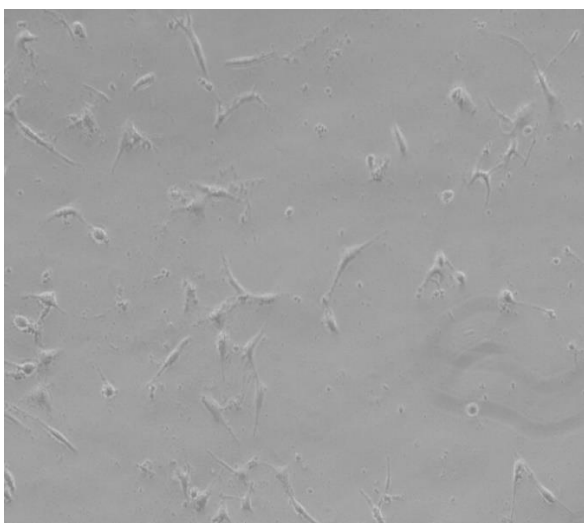
(a) Uncoated



(b) coated of 50wt% HAp- 50wt% BST



(c) coated of 60wt% HAp- 40wt% BST



(d) coated of 70wt% HAp- 30wt% BST

Fig 5.15: Study of Optical microscopy images of growth of cells on coated HAp- BST

(coated samples (a) Uncoated (b) coated of 50wt% HAp- 50wt% BST (c) coated of 60wt% HAp- 40wt% BST (d) coated of 70wt% HAp- 30wt% BST)

5.6 BIOACTIVITY STUDIES

5.6.1 Biomineralization

The deposition of calcium phosphate is shown in the given figure. Ca, p ion concentration changed in the SBF solution as a function of time, as shown in *Figure. 5.16*. After soaking in SBF for 4,8,14 days, the HAp-BST coated are partially covered with a new apatite after soaking, the weight gain is gradually increased from day 4 to day 14 and it could be due to the deposition of dissolved calcium and phosphate which is present in the SBF solution. The results indicate that the coated samples exhibit the bioactive nature.

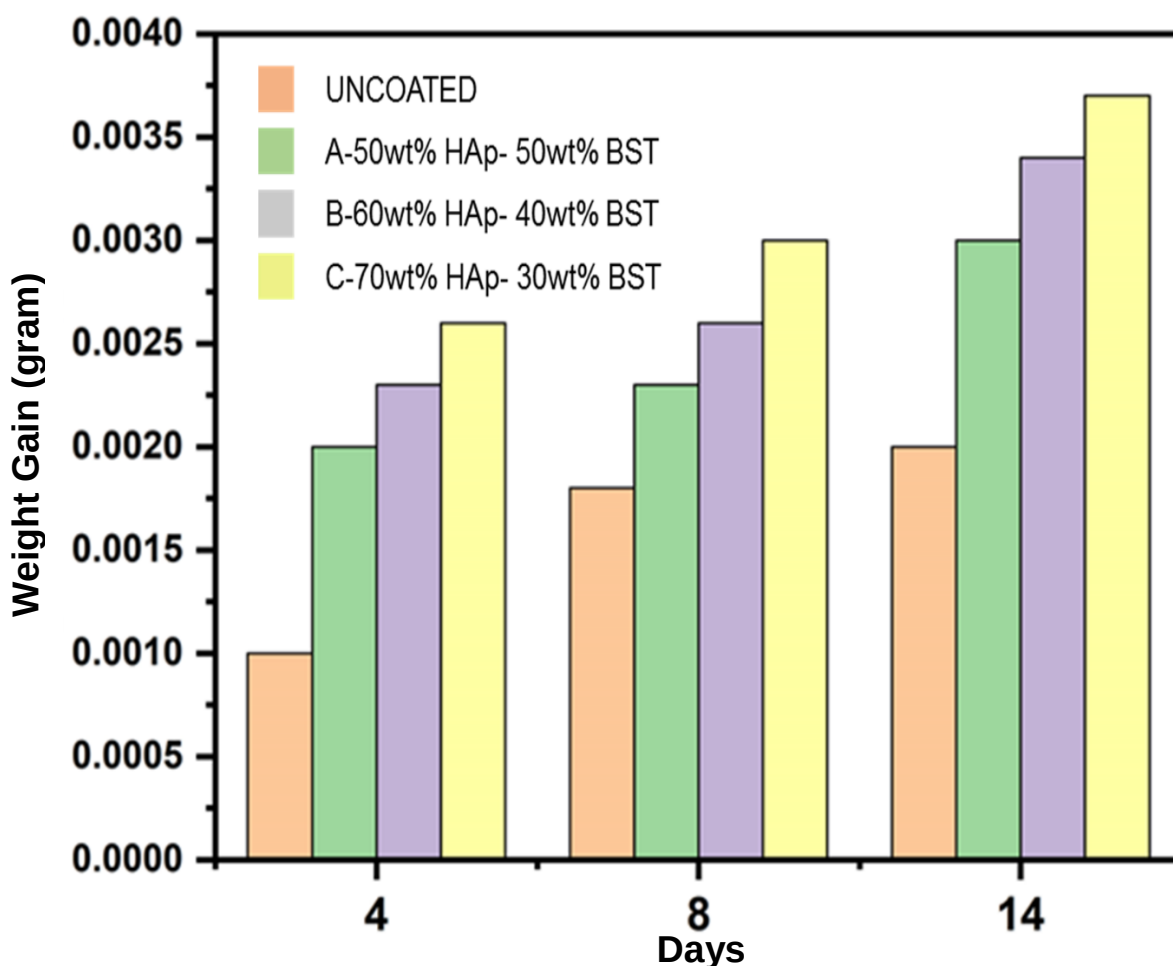


Fig 5.16: Behavioural study of Calcium mineralization behavior by MG-63 osteoblast cells onto HAp-BST coating.

CHAPTER- 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

The proposed project summarized that HAp and BST materials were prepared for potential bioimplants using dip coating technique. Initially the HAp is prepared through calcination of goat femur bone and under ball milling the HAp thus obtained as fine powder. Then, HAp-BST were prepared using certain methodologies were applied. For this HAp-BST undergoes FESEM analysis test which ensures the surface morphological characteristics and composition of HAp-BST is confirmed in EDX. Further, procedure include platelets adhesion test through this the adhesion and activation behavior of platelet in HAp-BST. It is also ensures the haemocompatibility of the material. Then, the higher amount of protein were observed by protein adsorption test. Followed by contact angle measurement is taken to ensure that the coated sample as wettability behavior. Further, Piezoelectric effect of BST-HAp nanocomposite coatings were determined to induce bone integration. In addition, the biocorrosion behaviour of the coated substrate was examined. Finally, we improvised the nature of the osteoblast through mineralization test to improve the calcium precipitation rate.

6.2 CONCLUSION

Our study has proved that is the deposition of three different compositions of 50wt% HAp- 50wt% BST, 60wt% HAp- 40wt% BST, 70wt% HAp- 30wt% BST nanocomposites by dip-coating method on medical-grade Titanium alloy was successful and it was achieved the highest rate. The surface morphology of the coated samples was characterized by FESEM equipped with EDX, which showed the presence of O, P, K, Ca, Ti, Sr and Ba elements. The XRD analysis is well agreed with the JCPDS number of HAp and BST showing crystallographic structures with a slight change of intensity peaks. The coated substrate exhibited super hydrophilic nature. The biomineralization ability of the samples is evaluated in the SBF solution. The coated sample displays superior biomineralization activity with an increase in the number of days improving calcium precipitate rate. The protein adsorption study

showed a high amount of protein adsorbed onto the coated samples. The platelet adhesion study exhibited less amount of platelets adhesion thereby no activation of platelets. Antibacterial activity of the coated substrate with *Pseudomonas aeruginosa* showed the least bacterial adhesion. HAp-BST coatings were found to be non-cytotoxic when tested with both Mg63 osteoblasts showing high cell viability. The mechanical analysis explained the good wear-resistant properties of the coated substrates. It is seen that the piezoelectric effect is increased with increasing concentration of BST indicating that 50wt% HAp- 50wt% BST nanocomposites exhibited better piezoelectric response (surface barrier) which is suitable for biological responses. HAp-BST coatings exhibited corrosion-resistant properties. To conclude, HAp-BST coatings display better bioactivity and biocompatible abilities with an increase in HAp content and BST content. The coated substrate is suitable for load-bearing implant application. Hence HAp-BST coated titanium alloy may be a suitable degradable implant for orthopaedic applications.

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