1 Introduction

1.1 Backgrounds

Biomaterials are defined as any biological or synthetic substance or combination that is used in the human body to restore and improve body function. They can be serving in the human body for a period, as a whole, or as a part (Narayan, 2018). With the evolution in the field of biomedical science, biomaterials have been known as an important area of research. For a biomaterial, it should be compatible with the body to perform the function properly. If the material is incompatible, it induces an unfavourable reaction or undesirable reaction with the blood and body tissue which is not preferable (Black et al., 2013). The term "compatible" is defined as the ability of a biomaterial to perform a beneficial host response in a clinical application. In other words, this term defines that material is not toxic or harmful to neighbouring tissues. For a biomaterial, biocompatibility is an essential property (Navarro et al., 2008). Biocompatibility is used to describe biomaterials and is characterized as an ability of a material to exist in the human body as well as its compatibility. Biocompatibility has historically been a concern for implantable devices that are meant to stay inside a person for an extended time (Rezaie et al., 2015). For biomaterials application and the investigation of their biocompatibility, three considerations should be considered. The first was that the response to particular materials may differ depending on the application location. As a result, biocompatibility may not be determined purely by material properties, but rather by the context in which the material is used. Second, a growing number of applications demanded that the substance reacts with the tissues directly rather than being neglected by them, as is the case with inert materials. Finally, certain applications allowed the substance to decay in the body over time rather than remain permanently. The term "biocompatibility" was redefined in 1987 as "a material's capacity to perform with an acceptable host reaction in a particular situation" (Williams, 2008).

Biomaterials were not feasible until an aseptic surgical technique was developed. Earlier surgical operations, regardless of whether biomaterials were used, were usually ineffective due to infection. Infection problems are compounded when biomaterials are present, as the implant may include an area that is unavailable to the body's immunologically capable cells (Parida et al., 2012). Bone plates were first used to help repair long bone breaks in the early 1900s. Many of these plates cracked due to poor mechanical design; they were too brittle and had stress-inducing corners. Products such as vanadium steel, which was selected for its strong mechanical qualities, corroded quickly in the body and had detrimental effects on the healing processes. Better performance was obtained in fracture fixing with the invention of stainless steel and cobalt-chromium alloys in the 1930s, and the first joint repair surgeries were performed (Pramanik et al., 2005).

In the case of polymers, it was discovered that warplane pilots in World War II who were wounded by plastic (polymethyl methacrylate) aircraft canopy fragments did not experience any adverse chronic reactions as a result of the fragments' inclusion in the body. After that, polymethyl methacrylate was commonly used for corneal repair and the replacement of broken skull bone portions. Blood vessel replacements were attempted in the 1950s, followed by heart valve replacements and cemented joint replacements in the 1960s, due to advancements in materials and surgical practice. Even further advancements have been made in recent years (Batista et al., 2004, Body, 2009). Here **Table 1.1** shows the uses of biomaterials in different body parts and the replacement of different parts.

Table 1.1 Uses of Biomaterials in Organs (Parida et al., 2012).

Organ	Examples
Heart	Cardiac pacemaker, artificial heart valve, total artificial heart, blood vessels
Ear	Artificial stapes, cochlea implant
Eye	Contact lens, intraocular lens
Bone	Bone plate, intramedullary rod
Kidney	Catheters, stent, Kidney dialysis machine
Bladder	Catheter and stent

Table 1.2 presents the use of biomaterials in different field of medical industries .

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Problem Area	Examples
Replacement of diseased or damaged part	Artificial hip joint, kidney dialysis machine
Assist in healing	Sutures, bone plates, and screws
Improve function	Cardiac pacemaker, intraocular lens
Correct functional abnormality	Cardiac pacemaker
Correct cosmetic problem	Augmentation mammoplasty
Aid to diagnosis	Probes and catheters
Aid to treatment	Catheters drain

1.2 Selection criteria of material for biomedical application

To select a biomaterial, generally, two criteria are considered in which one is a functional requirement and another one is interaction with neighbouring tissue. The functional requirements involve a physiologic role that will replace the implant, as well as the time intended to attain that role. The relation of the body and the inserted material should be studied from two different viewpoints: the biological environmental impact on the material properties, the material's effect, and any deterioration that may occur upon the body's local and systemic physiology (Kutz, 2002).

Table 1.3 Various factors of importance in material selection for biomedical applications (Ramakrishna et al., 2001)

Factors	**Chemical/biological characteristics**	**Physical characteristics**	**Mechanical/structural characteristics**
1st Level material properties	Chemical composition	Density	Elastic modulus Shear modulus Poisson's ratio Yield strength Compressive strength
2nd Level material properties	Adhesion	Surface topology, Texture, Roughness	Hardness, Flexural modulus, Flexural strength
Specific functional requirements (based on applications)	Biofunctionality Bioinert Bioactive Biostability Biodegradation behavior	Form and geometry, Coefficient of thermal expansion, Electrical conductivity, Color, Refractive index, Opacity or translucency	Stiffness or rigidity, Fracture toughness, Fatigue strength, Creep resistance, Friction and wear , resistance, Adhesion strength, Impact strength, Proof stress, Abrasion resistance
Processing & Fabrication: Reproducibility, quality, stabilizability, packaging, secondary processability			
Characteristics of host: tissue, organ, species, age, sex, health condition, activity, systemic response			

1.3 Progression of biomaterials

The progress of biomaterials is categorized into three generations. The first generation is bioinert materials, such as metals, zirconia, alumina, and polyethylene are not capable to combine with neighboring tissue which failed to integrate with native bone tissue, and become unstable and detached from the surrounding tissues with time (Wozniak et al., 2007). The second-generation biomaterials are bioactive, biodegradable, and resorbable materials, such as Tri-calcium phosphate, hydroxyapatite, bioactive glass, bioactive glass ceramics, and composites. In the physiological system, these materials induce action and reaction with the tissues through which they were implemented to attain the desired therapeutic impact (Hench et al., 2010). The third generations of biomaterials can stimulate specific cellular responses at the molecular level. The concepts of bioactivity and biodegradability are combined in this generation, and material properties are combined with their ability to signal and enable cellular activity and behaviour (Patel et al., 2012, Nie et al., 2011). The goal of the third generation of biomaterials, which is a logical extension of the rapidly evolving state-of-the-art, is to promote and stimulate functional tissue regeneration. The capacity of the physician or healer to restore tissues and organs lost due to disease or trauma was practically non-existent in human history; the physician's function was palliative-to make life easier (Ratner et al., 2013).

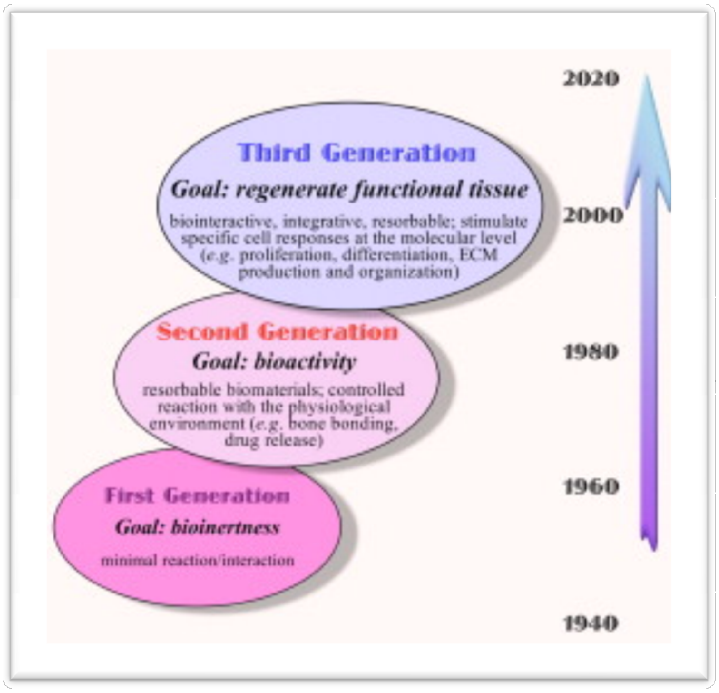

Figure 1.1 Progression of biomaterials (Ratner et al., 2013).

1.4 Classification of Biomaterials

Table 1.4 Classification basis for biomaterials.

Classification Basis	Classes of Biomaterials
Chemical composition	Metallic, Polymeric, Ceramic, Composite
Origin	Natural, Synthetic, Hybrid (Semisynthetic)
Dimensions	macrometric, Micrometric, Nanometric
Interaction with living tissues	Bio inert, Bioactive
Biodegradability	Biostable, Biodegradable
Structural integrity	Porous, Nonporous
Aim of application	Diagnostic, Therapeutic, Preventive, Restorative, Regenerative
Site of application	Extracorporeal, Extracorporeal,
Duration of contact with body	Limited (≤ l d), Prolonged (> l d and <30 d), Permanent (>30 d)

In the above-described **Table 1.4** the left column shows the classification basis for biomaterials, while the right column indicates the biomaterial groups based on the classification basis shown in the adjacent section (Masaeli et al., 2019).

Table 1.5 Summarized the different responses produced due to biomaterial type and tissue interaction (Rezaie et al., 2015).

(i) If the material is toxic, the surrounding tissue dies
(ii) If the material is non-toxic and biologically inactive (nearly inert), fibrous tissue of variable thickness forms, and if the material is non-toxic and biologically active (bioactive), an interfacial bond forms
(iii)If the material is non-toxic and dissolves, the surrounding tissue replaces it

1.5 Role of Bioceramic Characteristics for their Applications

Bioceramics have biological and physicochemical properties that enable them to be used in specialized applications.

1.5.1 Bioactivity

Bioceramics are bioactive materials that interact with the bone tissue when placed within the bone (Frayssinet et al., 1993). They integrate completely over time and are later replaced by neoformed bone. This property assigns these materials to the transient transfection of bone cells, especially osteoblasts and/or osteoclasts, which are functionally deficient in certain genetic diseases such as osteogenesis imperfecta or aging diseases such as osteoporosis (Demento et al., 2009).

1.5.2 Degradability

Bioceramics degrade and are replaced by bone in a resorption/reconstruction mechanism that is similar to that of natural bone. The ceramic grain boundaries are resorbed firstly. The degradation of materials allows for the release of micro- or even nanoparticles

that cause biological reactions, which may be useful for specific applications (Frayssinet et al., 1993).

1.5.3 Physicochemical Properties

The Biological activity of bioceramic is related to their physical properties, especially the properties of their surface. Mechanical properties, corrosion/degradation resistance, and electrical optical properties are the most significant physicochemical properties of a substance. These characteristics are considerably related to the material's composition and structure (Frayssinet et al., 2017).

1.6 Basic concept of composites

Composites are defined as the heterogeneous combination of two or more materials having different compositions, morphology, and physical properties at a macroscopic level. The aim is to develop these materials having properties that cannot be obtained from a single material (Yadav et al., 2020). The word “bio-composite” is referred to as a compound of one or more biological components in which one or all component is biological components or compound of the naturally found element. The components of composite stay as separate phases in the composite system and keep their physical and chemical characteristics (Rajak et al., 2019). In the present thesis, the word “composite” is utilized in materials which completely applicable for biological application.

It is important to note that biological structural materials found in nature are almost composite. Wood, bamboo, bone, teeth, and shell are all common examples. Furthermore, the use of synthetic composite materials is not a new concept. In biblical times, straw-reinforced mud bricks were used. This material is known as an organic fiber-reinforced ceramic matrix composite in modern terminology. Polymers, metals, ceramics, and carbon, are the four types of solid materials. All four groups include both reinforcements and matrix materials. This

allows us to create an infinite number of new materials with specific properties that are difficult to achieve with a single monolithic material. Polymer matrix composites (PMCs), metal matrix composites (MMCs), ceramic matrix composites (CMCs), and carbon/carbon composites (CCCs) are the four major types of composites (Kutz, 2002) (Rajak et al., 2019). Bone is a composite substance made up of an organic matrix that is primarily made up of collagen type (I) that has been mineralized with hydroxyapatite. Bone's composite design has a complex microstructure that is difficult to mimic, which accounts for the bulk of its superior mechanical properties (Gowda et al., 2018).

Composite structures can be described in several ways. One common method is to classify composites based on the geometry of the reinforcing process. Particulate composites, fibrous composites, and laminated materials are also examples of this. Fibrous composites can be classified as either continuous fibers or short/chopped fibers based on the ratio of fiber length to diameter (the so-called "aspect ratio"). Continuous fibers have a ratio greater than $1*10^5$, whereas short/chopped fibers have a ratio between 5 and 200 (Rajak et al., 2019). Composites are also classified on the matrix material used. Metal matrix composites, ceramic matrix composites, and polymer matrix composites are the three types. Each part of a composite in biomedical applications must be biocompatible (Mishra et al., 2018). Composites are heterogeneous structures that are made up of two or more homogeneous phases separated by an interface (s). At the interface, chemical or physical bonds bind the distinct constituent phases together. Composites, unlike conventional materials, may have their physical, chemical, biochemical, and other properties customized to the needs of particular applications. A composite is made to combine the best properties of each component or to produce new materials with properties that cannot be obtained by existing materials. A composite usually has two phases: the matrix phase and the reinforcement phase (also known as the "second phase" or "dispersed phase"). The matrix is a continuous phase

that wraps around the other phases and gives them their overall shape. The reinforcement phase is more distributed than the matrix phase and is usually tougher and stiffer (Ibrahim et al., 2015).

The following are the main factors that affect composite structure:

- Reinforcement shape, scale, and distribution;
- Reinforcement properties and the volume ratio;
- The reinforcement's bioactivity;
- The molecular weight and grain size of the matrix;
- Interfacial state of reinforcement matrix.

Total joint replacements, spinal rods, discs, plates, dental posts, screws, ligaments, and catheters are only a few of the promising surgical uses of biomedical composites (Rezaie et al., 2015).

1.7 Classification of composites

As seen in **Figure 1.2**, composite materials are categorized according to the form of constituents used.

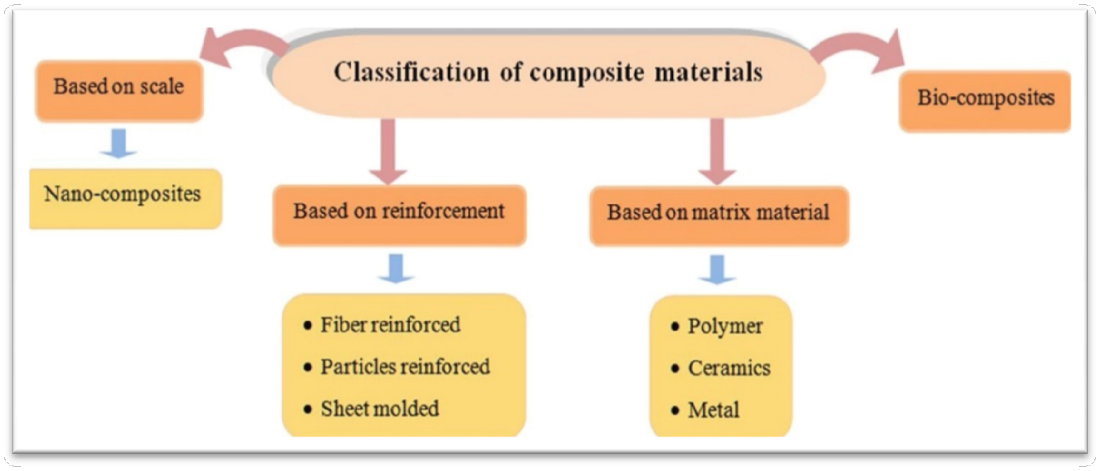

Figure 1.2 Classification of composite materials (Ibrahim et al., 2015) (Mishra et al., 2018).

1.7.1 Polymer matrix composites (PMCs)

PMCs are composed of a matrix of thermosets or thermoplastics with carbon, glass, Kevlar, and metal fibers as reinforcing (Ray, 2006). Due to their higher strength and tolerance to high temperatures, thermosets are more often used than thermoplastics. Thermosets are made by combining resin and hardeners. Easy handling and quick fabrication processes make PMCs low-cost composites (Thoppul et al., 2009) (Wisnom et al., 2006).

1.7.2 Ceramic matrix composites (CMCs)

CMCs are ceramics that are made up of carbon, silicon carbide (SiC), aluminum oxide (Al_2O_3), and silicon nitride (SiN) fibers incorporated in a ceramic matrix (Zhang, 2014). They were created to conquer the brittleness of monolithic ceramics. CMCs are produced through various manufacturing methods such as gas- or liquid-phase methods. The inner phase and matrix are formed around the fibers in this process using gaseous or liquid precursors (Kopeliovich, 2018) (Naslain, 2016).

1.7.3 Metal matrix composites (MMCs)

MMCs for biomedical applications have a metallic alloy matrix, and the reinforcement is dispersed ceramics like oxides and carbides. These composites are being studied specifically for their ability to provide the strength and stiffness required for load-bearing implants, which are supplied by the metal matrix. In most cases, the reinforced phase is a bioactive bioceramic (Hunt et al., 2004). Based on the temperature used in the manufacturing process, the methods for fabricating particulate metal-matrix composites can be divided into three categories: (1) liquid-phase process, (2) solid-state process, and (3) solid-liquid process. In the liquid phase process, the reinforced phase is dispersed in a molten metal matrix and solidified to form composites. Stir casting can be used in this process. For the solid-state process, both matrix and reinforced phases are kept in the solid-state but high

temperature and pressure are applied to form composites. For this, the powder metallurgy process is used. In the solid-liquid process, both phases are mixed at a region of the phase diagram where both liquid and solid phases exist in the matrix (Nayak et al., 2019) (Rajak et al., 2019).

1.7.4 Porous composites

For tissue engineering application, porous polymer, metal, or ceramic matrix composites (also known as "scaffolds") are developed and tested. Polymer matrix composite scaffolds are much more popular than metal or ceramic composite scaffolds (Kamel et al., 2017). Solvent casting, porogen leaching, phase separation, electrospinning, and 3D printing processes are used to make polymer scaffolds. Technical difficulties, such as homogeneous distribution of bioceramic particles in composite scaffolds and adequate quantities of bioceramic particles in the scaffolds, can occur when bioceramic particles (micro- or nano-size) are added to the polymer solution for scaffold fabrication. Composite scaffolds, on the other hand, hold promising potential for the regeneration of certain forms of human body tissues, and new and better techniques for producing novel nanocomposite scaffolds should be created (Mishra et al., 2018, Ratner et al., 2013).

1.8 Objective

The objective of this work was to develop and characterize the family of a ceramic-based composite system by incorporating the different reinforcing materials for their potential biomedical applications. For the succession of the work, the following procedures were carried out.

1. Synthesis and characterization of Zirconia substituted 1393 bioactive glass of the system SiO_2-CaO-Na_2O-P_2O_5-K_2O-MgO-ZrO_2.

   To produce this bioglass, the sol-gel method was used and produced materials were characterized with different techniques to get the properties of glass. Surface

properties, as well as bioactivity, biological, and mechanical properties, were also studied.

2. Synthesis and characterization of Hydroxyapatite ceramic

   For hydroxyapatite ceramic synthesis, the co-precipitation method was selected and characterized with different techniques.

3. Synthesis and characterization of calcium zirconium silicate (baghdadite) ceramic

   The solid-state method was selected for baghdadite ceramic synthesis and different characterization technique was used to obtain different properties.

4. Reinforcement of ceramics to prepare a composite system

   To prepare a composite system, ceramic was reinforced with other ceramics in different proportions and ball milling is carried out to get a homogeneous mixture.

5. Characterization of the composite system

   XRD, FTIR spectroscopy, and SEM coupled with EDX were performed to confirm the phases, structure, functional bond, morphology, and elemental analysis of the composite.

6. Analysis of In-vitro behaviour in terms of bioactivity, weight loss, and SBF absorption of the composites.

   The apatite formation ability, weight loss, water absorption, and pH value of composites during SBF immersion were carried out and compared with a base material. The Density of composites was also measured.

7. Hemolysis and In-vitro cell culture analysis

   Hemolysis behavior and In-vitro cell culture analysis of composites were evaluated in terms of proliferation, and viability on MG-63 cell lines.

8. Antibacterial behavior analysis

An antibacterial behavior of composites against both gram-negative bacteria such as E.coli and gram-positive bacteria such as S.aureus was measured.

9. Mechanical properties analysis

The mechanical properties such as compressive strength of composites were measured.