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Tools and Techniques Used for the Development of Scaffold for Bone Tissue Regeneration: A Detailed Review

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Abstract: Massive bone lesions present a significant surgical challenge and contribute to the socio-economic burden while reducing quality of life. Studies worldwide support the effectiveness of bone tissue engineering scaffolds in addressing bone tissue defects. The primary objective of tissue engineering is to develop biological replacements for failing organs or tissues due to accidents, aging, or trauma. The scaffold is vital in tissue engineering, providing an inductive environment for cell attachment, proliferation, and growth. It acts as a bioactive bridge, creating a microenvironment that promotes tissue regeneration. Tissue engineering finds applications in various medical specialties such as cancer, orthopedics, gastro-intestinal, skin, dental, musculoskeletal, urology, spine, vascular, neurology, and cardiology. Differentiated or stem cells are seeded onto a biomaterial scaffold, cultivated in a bioreactor, and then implanted in a living organism. An ideal scaffold should exhibit bioactivity, mechanical strength, porosity, pore size, and, most importantly, biocompatibility. These characteristics are crucial for scaffold selection and construction techniques. A combination of diverse biomaterials and appropriate scaffolding techniques is necessary to achieve these desirable characteristics, tailored to the specific medical and clinical requirements. This review provides a comprehensive understanding of the specific types of scaffolds needed for bone healing, repair, or regeneration, along with the suitable biomaterials and scaffolding techniques involved. It offers detailed insights into selecting scaffolds that facilitate the healing process and discusses the various biomaterial options available and the techniques used to construct the scaffolds.

Keywords: tissue engineering; bioactive scaffold; stem cell; scaffolding techniques; regenerative medicine.

1. Introduction

Tissue engineering is a process that exhibits the combination of biological and chemical agents along with the principles of engineering towards the repair, restoration, or regeneration of tissues with the help of cells, growth factors, and different kinds of biomaterials alone or in combination (Figure 1) [1].

There are various incidences like diseases, trauma, or other inherited defects wherein there is tissue or organ loss, but the body does not have the potential to regenerate the damaged or defective tissue as compared to the salamanders; thus, they permanently lose their organs. It would not be less than a miracle if the tissues and organs could be replaced on demand. Millions of people would benefit massively if tissue regeneration is possible [2]. In medicine, tissue

engineering is a novel and rapidly developing branch that was introduced in clinical practice only a few years ago. It is the imagination of scientists, physicians, and other healthcare professionals to soon regenerate the tissues and organs for entire human body sites. Artificial tissue generations are new tools for clinicians that have huge applications in different types of diseases related to the head and face of human beings. However, many challenges are posed in the process of tissue engineering that need to be addressed [3]. The potential of regenerative medicine is based on its ability and potential to repair, regenerate, and replace damaged or lost tissues or whole organs of the body [4]. Various studies have shown promising results of regenerative medicine for regenerating and replacing various tissues and organs like the heart, kidney, skin, and liver. They can potentially correct congenital defects [5,6]. There is a sharp increase in the incidents of bone disorders, which are expected to double by the end of the year 2023, especially in the aged people with increased obesity and poor physical activity. Tissue engineering is a broad term that works on all the tissues and organs of the body to repair, replace, or cure the damaged tissue or organ. In contrast, tissue regeneration focuses on regenerating the damaged tissue with proper functioning. The main objective of bone tissue engineering is to regenerate a new functional bone with a combination of synthetic biomaterials, cells, and other growth factors [7]. Repairing bone defects with the help of the tissue engineering process is viewed as a safer method, as in this technique, the reconstruction process starts using the patient's tissue until the restoration is complete [8]. In tissue engineering, autografts are considered a gold standard for bone grafting to date because, having histocompatibility and non-immunogenicity, there are lesser chances of graft rejection, and they offer all essential properties required for bone grafting. Autogrfts exhibit the necessary components to attain osteoinduction, osteogenesis, and osteoconduction: bone morphogenetic proteins and growth factors, osteoprogenitor cells, and three-dimensional porous matrix, respectively. Because autografts involve harvesting bone from the patient’s iliac crest, a secondary surgical procedure is required at the harvest site to ensure proper functioning and reduce discomfort. [9]. Autologous bone transplantation processes cause donor site injury, site deformity, and scarring and also have associations with surgical risks such as bleeding, inflammation, infection, and acute and chronic pain. However, it is a very expensive procedure [10].

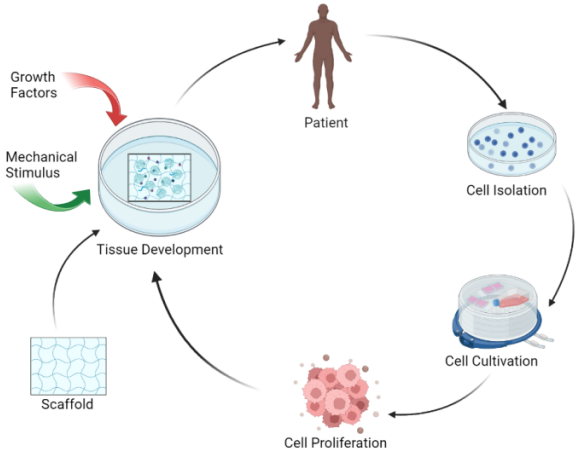

Figure 1. Process of tissue engineering.

There are some cases in which defect sites require a larger size of bone, which is not possible by this technique; thus, autografts are insignificant for such kind of treatment. On the other hand, allograft is the second most common bone grafting technique, which involves the transplantation of bone tissue from a donor, which is generally obtained from a dead body [11].

Depending on the host-site requirements, several allogeneic bones are available, such as demineralized bone matrix (DBM), morcellated, cancellous chips, cortical-cancel-lous, cortical grafts, osteochondral and whole-bone segments. Allogenic bone is also histocompatible. However, allografts have more risks of immunoreactions and infection transmission than autografts because the tissue is obtained from another body in the allograft, which acts as foreign material to the host immune system. As the donor tissues are devitalized by irradiation or freeze-drying techniques, they have lower osteoinductive properties and no cellular components [12,13]. Bone tissue engineering (BTE) is an emerging field that was initiated about three decades ago, and many studies show its huge progress and interest in the field of BTE over the years. BTE is a novel and alternative treatment that focuses on eliminating the issues with currently used organ transplantation techniques, which majorly include donor site morbidity, scarring of the donor site, limited availability of tissue, graft rejection, and infection transmission. It requires equal collaborative efforts of scientists, engineers, physicians, surgeons, and other healthcare professionals to attain this critical goal to regenerate and repair lost or damaged tissue [14]. There are basic requirements for bone tissue engineering, such as a biocompatible scaffold that plays a role like natural bone extracellular matrix niche, responsible for cell attachment, osteogenic cells play a role in laying the bone tissue matrix, morphogenic signals that direct the cells to the phenotypically desirable type and optimum vascularization to meet the sufficient requirement of nutrients supply for growing tissue (Figure 2).

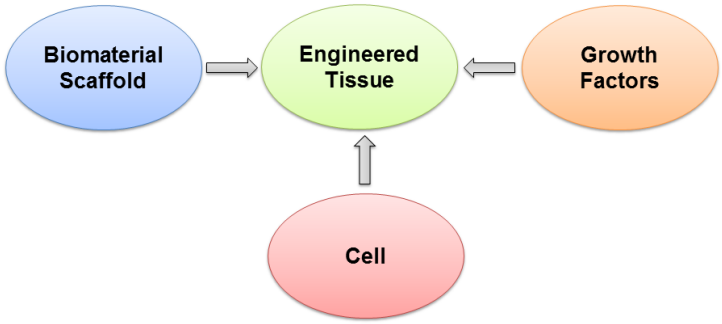

Figure 2. Basic requirements for tissue engineering.

The construct may influence the host by releasing osteogenic or vasculogenic growth factors based on the scaffold: growth factor-releasing scaffold, scaffold with growth factor analogs, or seeding with platelet-enriched plasma. It is also influenced by the cells genetically engineered to release the growth factors naturally after the scaffold implantation at the defect site [15]. After this process, cell homing, tissue vascularization, and bone regeneration of the defected sites occur. Much progress has been made in the field of bone tissue engineering, but many critical issues remain to be clear in order to become a true clinical reality.

2. Review of literature

2.1. Bone.

Bone is a highly complex and specialized connective tissue that has several critical functions in our body: maintaining the body structure, protecting the organs inside the body, providing mechanical support, movement of the body, production of blood cells, storage of minerals, homeostasis process, regulation of blood pH and also responsible for the housing of various progenitor cells. The term “Bone” ndicates the structures and the tissues by which they

are made [16]. If we talk about bone tissue, it is a natural hybrid nanocomposite that is composed of an organic matrix that is made primarily of collagen protein, which is supported by a layer of rod or plate-shaped hydroxyapatite particles, a type of inorganic ceramic that provides stiffness balance, toughness, mechanical strength, and damping properties to the bone. Bone tissues have characteristics that modify their structure and shape by forming new cells and removing the old ones to adapt to the changes that occur in the human body throughout life to maintain the structure-function relationship [17,18].

The human body is made up of more than two hundred bones, which are arranged in a wide variety of genomic and structural variations based on the different kinds of parameters like physiological functions, skeletal site, age, and sex of the individual [19].

2.2. Composition of bone.

In addition to water, bone is composed of inorganic and organic materials, and in terms of volume, the proportion of these materials is approximately 25%, 40%, and 35%, respectively. The weight contribution of the inorganic phase is approximately 60-70%, primarily composed of hydroxyapatite, a crystalline mineral. The ends of collagen fibrils contain small amounts, whereas the voided space between the collagen fibrils contains large crystals of hydroxyapatite crystals with a plate or rod-like shape (Figure 3). The thickness of these crystals ranges 2-10nm, and the length ranges from 20 to 50nm while the width falls about 15-30 nm [20,21].

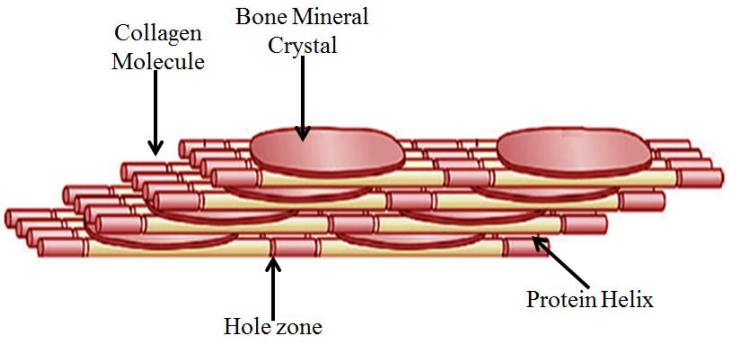

Figure 3. Mineral particles arrangement with collagen fibrils.

Various diseases and disease therapies can also affect the bone minerals and their structure, which leads to an alteration in the crystal's size and crystallinity too crystal's size and crystallinity, ultimately affecting the bone strength. Minerals like magnesium, potassium, strontium, and sodium are present in apatite crystals rather than calcium, while chloride and fluoride replaced the hydroxyl groups, and the phosphate group is replaced with carbonate, so hydroxyapatite is present in non- stoichiometric form in bone. This pattern of crystallinity or non-stoichiometry helps bone resorption by the osteoclast process by providing a mineral phase with optimum solubility [21,22]. Approximately 99% of calcium present in the body is stored in the mineral phase of bone, while the remaining 1% is present in extracellular and intracellular fluids; on the other hand, 85% of the phosphate anions exist in the mineral phase of bone, and teeth, 1% in extracellular fluid and remaining 14% is distributed in other tissues of the body. Calcium and phosphate are known biomarkers for the determination of the health of bone because they are responsible for bone growth and development and influence the storage of bone mineral homeostasis and metabolism [23,24].

25 to 30% of the weight of the bone is due to the organic phase, which includes around 90% type-I collagen and non-collagenous proteins. Collagen is a natural polymer made by

osteoblast that is responsible for the toughness and ductility of the composite scaffold [25]. On the other hand, the regulation of collagen formation, mineralization, cell attachment, and the control of fibril size is done with the help of non-collagenous protein [18,26]. The remaining 10% includes the cell's water, fluid, and bone apatite crystals, which help the collagen to bind with the mineral phase [27–29]. Other than these, growth factors and cytokines are also present but in minute amounts in the matrix of bone, which are responsible for regulating cell growth, cell activation, cell differentiation, etc. [21]. The composition of bone is enlisted in table 1; however, the variations can be seen from bone to bone [30].

Table 1. Composition of bone.

S.No.	Components	Weight %
1	Hydroxyapatite	60-70
2	Collagen	10-20
3	water	9-20
4	Non-Collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin, bone morphogenetic proteins, sialoprotein, serum	3-5
5	Carbonate	4-6
6	Sodium	~ 0.7
7	Magnesium	~ 0.5
8	Other inorganic ions (Cl^-, F^-, K^+, Sr^+, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+})	Traces
9	Other organic material (Polysaccharides, lipids, cytokines)	Traces
10	Primary bone cells: osteoblasts, osteocytes, osteoclasts	-

2.3. Bone fracture and healing mechanism.

2.3.1. Bone fracture.

Bone fractures occur due to trauma, such as sports injuries, road accidents, or any other mechanical injury, which causes a loss of the continuity and mechanical stability of the bone. These conditions may also develop due to other medical conditions like osteoporosis and bone cancer. There are two types of fracture: open fracture and closed fracture. The external surface of the body is exposed in direct contact with an object, which causes the fracture; it includes skin wounds and soft tissue damage. There is a high risk of contamination in open fractures. On the other hand, in closed fractures, there is a lower risk of infection,is a lower risk of infection, and the skin remains intact [31].

Fracture is also classified into another class based on the force that causes fracture, which includes:

2.3.1.1.Simple fracture.

This type of fracture, also known as closed fracture, generally occurs when a twisting or bending force is applied to the bone. This force causes the bone to break into two fragments, often characterized by transverse or oblique fracture lines.

2.3.1.2. Comminuted fracture.

In this type, the bone breaks down into fragments due tohigh-energy trauma, which may cause an everlasting deformation of the fractured part. Compared to simple fractures, this type of fracture is harder to heal.

2.3.1.3. Stress fracture.

When the repetitive low force is applied for a longer period, it causes micro-damage to the bone. The normal bone remodeling is enough to heal this type of fracture, but sometimes it may be a permanent microdamage that cannot be repaired if the continuous low force is applied to the bone [32].

2.3.2. Bone healing mechanism.

The healing process of fracture usually takes about 6-8 weeks. This period is enough for the full restoration of the functions and structure of bone. The healing process of a long bone is classified into two categories: primary or direct healing and secondary or indirect healing [33].

2.3.2.1. Primary (direct) healing.

This type of healing required the inhibition of callus formation and rigid stabilization of fractured bone. There are two different processes in primary healing: gap healing and contact healing. Two phases also exist in gap healing. In the first phase, the gap is filled by the formation of a woven bone layer. Then, the supportive lamellar bone formation occurs in a transverse orientation to the original bone. At the same time, in the second phase of gap filling, the remodeling of the fracture site occurs, in which the fractured ends are reconstructed, and the osteons are formed parallel to the original orientation of the bone. The second class of primary healing process is contact healing, in which the growth of osteons occurs in the same orientation of the original bone on the fractured ends that are in direct contact. The formation of the Harversian system across the fracture site results from osteoclasts' formation of cutting cones [34].

2.3.2.2. Secondary (indirect) healing.

Secondary healing is a prevalent bone healing method involving controlled movement between the fractured ends of the bone. It typically employs cast immobilization or external fixation techniques to stabilize the broken bone segments. These methods allow for a certain degree of motion while maintaining stability, aiding in healing. Examples of such controlled motion include using an external fixation device or a plaster cast [35]. The secondary healing process involves four spontaneous stages.

2.3.2.2.1. Inflammatory phase.

The inflammatory phase has a protective mechanism against fracture by forming the hematoma through the bone matrix, soft tissue, and vascular damage. The occurrences of swelling immobilize the fracture motion and help in healing. The duration of this phase is last up to 7 days [34]. Cells involved in inflammation release cytokines to initiate the repairing process. Platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), interleukin-1 (IL-1), interleukin-6 (IL-6), and fibroblast growth factor (PDGF) etc., are the cytokines involved in an inflammatory phase that cause local mesenchymal cells to differentiate into osteoblast [36].

2.3.2.2.2. Soft callus formation (cartilage formation).

The cells stimulated in the inflammatory phase produce vascularized reparative callus, improving fractured bone ends' stability. This phase's peak duration lasts 7-10 days after the day of fracture.

2.3.2.2.3. Hard callus formation.

The calcification of soft callus tissue occurs in woven bone. For this process, endochondral ossification and vessel invasion take place. This process happens with the intramembranous ossification process along with endochondral ossification in the periphery of the fracture site of bone [32].

2.3.2.2.4. Remodeling phase.

The final stage of the secondary healing process involves the replacement of woven bone with lamellar bone and the excessive resorption of callus. This phase typically begins around 3 to 4 weeks after the fracture occurs and can take several years to fully restore the bone to its original shape. During this period, the newly formed lamellar bone gradually replaces the initially formed woven bone while the body reabsorbs excess callus [34,37].

3. Concept of (bone) tissue engineering (TE)

Bone generation is a very important requirement in many clinical conditions; it includes bone defects due to trauma, tumor resection, infection, and skeletal abnormalities. Other than these, many cases like avascular necrosis, atrophic non-union, and osteoporosis require tissue regeneration. Approximately 2.2 million bone grafts are performed yearly worldwide. Due to the histocompatibility, non-immunogenicity, and properties of bone growth, autografts are considered as “gold standard” treatment for bone flaws. However, autografts have some drawbacks, like donor site morbidity and limited supply of blood. Other than autografts, allografts are also come into force in which the organ obtained from living donors. Allografts overcome the demand problem associated with autografts. A few limitations are also associated with allografts as the procedure is expensive, causes the host immune response, and increases the risks of transmitting disease from donor to recipient.

Bone tissue engineering is a developing field working towards the invention of alternatives to conventional treatment to overcome the problems associated with the previous [38,39]. The main objective of bone tissue engineering is to develop an artificial biological substitute that can restore or improve tissue function by combining biomaterials, cells, and growth factors. This process is based on understanding the bone structure, bone formation, and bone mechanics [7,40,41]. The research work on bone tissue engineering was initiated by an orthopedic surgeon W.T. Green, five decades ago. In 1970, W.T. Green generated a new cartilage by implanting chondrocyte cells into bone spicule implanted in mice. Vacanti brothers and Lange 1980 designed a method to develop a scaffold for delivering cells using synthetic and biocompatible polymers that could not be replicated [42]. Caplan, in 1991, assumed that the desired repair of bone tissue could be attained by the isolation of autologous stem cells via mitotic expansion and its desired delivery into the defective site. Quarto et al. published their first clinical paper in 2001, reported that autologous bone marrow cells can repair large bone defects [43]. The human study of Schimming and Schmelzeisen in 2004 reported the formation

of lamellar bone from osteoblastic cells obtained from the periosteum within three months after the transplantation. Until now, a large amount of progress has been seen in the field of bone tissue engineering, but many crucial difficulties remain to be cleared to become the clinical reality of this field [7,44].

The overall process of tissue engineering is based on the successful interaction between the following three main components; these are also known as the tissue engineering triad (Figure 4): Scaffold - A scaffold or matrix that works as a natural extracellular matrix. It facilitates the cell attachment during transplantation onto the desired site [45]; Implanted and cultured cells - These cells can make a completely new tissue; Biological signaling molecules - These molecules direct the cells to form desired tissue. These include growth factors, differentiation factors, and adhesion molecules [46,47].

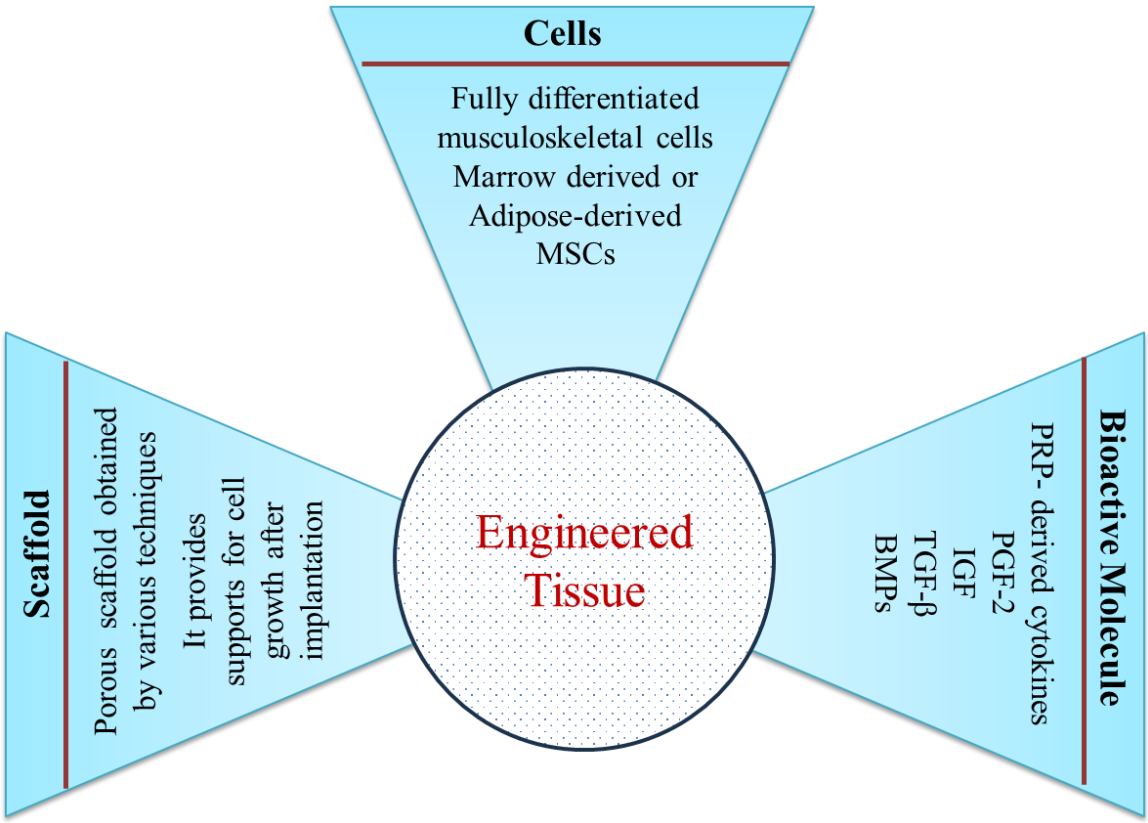

Figure 4. The tissue engineering triad

3.1 Characteristics of scaffold used in bone tissue engineering

The bone scaffold needs to satisfy each property at the macro and micro structure levels for the desired growth of bone tissue. The essential characteristics of scaffold material are explained below.

3.1.1. Biocompatibility and bioactivity.

The fabricating material used for the scaffold should not cause any immunological response to the recipient's body. The biocompatibility of scaffolding material produces superiority over allografts and autografts, with fewer graft rejection chances due to immunological responses in both previous techniques [48]. The biocompatibility of a scaffold, which is crucial for its suitability as an implant, is significantly influenced by the polymer used in its fabrication. Various chemicals involved in the polymerization process, such as stabilizers, cross-linking agents, organic solvents, initiators, or unreacted monomers, have the potential to

leach out after implantation. This leaching can lead to inflammation at the implanted site. To prevent such inflammation, the leachable substances need to be biocompatible, ensuring they do not cause adverse reactions within the body [49]. Biocompatibility of biomaterial is an essential requirement for bone tissue engineering than bioactivity. Bioactivity is also preferred for promoting bone healing faster because it facilitates the direct bonding of the implanted scaffold with the host tissue that forms bone-like apatite [50].

3.1.2. Porosity and pore size.

The porosity and pore size of bone scaffold also play a very important role in successful tissue regeneration. The porosity and pore size help uniform distribution of cells and facilitate the nutrient supply throughout the region of implanted cells. The pore size of the scaffold depends upon the size of the cells that have to be implanted and ranges from 100 to 400μm. The porous structure of the scaffold helps to migrate and proliferate cells, facilitates the proper supply of nutrients and oxygen to the implanted cells, and allows metabolic waste to be secreted from the cells. Although higher porosity decreases the mechanical strength of the scaffold, maintaining the optimal balance between porosity and mechanical stability remains a crucial parameter for bone tissue regeneration [51,52].

3.1.3. Surface properties.

The surface area of the scaffold provides a platform for the tissue of the implanting site and scaffold materials where the interaction between implanted cells and the tissue of the implanting site takes place. The chemical and topographical properties of the scaffold surface should help support cell adhesion, proliferation, and migration, as well as establish cell-to-cell contact. The roughness of the scaffold also directly impacts cellular morphology and phenotypic expression of cells. To increase the cellular attachment, modification is required on the scaffold's surface. The surface properties can be modified with the help of gas plasma or by growth factors and biologically active compound attachment [41,48].

3.1.4. Mechanical properties.

The mechanical property of the scaffold is a very important parameter for bone tissue engineering. To ensure the protection and mechanical integrity of the developing tissue, dynamic stress is needed; thus, sufficient strength and stiffness of the scaffold are required. The mechanical properties of a scaffold depend on the biomaterial by which it is made. Tissue scaffolds made from polymers show mechanical strength similar to cancellous bone, while scaffolds made from biodegradable ceramics like hydroxyapatite and tricalcium phosphate possess compressive strength and elastic moduli compatible with the cortical bone. However, the ceramics are brittle and exhibit a slow degradation rate. Cancellous and cortical bone have different mechanical properties, so the composition of ceramics and polymer could be a good option for fulfilling the mechanical strength requirement of cortical and cancellous bone in bone tissue engineering [7,53].

3.1.5. Biodegradability.

Biodegradability of scaffold material also plays a major role in bone tissue regeneration. An ideal scaffold should be degraded in a controlled manner in the human body to provide space for new growing tissue. The scaffold's degradation rate should be similar to the rate of

new bone formation, and its degradation property should also be variable according to the implantation site. For instance, the complete degradation of the scaffold used for maxillofacial should take 3-6 months, while the complete degradation of the scaffold used for spinal fusion should take 9 or more than 9 months [51]. The degradation rate of the scaffold can be controlled by varying the scaffold composition, and it can also be controlled by various scaffolding techniques [54].

4. Biomaterial

The biomaterials used in tissue engineering were developed approximately 60 years ago. Several limitations in allografts and morbidity were also present during tissue implantation into the human body[55]. The first definition of biomaterial was given by David F. Williams in 1987, and he defined biomaterials as "A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems [56]."

David F. Williams modified his definition in 1999 and defined biomaterial as "A material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body."

In 2003, Miller-Keane and O'Toole defined biomaterials in another way, "any substance (other than a drug), synthetic or natural, that can be used as a system or part of a system that treats, augments, or replaces any tissue, organ, or function of the body; especially, material suitable for use in prostheses that will be in contact with living tissue" [57]. Williams in 1987 defined the other important factors related to biomaterials such as mechanical properties, biocompatibility as, "the ability of a material to perform with an appropriate host response in a specific application" [58] and the biodegradability as explained before that the biomaterials used for bone regeneration should be degraded by enzymatic or body fluids after a certain period [59]. Biodegradable biomaterials have various advantages in the tissue engineering process as they excrete out from the body naturally without producing any harmful effect, which facilitates the new tissue to grow freely and helps it to restore normal function. Besides this, biodegradable biomaterials also help prevent graft rejection due to immunogenic reactions and reduce the risk of device failure [60].

4.1. Types of biomaterials.

The foreign material implanted in the body is not completely compatible with the body's immune system. The host-tissue immune responses begin to start after the implantation of foreign material into the body, which leads to the rejection of the implant. To overcome this problem, the biomaterials are classified into three classes based on tissue response. They are following:

4.1.1. Bio-inert biomaterial.

There is no or minimal interaction of bio-inert biomaterial with the tissue of the implanting site to the implanted scaffold. The tissue of the implanting site makes a fibrous capsule around the implant to prevent the interaction with it. Example: titanium, alumina, stainless steel, ultra-high molecular weight polyethylene, and partially stabilized zirconia.

4.1.2. Bioactive biomaterial.

The bioactive biomaterial forms a surface layer of biological apatite on the implanting scaffold to prevent direct interaction with host tissue at an atomic level, resulting in chemical bonding between the implant and host tissue. Example: glass ceramics, hydroxyapatite, and bioactive glass.

4.1.3. Biodegradable biomaterials.

This type of biomaterial is designed to degrade after a certain period of time after implanting. The degradation of these biomaterials facilitates the provision of space for new tissue to grow without causing any harmful effects on the body. Example**:** tricalcium phosphate, polylactic acid, polyhydroxybutyrate, polylactic–polyglycolic acid copolymers[61,62].

Based on the source of isolation, biomaterials are also classified into different categories (Figure 5).

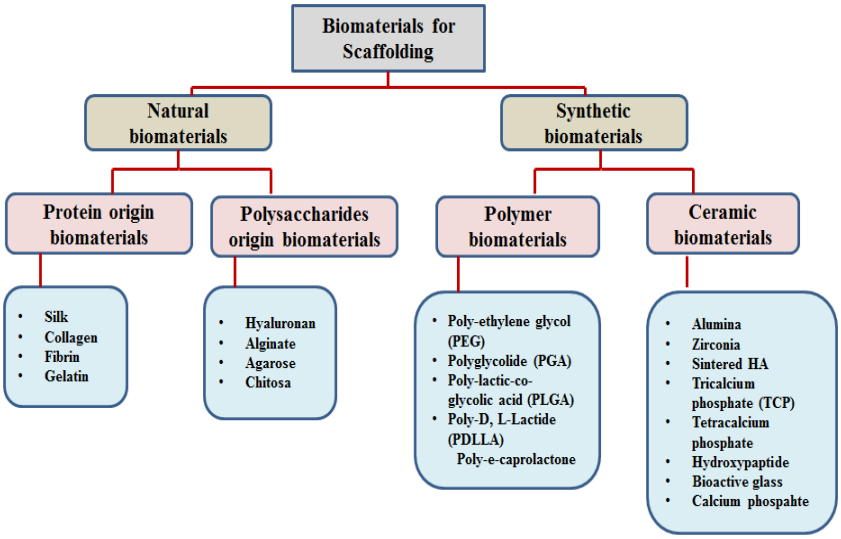

Figure 5. Classification of biomaterials used for scaffold fabrication.

4.2. Metals and alloys.

This type of biomaterial has various applications in tissue engineering. These are well known for their load-bearing capacity and are broadly used in artificial joints for hips, knees, ankles, and shoulders. Other than this, it is also used in fracture fixation as plates, screws, and pins. Other than these, metallic biomaterials have a number of applications, and they are widely used in maxillofacial surgery, cardiovascular surgery, and dental materials. Metals have a very good tensile strength due to the strong inter-atomic bonds. Metals deform under high load without any breakage in the structure. Metals have some limitations, such as living tissue, which has a low corrosion resistance due to the higher variation in physiology and mechanical properties. Commercially pure titanium alloys are widely used due to their higher strength and corrosion resistance. Titanium and titanium alloys, 316 and 316L stainless steel, and cobalt-chromium alloys are examples of widely used orthopedic materials [63]. The mechanical properties of metallic biomaterials in human cortical bone are mentioned in Table 2.

Table 2. Mechanical properties of metallic implants [63,64].

S.No.	Material	Modulus of elasticity (GPa)	Yield strength (MPa)	Tensile strength (MPa)
1	Cortical Bone	15-30	30-70	70-150
2	Stainless steel	190	221-1213	586-1351
3	Co-Cr alloy	210	448-1606	655-1896
4	Titanium (Ti)	110	485	760
5	Ti alloy	116	896-1034	965-1103

4.3. Bioceramics.

These are non-metallic, inorganic solid materials containing crystalline ceramics and amorphous glass compounds. Bioceramics have great applications in either dense or porous forms in maxillofacial prosthetics, dental implants, orthopedic coatings, bone fillings, and bone scaffold fabrication. Physically, bioceramics are hard and stiff, and they have a very biocompatibility with the human body. Their bioceramics are also brittle in nature and weak in tension. Based on the host immune response, bioceramics are also categorized into three types: (i) Bio-inert, (ii) Bioactive, and (iii) Biodegradable [65,66].

4.3.1. Bio-inert bioceramics.

This class of bioceramics has high chemical stability with high mechanical strength. The tissue of the implanting site forms a non-adhesive fibrous capsule around the implant to prevent interaction with the implant [67]. They have a high hardness and good corrosion resistance. Examples of bio-inert bioceramics are alumina, pyrolytic carbon, and zirconia [63].

4.3.2. Bioactive bioceramics.

The bioactive bioceramics form bone-like hydroxyapatite layers on the surface of implant material, which prevent the direct interaction between the implant and host tissue at the atomic level and lead to the establishment of chemical bonding between the implant and host tissue. Hench et al. reported direct bone bonding with silicate-based implants in 1970. After that, other glasses, ceramics, and glass-ceramics were reported to have bone-bonding capabilities [65,66,68,69].

4.3.3. Biodegradable biomaterials.

This type of bioceramics is designed to degrade after a certain period of time after implant. The degradation of these bioceramics enables the formation of a conducive environment for new tissue ingrowth without eliciting harmful effects on the body. Tricalcium phosphate is a widely utilized biodegradable bioceramic in biomedical applications [61,62].

Commonly used bioceramics are discussed below:

4.3.3.1. Bioactive glass.

Bioactive glass is amorphous, having a random arrangement of atoms. Generally, SiO_2, Na_2O, CaO, and P_2O_5 are the basic components of bioactive glass. The bioactivity, osteoconductivity, and biodegradability of bioactive glass depend on its composition. For example, the silica percentage ranged from 42-53%, showing fast bonding to the bone, while it took 2-4 weeks when the silica percentage ranged from 54-60%, and there was no bonding occurred when the silica percentage was more than 60% in bioactive glass [70]. A specific composition of bioactive glass (45S5 bioglass) represented the osteoconductive and

osteoinductive properties after the implantation [71]. Several bioactive glasses have been invented in the last three decades; some of them are silicate-based, phosphate-based, and borate-based, and they have been used as bone scaffolds for the replacement of middle ear and tooth roots. The load-bearing capacity of these scaffolds was low due to their amorphous structure; hence, they had limited applications in bone tissue engineering [72]. The bonding of bioactive glasses with bone on the material surface is established through a sequence of reactions; cellular reactions occur for further growth. For instance, the dissolution of the glass network, precipitation, and growth of a calcium-deficient carbonate apatite occur after the ion exchange on the surface of implanted material. The above reactions lead to the occurrence of biochemical adsorption of growth factors, and the osteogenic precursor leads to the formation of osteoblasts. Then cellular reactions result in quick new bone formation [65,68,70,73]. Glass ceramics have been developed to overcome the limitations of bioactive glass as they have stronger mechanical stability and bioactivity than bioactive glass. These are crystallized glasses containing a crystalline phase having crystal sizes ranging from 0.1 to 10μm and a residual glassy phase. The glass-ceramics are prepared by applying heat treatment in which a base glass is converted into a mixture of the glass-crystal mixture by the induction of controlled crystallization. The purpose of heat treatment is to stimulate the nucleation and growth of different kinds of fine-grain-sized crystalline phases. This crystalline phase has a combination of special properties like bioactivity, machinability, and mechanical properties that are better than bioactive glass [30,65,70,74].

4.3.3.2. Calcium phosphate.

Calcium phosphate is another class of bioceramics that is a main constituent of bone and teeth. Researchers have tried to make calcium phosphate bioceramics scaffolds for tissue engineering because they have a major role in our daily lives. Today, many calcium phosphate-based tools are used for bone replacement (Table 3). Hydroxyapatite, tricalcium, phosphate, and biphasic calcium phosphates are widely used. Based on the application, these phosphate-based bioceramics can be prepared in porous, dense powders, scaffolds, or in granule form [65,75,76].

Table 3. List of calcium phosphate compounds with Ca/P molar ratio.

S.No.	Compounds	Ca/P molar ratio	Solubility at 25°C, -log Ks	Chemical Formula	Typical abbreviations
1	Dicalcium phosphate (montite)	1	6.9	CaHPO4	DCPA or DCP
2	α-Tricalcium phosphate	1.5	25.5	α -Ca3(PO4)2	α- TCP
3	β-Tricalcium phosphate	1.5	28.9	β -Ca3(PO4)2	β- TCP
4	Monocalcium phosphate monohydrate	0.5	1.14	Ca(H2PO4)2.H2O	MCPM
5	Tetracalcium phosphate, mineral hilgenstockite	2	38-44	Ca4(PO4)2O	TTCP or TetCP
6	Fluorapatite	1.67	120	Ca10(PO4)6F2	FA or FAp
7	Calcium-deficient hydoxyapatite	1.5-1.67	~85	Ca10-x(HPO4)X(PO4)6-X(OH)2-X(0< X <1)	CDHA or Ca-def HA
8	Hydroxyapatite	1.67	116.8	Ca10(PO4)6(OH)2	HA or Hap or OHAp
9	Oxyapatite	1.67	~69	Ca10(PO4)6O2	OA, OAp or OXA
10	Dicalcium phosphate dihydrate (brushite)	1	6.59	CaHPO4.2H2O	DCPD
11	Monocalcium phosphate anhydrous	0.5	1.14	Ca(H2PO4)2	MCPA or MCP
12	Octacalcium phosphate	1.33	96.6	Ca8(HPO4)2(PO4)4.5H2O	OCP

Due to the similarity in chemical and crystal configuration of calcium phosphate to bone, it has excellent biocompatibility with the human body. The calcium phosphates exhibit osteoconductive activity, whereas some calcium phosphates, such as porous synthetic and coralline [75,77,78]. The hypothesis behind the bioactivity of calcium phosphates and the formation of new bone on their surface is directly related to the release of ionic and partial dissolution of their biological product; this leads to an increase in the local concentration of calcium and phosphate ions, which helps to form the precipitation of apatite microcrystals on the surface of the scaffold. Other ions like carbonate and magnesium are also combined to apatite microcrystals released from biological fluids, and the micro-environmental growth factors and proteins also combine to precipitate apatite, which promotes cell attachment and functions [75,79]. The chemical composition of calcium phosphates is a very important parameter for its dissolution rate, and the stability of this compound is based on the ratio of Ca/P. The ratio of Ca/P below one shows higher solubility; hence, it is not acceptable for a biological implant. Other parameters such as basicity, acidity, porosity, particle size, local acidity, temperature, pH of media, and solubility also affect the dissolution rate of calcium phosphate compounds. Not only the resorbability but the mechanical properties and bone growth are also influenced by the porosity of calcium phosphates. For the improvement in cellular adhesion, proliferation, and differentiation, the calcium phosphates are used in different-sized porosities such as macro- (>100μm), micro- (<10μm), and nano- (<100nm). Various studies prove that an increment in the surface area and pore volume of calcium phosphates enhances the biological apatite accumulation, resulting in the rapid formation of bone. The active resorption through the cellular activity of macrophages and osteoclast leads to the biodegradation of calcium phosphates, also called “cell eating”. The natural structure of calcium phosphates is brittle and has low load-bearing capacity, but the compressive strength is better than natural bone. These compounds are commonly used in middle ear surgery fillings of bone defects in the oral cavity. Also, they can be used as a dental and orthopedic implant coating due to their brittle nature and lower load-bearing capacity. An increase in the amorphous phase, grain size, and microporosity lead to a decrease in the mechanical properties of calcium phosphates, while higher crystallinity, low porosity, and small porosity result in enhanced compressive and tensile strength and higher stiffness with higher fracture toughness [80]. Thus, the chemical composition, crystallinity, pore size, grain size, and shape directly influence the mechanical properties of calcium phosphates. [30,66,69,75,79,81]. The mechanical properties of the common ceramic biomaterials are listed in Table 4.

Table 4. The mechanical properties of the common ceramic biomaterials.

S.No.	Material	Young’s modulus (GPa)	Compressive strength (MPa)	Tensile strength (MPa)
1	Alumina	380	4500	350
2	Zirconia	150-200	2000	200-500
3	Pyrolytic carbon	18-28	517	280-560
4	Bioglass-ceramics	22	500	56-83
5	Calcium Phosphate	40-117	510-896	69-193

Commonly used calcium phosphates are explained below.

4.3.3.2.1. Tricalcium phosphate (TCP).

The chemical formula of TCP is $Ca_3(PO_4)$. It is a biodegradable bioceramic with a 1.5Ca/P ratio. Three types of polymorphs exist in TCP, including β-TCP, α-TCP, and α`-TCP. The first is β-TCP, which is stable at low temperatures (<1125°C), whereas the stability of α-

TCP is between 1125-1430°C and the third is α`-TCP, which is stable at a very high temperature (>1430°C)[79,82]. α`-TCP is not of reasonable enthusiasm since it just exists at high temperatures and returns momentarily to α-TCP after cooling underneath the changing temperature. β-TCP is stable at room temperature and is converted into α-TCP at ~1125°C which can be held during cooling to room temperature; that's why three α-TCP and β-TCP have large applications for clinical purposes like; both are widely used in dentistry maxillo-facial surgery and orthopedics [83–85]. Although both have the same chemical composition, there is a higher difference in the solubility due to the crystal structure and specific energy. The solubility of α-TCP and β-TCP is 2.5mg/L and 0.5mg/L 0.5mg/L at 25°C. Due to the higher solubility of α-TCP, it is not suitable for biological implants but can be used as a component for bone cement [79,84]. After the implantation in the human body, TCP reacts with human body fluids, resulting in HA formation on an uncovered surface. This interaction is justified by the below equation [85–87].

$$4Ca_3(PO_4)_2\ (surface) + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2\ (surface) + 2Ca^{2+} + 2HPO_4^{2-}$$

There are a number of methods to synthesize β-TCP, each of them having different advantages and disadvantages. Wet chemical co-precipitation is the most used method for the preparation of β-TCP. The reason behind the popularity of this method is its simplicity, low operational temperature, and purity of synthesized compound at a low cost. There is a higher chance of undesirable impurities if the reaction is incomplete. Other than this, sol-gel and solution combustion methods also used for β-TCP synthesis in the sol-gel method have the advantages over co-precipitation due to the homogenous molecular mixing and also have the ability to form nano-sized particles. In contrast, the solution combustion method is a fast exothermic and energy-saving process. In this process, a self-sustaining chemical reaction occurs between an oxidant and suitable organic fuel in an aqueous solution. The particle size, crystal size, densification behavior, and substance morphology vary with different synthesis techniques [88–90]. As previously explained, the tensile strength of calcium phosphates is low and brittle, hence providing limited biomechanical support. However, these are less brittle than hydroxyapatite. The fast degradation rate of TCPs leads to a faster loss in mechanical strength with time [69,91].

4.3.3.2.2. Hydroxyapatite (HA).

This is another example of calcium phosphates, and after the fluorapatite (FA), it is the most stable compound with the least solubility. The chemical formula of HA is $Ca_5(PO_4)_3(OH)$. Still, it is commonly written as $Ca_{10}(PO_4)_6(OH)_2$ to indicate that the hexagonal unit of HA is composed of two molecules, and the ratio between Ca/P is 1.67 [79,92]. Although the stoichiometric of HA is not present in the human biological system, it is widely used in human bone grafts or as a coating material for orthopedic devices as; well it is also used in dental implants due to the similarity of structure with natural bone material [71]. Various ions can undergo cationic and anionic substitutions within the crystal structure, replacing Ca^{2+}, PO_4^{3-}, and OH^- hydroxyapatite (HA) groups. This substitution process changes HA crystals' morphology, crystallinity, thermal stability, and solubility. Furthermore, these reactions significantly impact the response of osteoblasts and osteoclasts, as well as bone degradation and regeneration in laboratory settings and within living organisms [70,93]. Bone contains carbonate substituted apatite, which is abundant with carbonate ranging from 3-8%. So, the

Ca/P ratio is below 1.67, which makes it more soluble than pure HA [65,70,94]. The percentage composition of human enamel, bone, and pure HA is mentioned in Table 5. Mechanical properties are also important parameters that depend on particle size, physical form, synthesis technique, and the presence of a non-HA phase. The mechanical properties of HA are summarized in Table 6.

Table 5. Composition percentage of human enamel, bone and pure HA ceramic [79,95].

S.No.	Constituents (weight %)	Stoichiometric HA	Enamel	Bone
1	Potassium	-	0.08	0.03
2	Ca/P molar ratio	1.67	1.62	1.71
3	Magnesium	-	0.44	0.55
4	Fluoride	-	0.01	0.02
5	Calcium	39.6	36	34.8
6	Phosphorous	18.5	17.7	15.2
7	Carbonate	-	3.2	5.8
8	Chloride	-	0.3	0.1
9	Ash (total inorganic)	100%	97%	65%
10	sodium	-	0.5	0.7

Table 6. Mechanical properties of HA bioceramics[79,96].

S.No.	Parameter	Property	Value
1	Bending strength	Dense HA	38-250 MPa
		Porous HA	2-11 MPa
2	Tensile strength	Dense HA	38-300 MPa
		Porous HA	~ 3 MPa
3	Vickers hardness	Dense HA	3-7 GPa
4	Compressive strength	Dense HA	120-900 MPa
		Porous HA	2-100 MPa
5	Young' modulus	Dense HA	35-120 GPa
6	Poisson' ration	Synthetic HA (bone ~0.3)	0.27
7	Fracture toughness	Decreased almost linearly with porosity	0.7-1.2 MPavm

4.3.3.2.3. Mesoporous bioactive glass (MBG).

MBG is a new bioactive material that has gained popularity in the recent few years due to its advantages over non-mesoporous bioactive glass (NBG). MBG shows improved surface area and nanopore volume, increasing bioactivity and biodegradability. *In vitro* studies proved an improvement in the degradation, bioactivity, and drug delivery properties of MBG compared to NBG concerning bone regeneration and drug delivery [97]. Zhao and coworkers synthesized MBG using the sol-gel technique, which has shown pluripotency due to its multiple applications in soft and hard tissue engineering [98,99]. MBG has served as an efficient vector for anti-osteoporotic, anti-inflammatory, antibacterial, and anticancerous drugs. It also serves other therapeutic molecules like growth factors, genes, and apatite [100–102]. Bioactive glasses also have multiple applications in tissue engineering, but their clinical uses are restricted due to their limited preparation methods. There are various methods available to synthesize MBG, ranging from the sol-gel process and evaporation-induced self-assembly to the bioinspired route, based on the application [98,103,104]. The bioinspired approach is a new procedure to synthesize MGB, which is superior to previous techniques as this procedure works at ambient temperature and pressure rather than higher temperature; thus, it is an eco-friendly and cost-effective procedure. Various factors, such as pore size, pore volume, and surface area,

affect the performance of implants' mechanical properties, cell adhesion properties, cell differentiation, proliferation, and therapeutic delivery system for healthy bone growth [100,101]. The characteristics of an ideal bioglass are not limited to its bone regeneration properties; it should also extend its functionalities to the goodness of bone growth and health. Achieving these characteristics, bioactivity, and multi-functionality on the microstructure level is a big challenge. A few studies by researchers have shown that highly ordered micro-structured material can help achieve these characteristics. The surface characteristics of scaffolds also have a crucial role in loading biological cells and their corresponding interactions with biological systems.

4.3.3.2.4. Importance of porosity, specifically mesoporosity.

Pore size, pore diameter, pore volume, and surface area of bioglass play an important role in the texturing of it. The pore size of bioglass ranges from 2-50nm and is categorized as a mesoporous biomaterial. Zhao and coworkers in 2004 explained the mesoporosity of multicomponent bioglass in tissue engineering [98]. Several techniques for synthesizing MBG and its application have been developed up till now from the time it was first discovered. Research has shown that the difference in composition of MBG influences the mesoporosity, and the variation in the technique with the same composition also influences the porosity of bioglass. However, MBG always exhibits higher surface area and pore volume than NBG [105]. The pore volume and surface area affect the scaffold's cell-carrying capacity and biodegradation. For instance, larger pore volume and surface area carry a large amount of cells while reducing the degradation time of the scaffold with enhanced bioactive behavior and improved carbonated hydroxyapatite formation. The texture of the bioglass also depends upon the concentration of network modifiers. For example, the increased concentration of network modifier calcium oxide causes a decrease in the pH solution, resulting in the decrease of nuclei, ultimately causing form the smaller nanoparticles, and it also transforms the mesostructure into 2D-hexagonal from 3D-cubic due to the increment in the inorganic/organic volume ratio. The mesoporosity of bioglass is also influenced by controllable parameters like temperature, time, and type of reaction, as well as the template and dopants [106–111]. Other parameters also should be kept in mind before the start of the synthesis of bioglass as the bioactivity is a surface process and surface characteristics regulate all the responses of living tissue; however, the shape and size of bioglass are influenced by the interaction with simulated body fluid for bioactivity evaluation studied by Zhu et al. in 2008. Thus, surface functionalization influences the texture of the biological implant [112].

4.4. Polymers.

These are organic macromolecules composed of monomers. A number of monomers join with the help of covalent bonds and form a large macromolecule called “mers”. These macromolecules join together with the help of hydrogen bonds and Van der Waals bonds, forming an entanglement structure called “polymer” [113]. Polymer possesses low thermal and electric properties due to the covalent bonding between molecules. However, several factors play a crucial role in affecting their thermal and mechanical properties, including the composition of the primary structure, side chain functional group, the structure of the molecule, and the molecular weight of the polymer. Polymers are popular biomaterials due to their large applications in biomedical science, including contact lenses, dental implants and fillings, drug

delivery, tissue engineering, and surgical devices. It is easy to manufacture polymers in the desired shape and size compared to ceramics and metals. Varieties of polymers in terms of physical and chemical properties are available with good biocompatibility and flexibility [114,115]. Based on the degradability, polymers are categorized into two classes:

4.4.1. No-degradable polymers.

These types of polymers have various advantages such as stability, durability, and biocompatibility and also have good mechanical properties. Such advantages allow these polymers to be used in various clinical purposes, including dental fillings, heart valves, ocular lenses, and drug delivery systems. Examples**:** Poly(ethylene) (PE), poly(propylene) (PP), poly(sulfone) (PS), poly(tetrafluoroethylene) (PTFE), poly(methyl methacrylate) (PMMA), poly(dimethylsiloxane) (PDMS), poly(ethylene terphthlate) (PET) and polyurethanes [116].

4.4.2. Degradable polymers.

These polymers degrade inside the body enzymatically or hydrolytically without producing any toxic effect on the body. Degradable polymers are also divided into two parts: natural and synthetic polymers [117].

Generally, natural polymers are bioactive and degrade enzymatically inside the body, so the *in vivo* degradation of these polymers depends on the site of implantation and the concentration of the degradation enzyme at the implantation site. The degradation rate of polymers can be altered by chemical modification. Bioactivity, the ability to present receptor-binding ligands to cells, susceptibility to cell-triggered proteolytic degradation, and natural remodeling are the advantages of natural polymers. In contrast, they have some disadvantages also, like possible immunogenicity due to their bioactivity, structure complexity as well as poorer biomechanical properties [118]. Examples of natural polymers: Polysaccharide-based polymers (Chitosan, cellulose, alginic acid, and hyaluronic acid) and Protein-based (Collagen, gelatin, silk, elastin, fibrin, albumin, and keratin) [119,120].

On the other hand, synthetic polymers are bioinert and have more anticipated properties. These types of polymers show better batch-to-batch uniformity than natural polymers. The degradation of these polymers occurs via a hydrolytic degradation process, and the behavior can be changed according to the application of implants. Natural polymers exhibit lower site-to-site and human-to-human variations than enzymatically degradable polymers. However, some synthetic polymers can also be degraded through enzymatic degradation. Ese polymers are commonly used in soft and hard tissue engineering for scaffold fabrication, drug delivery systems, and gene therapy. Biodegradable polymers with their application in clinical applications are enlisted in Table 7 [119–122]. The main properties of natural and synthetic biodegradable polymers are defined in Figure 6.

Table 7. Biodegradable polymers with their research applications.

Polymers	Applications	Reference
Synthetic degradable polyesters		
Polycaprolactone (PCL)	Long-term drug administration, implantable devices for contraception drugs, medical applications in orthopedics, fasteners, and medical devices for maintaining vessel or organ patency	[123]
Poly(hydroxybutyrate) (PHB), poly(hydroxyvalerate) (PHV) and copolymers	Long-lasting drug administration, medical uses in orthopedics, devices for maintaining vessel or organ patency, synthetic skin, materials for patching congenital heart defects during surgery, and stitching materials.	[124]
Polydioxanone (PDS)	Fixing fractures in bones that don't bear weight, stitching materials, and clips for closing wounds	[125]

Polymers	Applications	Reference
Poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and copolymers	Barrier membranes for controlled substance release, delivery of hormones, promoting tissue regeneration in dental applications, medical uses in orthopedics, stents for vascular and urological purposes, fasteners like staples and sutures, injectable substances for filling, substitutes for dura mater, materials for replacing skin, and advancements in tissue engineering	[126,127]
Natural degradable polymers		
Elastin	Drugs can be administered through a delivery system and a coating can be applied to vascular grafts.	[128]
Collagen	Administering drugs and genes through delivery mechanisms, developing synthetic skin, applying coatings to enhance cellular adhesion, promoting tissue regeneration in dental procedures, repairing spinal dural defects, medical uses in orthopedics, enhancing soft tissue volume, advancing tissue engineering, constructing scaffolds for reconstructing blood vessels, closing wounds, and developing agents to promote hemostasis	[128]
Hyaluronic acid	Utilizing dressings for wound treatment, delivering drugs, advancing tissue engineering, creating artificial bone grafts, and developing substitutes for synovial fluid	[129]
Fibrinogen and fibrin	Cell delivery and tissue Sealing	[130]
Gelatin	Coating capsules for delivering drugs orally and developing a substance to stop bleeding	[131]
Polysaccharides such as chitosan, alginate	Delivering drugs and vaccines, encapsulating cells, using stitching materials, and promoting wound healing through dressings	[132]
Other Synthetic Degradable Polymers		
Poly(amino acid)s and “pseudo”-poly(amino acid)s	Administering drugs, advancing tissue engineering, utilizing medical applications in orthopedics, developing stents, and creating barriers to prevent adhesion.	[133]
Polyphosphazenes	Blood contacting devices, drug delivery, skeletal reconstruction, vaccine adjuvants	[134]
Polyanhydrides	Drug delivery	[135]
Poly(ortho ester) (POE)	Drug delivery and stents	[136]
Poly(propylene fumarate) (PPF)	Orthopaedic applications	[137]

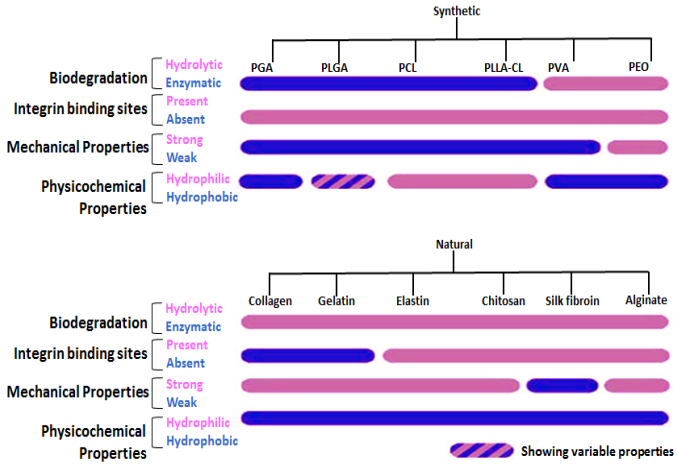

Figure 6. Commonly used biodegradable polymers, comparing the activity levels of different properties [138].

5. Cells for bone tissue engineering

The main objective of bone tissue engineering is to deliver the stem cells/progenitor cells to the skeletal system to repair, replace, or regenerate the damaged tissue or complete organ. However, the material used for the scaffold plays a crucial role in the quality of the implant and in retaining the cells in it. Cells play the most important role in bone regeneration; hence, a reliable cell source is the primary requirement in tissue engineering [139]. The autologous osteoblastic cells were isolated from the same patient and had to be implanted later. It is the first choice for tissue engineering due to their low risk of immunogenic reactions.

However, it has some restrictions, such as time consumption and the number of cells isolation after the tissue dissociations. Besides this, a low expansion rate is also usually seen after isolating autologous cells, which influences the scaffold's seeding after new cells are available [140]. Another source of cells also can be used to solve this problem. Xenogeneic cells can also be a good choice to fulfill the demand for low cell availability. These cells are isolated from a non-human species; hence, there are more chances for immunogenic reactions. There is also a high risk of infection transmission as xenogeneic cells are isolated from non-human species. The ethical and social issues also are there that reduce the enthusiasm for this methodology [41]. The above-mentioned disadvantages can be overcome by using stem cells. The self-renewal ability, high proliferation ability, and multilineage differentiation of stem cells result in tissue regeneration, which makes it the most preferred choice for tissue engineering [41]. These cells also regulate bone remodeling by maintaining the osteoblast-osteoclast ratio. Various types of stem cells exhibit distinct differences in their capabilities for bone repair and regeneration. These included adult stem cells, embryonic stem cells, induced pluripotent stem cells, human exfoliated deciduous teeth stem cells, and umbilical cord blood mesenchymal stem cells. For the vascularization of the implants, the adipose-derived stromal vascular fraction is an efficient, abundant source that plays a crucial rolein tissue regeneration [141]. The types of stem cells for tissue engineering applications, as well as their advantages and disadvantages, are listed in Table 8.

Table 8. Sources of stem cells for bone tissue engineering [141].

Cell Source	Advantages	Disadvantages	Reference
Umbilical cord blood mesenchymal stem cells (UCB-MSCs)	(i) High availability (ii) Broad differentiation and proliferation potential (iii) Higher in vivo safety than embryonic stem cell	(i) More difficult to isolate than MSCs from the marrow (ii) More studies are needed to test their use in bone repair	[142]
Induced pluripotent stem cells (iPSCs)	(i) Pluripotency (ii) Capable of differentiating into all bone cell types	(i) Reprogramming efficiency is low (ii) Require extensive expansion (iii) Safety concerns; limited clinical application	[143]
Bone marrow-derived mesenchymal stem cells (BM-MSCs	(i) Has demonstrated a strong capacity for osteogenesis (ii) Has been extensively researched and investigated	(i) Limited availability necessitates extensive in vitro amplification.	[144]
Adipose-derived stem cells (ASCs)	(i) Exhibits comparable osteogenic traits to that of BM-MSCs (ii) Readily available in large quantities and can be easily obtained through surgical procedures	(i) Further investigations are required to evaluate their potential for bone repair applications	[145]
Adipose-derived stromal vascular fraction (SVF)	(i) Plentiful in supply and can be easily collected through the liposuction procedure (ii) Capable of generating vascularized bone structures	(i) The composition of cell populations differs among donors. (ii) A multistep isolation process that typically takes around two to three hours	[145]
Embryonic stem cells (ESCs)	(i) Possessing pluripotent characteristics. (ii) Exhibits the ability to differentiate into all cell types found in bone tissue	(i) Limitations imposed by ethical and regulatory considerations. (ii) Has the potential to generate teratomas upon *in vivo* transplantation	[146]

The normal healing process is discussed earlier, but if we talk about the regenerative healing process, it is something different from that one. The mesenchymal stem cells migrate toward the injury site with the bloodstream to participate in the regeneration process in the natural healing process of tissue regeneration. The combination of growth factors and cytokines

initiates this stem cell homing and mobilization process by providing the signal gradient to bone marrow stem cells through activated platelets and vascular endothelium. The term "stem cell homing" is related to organizing the stem cell population to the injured site [147]. Two main approaches are widely used for tissue engineering to attain the desired cell homing at the defect site. (i) Cell-based approach (ii) scaffold-based approach

In a Cell-based approach, cells are engineered in such a manner that a marker guides the cells to the regeneration site, whereas the chemokines and growth factors are released from the implanted scaffold in a scaffold-based approach, which is responsible for mesenchymal stem cell homing. Both approaches are widely used and may be important for bone regeneration [7].

6. Growth factors

These are the family of protein molecules that control cellular growth, cellular differentiation, cellular migration, cellular metabolism, and cellular apoptosis. Growth factors play a vital role in tissue regeneration to accelerate the functional restoration of damaged tissue as they transmit the signals between the cells and transfer information between cells and their micro-environment [148]. There are a number of growth factors available for tissue engineering purposes (Table 9) like bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF),), insulin-like growth factors I and II (IGF I and IGF II), and transforming growth factor beta (TGFβ). Among those, BMPs are of great interest due to their induction of mitogenesis of mesenchymal stem cells and another osteoprogenitor, as well as their differentiations in osteoblastic cells. More than 15 types of BMPs have been identified so far in the human body, but among those, BMP-2 has been found to be the most effective inducer of bone formation [39].

Table 9. Different types of growth factors useful in bone tissue engineering.

Type	Advantage	Disadvantage	Reference
Insulin-like growth factor (IGF)	(i) Participates in the process of adult neovascularization.		[149]
Transforming growth factor-β (TGF-β)	(i) Synthesis of the extracellular matrix (ECM)		[150]
Bone morphogenic protein (BMP-2)	(i) Stimulates the proliferation of osteoblasts and promotes the differentiation of mesenchymal cells (MSCs). (ii) Triggers the secretion of VEGF-A, thereby playing a role in promoting angiogenesis	(i) Controlled delivery is required to achieve the desired effects. (ii) Human studies have shown diverse outcomes and varying responses (ii) Exhibits limited ability to initiate vascular proliferation	[151]
Fibroblast growth factor (FGF)	(i) Participates in the process of neovascularization, contributing to the formation of new capillaries		[152]
Vascular endothelial growth factor (VEGF)	(i) Stimulates the mitogenesis of endothelial cells (ii) Attracts mesenchymal stem cells (MSCs) and induces their differentiation		[153]
Platelet-derived growth factor (PDGF)	(i) Attracts cells involved in stabilizing the developing vasculature (ii) Attracts and mobilizes mesenchymal stem cells (MSCs) to the target site (iii) Increases the production of VEGF (vascular endothelial growth factor)	(i) When delivered independently, they result in a lack of organized bone regeneration	[154]

The main purpose of introducing growth factors in bone tissue scaffold is to increase the healing mechanism and fasten regeneration. Even though due to their half-life, they require a high dose and also diffuse other tissues, which results in the treatment being expensive and unsafe. They also have demerits after implantations, such as ectopic bone formation and swelling of the implantation site, and also have neurological side effects after spinal fusion applications of BMPs. To overcome these problems, researchers are continuously working towards developing new techniques for formulating growth factors and improving their delivery to make them available for skeletal defects [155]. There are two main approaches generally used for the fabrication of growth factors-loaded scaffold. The first is the chemical immobilization of growth factors onto the matrix, and the second is the physical encapsulation of growth factors in the delivery system. In the first approach, the growth factors bound with the polymer either covalently or through biomimetic interactions. This binding makes the matrix control and regulate the loading, stability, distribution, and delivery of growth factors. This method facilitates the sustained release of growth factors and minimizes burst release. Some precautions should be taken to design the linkage strategies to preserve their bioactivity [156]. The second one is the physical encapsulation of growth factors. This is the simplest method for the delivery of growth factors that involves physically incorporating blended growth factors directly into the scaffold. Initially, the burst release of proteins or DNA occurs and then controlled diffusion and degradation of the scaffold results the slower release of these molecules. The release of these compounds depend on the scaffold properties including porosity, rigidity, hydrophobicity, hydrophilicity, charge density and swelling behavior of scaffold. The 3D polymeric and composite scaffold is attracting the attention to finding the hybrid approach for bone tissue engineering to facilitate the multi-agent delivery [157].

7. Techniques for scaffold fabrication

In the body, cells and tissue are organized into three-dimensional architecture. Thus, to engineer these functional tissues and organs, scaffolds have to be fabricated into three-dimensional space that mimics the architecture of the native extracellular matrix to facilitate cell distribution and guide their growth for the regeneration of new tissue [158]. Over the years, various methods to design and fabricate 3D biomimetic scaffolds have been developed for tissue engineering and regenerative medicine, and choosing the appropriate technique depends on several factors, including the required shape and properties of the scaffold, types of materials used in the scaffold, shape, and size of pores as well as their interconnectivity, and the distribution of the materials in the scaffold [159]. Various techniques for scaffold fabrication are mentioned here.

7.1. Conventional methods.

Conventional tissue engineering methods utilize scaffolds made from biocompatible materials to facilitate tissue growth and regeneration. These scaffolds provide a framework for cells to adhere and organize, forming functional tissue. However, there are certain drawbacks associated with this approach. One limitation is the difficulty in precisely controlling scaffold degradation, which can impede tissue integration and remodeling. Another challenge lies in the static mechanical properties of conventional scaffolds, which fail to mimic the dynamic mechanical conditions of natural tissues. Additionally, incorporating bioactive signals into the

scaffold matrix can be problematic, restricting their ability to promote cell behavior and tissue regeneration effectively [160].

7.1.1. Solvent casting.

It is a very simple and inexpensive technique that does not require big equipment. This technique involves the evaporation of solvent to form a scaffold. Generally, two methods are used to fabricate scaffold, briefly discussed here. The mold is dipped into a polymeric solution for a period of time until the mold draws off the solution, forming a polymeric layer [161]. Another method is opposite to the first one, in which the polymeric solution is filled into a mold and set aside for a period to evaporate the solvent. After evaporation, a polymeric scaffold is obtained [162]. However, this technique has a big disadvantage: the denaturation of proteins by toxic solvents can also affect other solvents. There is a high risk of retaining the toxicity by scaffold. This problem can be overcome with the help of a vacuum drying process in which toxic solvents are removed, but this is very time-consuming. Researchers combined this technique with particulate leaching to overcome the problems generally faced in scaffold fabrication using the solvent casting method [163,164]. The solvent casting process is shown in Figure 7.

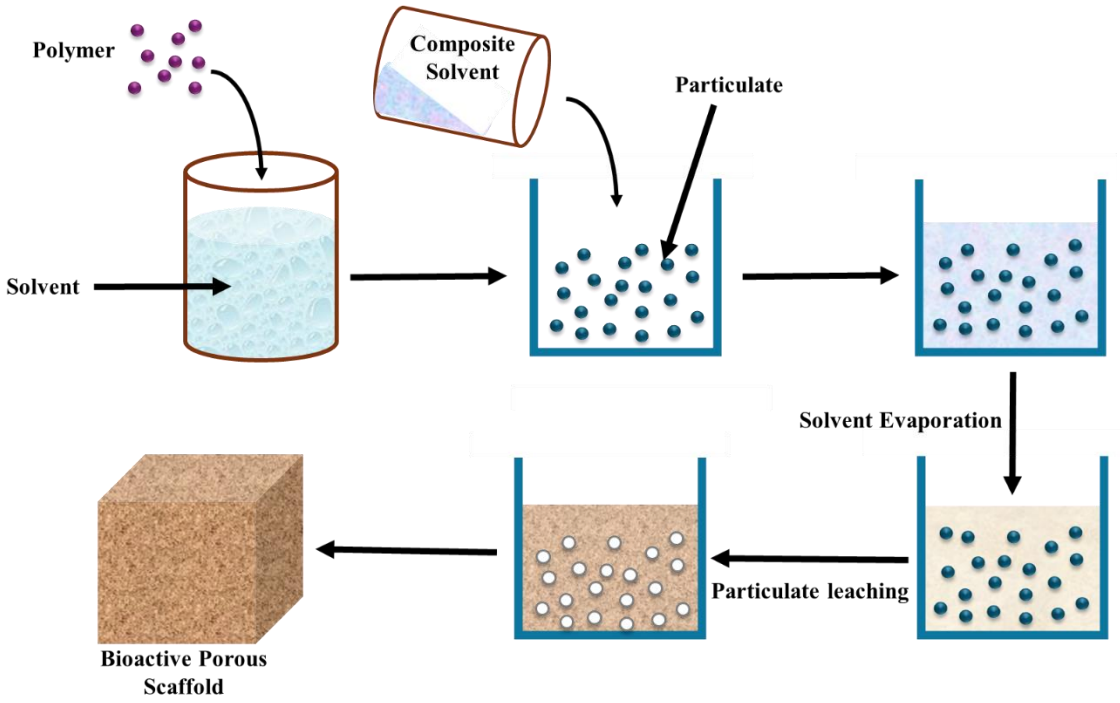

Figure 7. Solvent casting process of scaffold fabrication

7.1.2. Particulate-leaching techniques.

This is a very popular and widely used technique for scaffold fabrication in tissue engineering [165]. The pores or channels are created by salt, wax, or sugar, which is usually known as porogen. One of these compounds is grounded into small particles, and the desired-sized particles are poured into the mold. Porogen-filled mold is cast with a polymeric solution and evaporation starts. The porogen crystals leached out with the water and formed a porous scaffold [166–168]. This is a very easy process to obtain the desired pore size scaffold in which pore size can be controlled by controlling the porogen amount, porogen crystal size, and shape [169]. The pore size of the scaffold developed using the particulate technique is usually around 500µm, and porosity ranges from 94-95% with desired crystallinity [170,171]. The requirement

of less polymer for the fabrication of scaffold is the main advantage of this technique. However, it also has some disadvantages, such as this technique does not control pore shape and inter-pore openings [172]. New techniques are being developed to overcome these drawbacks.

7.1.3. Gas foaming.

Organic solvents and high temperatures are the basic requirements for scaffold fabrication using many techniques. The remaining residue after the completion of the process may harm the tissue and nearby cells by its toxicity. There are high risks of denaturing the active biomolecules within the scaffold. These problems can be avoided using gas foaming techniques for scaffold fabrication as there is no need for organic solvents and high temperatures in this technique [173].

A highly porous scaffold is prepared using high-pressure carbon dioxide gas using the gas foaming technique. A highly porous polymer is exposed with high pressure (800psi) carbon dioxide gas to make it saturated with gas. The phase separation occurs between the carbon dioxide and polymer molecules due to the instability of carbon dioxide gas. Cluster formation of carbon dioxide molecules occurs to minimize the free energy, resulting in the creation of pores in the polymer. These pores help to expand the polymeric volume and decrease the polymeric density. After completion of the process, a -dimensional scaffold with a porous structure is obtained. The amount of carbon dioxide gas controls the scaffold's porosity and can be controlled using porogens such as sugar, salt, and wax [174]. During the foaming process, the expanded polymer fused around the porogen, producing a polymeric matrix and entrapping other molecules in the mixture. The porogen and polymer are exposed to the highly pressurized carbon dioxide gas until full saturation of the polymer with the gas. After this, the foaming process is removed, and a highly porous scaffold is obtained [175].

7.1.4. Phase separation.

This technique required a change in temperature for scaffold fabrication. The polymeric solution is separated into two phases as a result of a temperature change. The low polymeric concentration is called the polymer lean phase, while the highly polymeric concentration polymer is called the polymer-rich phase [176].

To initiate this process, the phenol is dissolved in phenol or naphthalene, and then the biologically active molecules are dispersed in this mixture of solutions. The liquid-liquid phase is separated by lowering the temperature. Upon quenching this phase-separated liquid, a two-phase solid is obtained. Then the solvent is removed by extraction, evaporation, and sublimation process to obtain a porous scaffold containing bioactive molecules [177,178]. This technique has advantages over other techniques as it can easily combine with other techniques, especially particulate leaching, to fabricate the desired pore-size scaffold. Nanofibrous scaffold can also be produced by combining this technique with the phase separation technique [179].

7.1.5. Electrospinning.

Electrostatic force plays a very important role in this technique for producing polymeric fibers from nano to micro scales. An electric field of high intensity is developed between two electrodes of opposite charge polarity to control the whole process. One of the electrodes is kept in the polymeric solution, while another is placed in the collector. A drop of polymeric solution is pumped out, and the electric field is applied to force it on droplets. Introducing force

leads to overcoming the surface tension of the droplet solution. A polymer jet is ejected, which forms the fibers, and at the same time, the solvent starts evaporating. This process continues until the nanofibers are deposited into the collector [180,181]. Widely available polymers like collagen, gelatin, silk fibroin, and chitosan are commonly used for electrospinning scaffolding technique.

Several polymers are available for electrospinning techniques, such as collagen, gelatin, silk fibroin, and chitosan [182]. This is a versatile procedure in terms of polymer use, non-invasive, and it does not require a high temperature for fiber production. This process involves the high voltage current to create an electrically charged jet of polymer solution, which produces polymer fibers after drying or solidifying this solution [183,184]. The main advantage of this method is that a scaffold of desired characteristics in terms of cell attachment and cell growth can be produced [185–187]. Ultra-fine fibers with a specific orientation, high aspect ratio, and high surface area can be formed by controlling pore geometry. These characteristic parameters directly affect cell adhesion, cell expression, and transportation of oxygen and nutrients to the cell, promoting better cellular growth *in vitro* and *in vivo*. An optimum environment is created by these fibers to perform appropriate physiological functions and better growth for new tissue [55]. The main disadvantage of this technique is the difficulty of cell seeding, but this problem can be overcome by using a sacrificial biopolymer or cryospinning. Cryospinning facilitates the creation of the desired-sized hole in electrospun matrices [187,188].

7.1.6. Fiber mesh.

This technique contains only one woven or interweaved fiber, with three-dimensional structures with variable pore sizes [189]. Polyglycolic acid (PGA) is the first biocompatible and biodegradable material that is used to spun into fiber. This fiber is used as a suture thread. A polymer solution is deposited on a nonwoven mesh of another polymer, and then the evaporation process occurs [174]. This technique facilitates the large surface area of the cells for attachment, and the rapid diffusion of nutrients occurs, which is beneficial for cell survival and cell growth [190]. The lack of structural stability is the considerable disadvantage of this technique. However, this problem can be reduced by hot drying poly-L-lactide fiber to the extent that it helps to improve the structural orientation and crystallinity [164].

7.1.7. Fiber bonding.

Mikos and his coworkers developed this technique to fabricate scaffolds [163]. To initiate the process, PLLA dissolved in chloroform, and after that, a non-woven mesh of PGA fiber was added. A composite material was obtained by removing solvent using an evaporation process. Non-bonded PGA fibers embedded in the PLLA matrix are seen in this composite [190]. A collagen matrix was attached to the PGA polymers with the help of collagen fiber stitches to obtain a scaffold [191]. The temperature above the melting temperature of PGA results in the fiber bonding during the process. When the composite is dissolved in methylene chloride, it is the PLLA matrix because PGA is insoluble in methylene chloride [178]. After this process, a PGA scaffold structure consisting of PGA fibers bonded with each other by heat treatment is obtained. PGA fibers are responsible for their high porosity and surface area to polymer mass ratio [192]. PGA fibers are also responsible for its better mechanical strength and tissue growth. The bigger surface area of the PGA scaffold is the main advantage of this

technique. Thus, this technique facilitates a large surface area, which is favorable for a large number of cell attachments and also provides enough space for extracellular matrix regeneration [193].

7.1.8. Self-assembly.

It is a spontaneous arrangement of biomolecules into a predefined ordered structure used to perform a specific function [194]. This technique is used to produce nano-sized fibers known as nanofibers. The three-dimensional nanofibrous scaffold is commonly synthesized by amphiphilic peptide sequence. Within these peptides, the hydrophilic and hydrophobic phases link together with the help of weak covalent bonds that produce hydrogels[193,194]. Other than this, the self-assembly of di-block polymer (AxBy) can also be used to prepare the nanofibers. The B domain of cylindrical shape with nanoscale diameter embedded in matrix A is obtained by controlling the volume of A and B. Nano-fibers can also be prepared by polymeric dendrimers[55]. The rod-shaped nano-fibers are self-assembled using di and tri-bock peptide ampholites (PAs). In this technique, the nano-fibers of PAs are prepared using self-assembly by controlling their pH and modifying the peptide head group of Pas [195]. This modification in the PAs structure facilitates the number of techniques to the self-assemblies, including layered and lamellar structures. The reversibility properties of these polymers are responsible for the system's flexibility. Thus, various scaffolds can be obtained by self-assembly for tissue engineering purposes. This technique has various advantages over the electrospinning technique, such as the nano-fibers prepared by self-assembly are thinner than electrospinning. The amino acid residue of these nano-fibers can be modified chemically by adding bioactive compounds. This technique is also free from the use of organic solvent, and the process occurs in aqueous salt solution or physiological media, hence showing reduced cytotoxicity. However, the process is very complicated and lengthy [196].

7.1.9. Melt molding.

This technique is intended to involve PLGA powder, which is filled into the Teflon mold with specific diametric gelatin microspheres. Then, the temperature above the PLGA transition is applied to heat the mold with a specific pressure on the mixture [197,198]. This process results in the attachment of PLGA particles together. After this process, the PLGA structure is removed from the mold and transferred into the water to dissolve the microsphere. Finally, the scaffold is dried to form a porous structure. The obtained shape of the scaffold is the same as the shape of the mold. HA, which can be modified by incorporating it with PLGA. This modification involves the uniform distribution of HA throughout the PLGA scaffold, combined with the solvent casting technique. This modification of the melt molding technique facilitates the preparation of the composite material of HA fiber, PLGA matrix, and gelatin, or sometimes, porogens are also used in the melt molding process to produce a porous scaffold [199].

7.1.10. Membrane lamination.

This is another solid, free-form technique used to prepare precise anatomical shape scaffold with 3D polymeric foam. This technique involves the solvent casting and particulate leaching technique, introducing peptides and proteins layer by layer. The specific shaped membranes are soaked with solvent and loaded with 3D assemblies having porous structures.

The generated scaffold shows the bulk properties, the same as the properties of a membrane soaked in solvent [200]. A defined anatomical-shaped scaffold of polymer foam with a 3D porous structure is formed, while computer-based modeling makes it possible to design the desired template according to the implant shape. The layering of porous sheets is a considerable drawback of this technique, which causes a reduction in the pore interconnectivity. This technique is also time-consuming because only thin fibers can be used to perform it [201,202].

7.1.11. Freeze drying.

This technique is also used to develop the porous scaffold for bone tissue engineering [203]. The basic principle used in this technique is sublimation. The process begins by dissolving the polymer in an appropriate solvent to make a solution with the desired concentration. The solvent is removed under a high vacuum using lyophilization to get a scaffold with high porosity and higher interconnectivity between the pores of the scaffold [204]. Various polymers can be used, such as silk proteins, PGA, PLGA, PLLA, PLGA/PPF, and many more, according to the desired requirement of the scaffold [168,205]. The pore size of the scaffold can be controlled by controlling the freezing rate as the higher freezing rate decreases the size of pores, and adjustment in the pH also influences the porosity [203]. In this technique, there is no requirement for high temperature, and separate leaching is also not required [206]. Smaller pore size and lengthy process are the main disadvantages of this technique [207].

7.2. Rapid prototyping (RP).

The term “rapid” indicates the fast manufacturing rate of three-dimensional scaffolds using a layer manufacturing process. This technique is free from solids and is totally controlled by a computer. This is a very advanced technique used for the fabrication of scaffolds. The scaffold model is designed using computer-aided design (CAD), and then a series of cross-sections is expressed [208]. RP machine prepares a scaffold by laying down a layer corresponding to the cross-section from the bottom to the top. For instance, the defective bone image is taken, and then the 3D CAD model is designed by computer. After this, the images are divided into layers, and then 3D objects are generated layer by layer using RP techniques. RP techniques involve selective laser sintering (SLS), fused deposition modeling (FDM), 3D printing (3D-P), or stereolithography. RP is a very advanced technique for developing a desired property scaffold. A scaffold with reproducible composition and architecture can be generated with this technique. This technique has advantages over other scaffolding techniques as it can control the architecture of the matrix, including geometry, size, shape, interconnectivity, and orientation, which produce a biomimetic structure with variations in design and composition. Various advantages are also there, such as the mechanical properties can be controlled with RP, and the scaffold's biological effects and degradation rate can also be controlled [201,209]. However, RP also has drawbacks, such as achieving low resolution by existing systems and types of polymeric materials [201].

7.2.1. Selective Laser Sintering (SLS).

Selective laser sintering (SLS) is an adaptable rapid prototyping (RP) technique that leverages the power of laser beams to selectively fuse powdered materials, creating intricate three-dimensional objects. This advanced method allows for precise control over the object's

architecture, enabling the formation of complex structures based on designs derived from data acquired through computer-based medical imaging technologies. By employing SLS, researchers and engineers in the biomedical field can fabricate customized objects with enhanced detail and accuracy, paving the way for breakthroughs in various applications such as tissue engineering and medical device development [210]. A comparable *in vitro and in vivo* study was performed based on a sintering technique in which a novel sintering technique utilizing Ti6Al4V powder suspension was employed to create a porous Ti6Al4V alloy with 75% porosity, while a selective laser melting technique was used to fabricate a control group with the same porosity. The mechanical and biological properties of both porous Ti6Al4V alloys were assessed through mechanical tests, in vitro cell analysis, and implantations. The findings revealed that both groups exhibited favorable biocompatibility and osteogenic ability. However, the sintered porous Ti6Al4V displayed microstructure and mechanical properties more akin to cancellous bone, minimizing stress shielding and potentially offering better early stability post-implantation. These results suggest that this new type of porous alloy holds promise as a biomaterial, particularly for bone defect repair and orthopedic arthroplasty [211].

7.2.2. Stereolithography.

This technique was introduced in the late 1980s and is a leading and adaptable solid freeform technique (SFF). Despite newer techniques emerging, it maintains exceptional fabrication accuracy and an expanding range of processable materials [212]. Stereolithography is a valuable technique for fabricating biocompatible tissue engineering scaffolds with precise internal structures and external geometries resembling human tissue. This rapid prototyping method uses controlled UV light exposure to solidify liquid resins in a layer-by-layer approach. UV light follows CAD-defined patterns on the resin surface, initiating polymerization and forming a solid material. Each photopolymerized layer adheres to a supportive build platform. After polymerization, the platform moves incrementally for subsequent layer polymerization. This iterative process continues until the three-dimensional structure is constructed [213]. Stereolithography relies on four crucial components: a UV laser that emits radiation onto the resin, a photosensitive liquid resin, a transferable build platform, and a dynamic mirror system. These elements collaborate harmoniously to facilitate the creation of intricate structures. The UV laser plays a key role by shining radiation onto the photosensitive resin, triggering the polymerization process. The resin reacts to the UV light through its formulation, solidifying and forming the desired structure. The transferable build platform is a stable foundation, ensuring the object's stability throughout fabrication. The dynamic mirror system also guides the UV laser beam, controlling its movement and enabling precise layer-by-layer construction. Collectively, these components synergize to successfully implement stereolithography across a broad range of applications [214].

7.3. Extrusion-based system.

Extrusion-based bio-printing shows immense promise as a method for manipulating biomaterials and living cells to fabricate three-dimensional (3D) scaffolds that aid in tissue repair and restoration of functions. In recent years, significant progress has been made in engineering techniques and life sciences, transforming extrusion-based bio-printing from a basic approach to a sophisticated method capable of producing diverse tissue scaffolds using various biomaterials and cell types. Nevertheless, synthesizing materials for bio-printing and

manipulating multiple materials and cells present considerable challenges in scaffold fabrication [214]. Various scaffolding techniques based on extrusion-based systems are explained below.

7.3.1. Multiphase jet solidification.

Multiphase jet solidification (MJS) is a technique developed by the renowned research organization Fraunhofer-Gesellschaft in Germany [214]. It enables the production of metallic or ceramic parts through the controlled extrusion of low-melting point alloys, powder-binder mixtures, or semisolid metals. The process involves a computer-controlled nozzle that precisely deposits the material onto a building platform, layer by layer, following the 3-axis table movement. MJS can be integrated with other prototyping techniques to facilitate small production runs of ceramics. The MJS apparatus comprises a personal computer, a computer-controlled positioning system, a heated chamber with a jet, and a hauling system. Materials in the form of powder, pellets, or bars are supplied, and the extrusion temperature can reach up to 200°C. The diameter of the extrusion orifices ranges from 0.5 to 2.0mm [215].

In the MJS process, a powder-binder mixture feedstock is heated above the binder's melting point in a process chamber. Only the binder liquefies during the process, while the other components remain solid. The low-viscosity mixture is then extruded through a jet controlled in the x-y-z directions by a piston. The piston's feed rate regulates the material flow. As each layer is deposited, it solidifies upon contact with the base platform or the previous layer due to a decrease in temperature. While stainless steel powder-binder mixtures have predominantly been used for building metallic and ceramic parts, the MJS process can also be adapted for medical applications. In such cases, a polymeric material is employed as the modeling material instead of a powder-binder mixture, and it is supplied in the form of powders, pellets, or bars. The MJS technique offers a versatile approach to producing high-density metallic and ceramic parts, catering to various industry needs and applications [214].

7.3.2. Fused deposition modeling.

Fused Deposition Modeling (FDM) is a widely used rapid prototyping (RP) technique initially developed by Stratasys. It allows for the creation of physical objects directly from 3D computer-aided design (CAD) models. This is achieved using a computer-controlled robotic system to extrude thin layers of polymer material in a precise and additive manner. The layers are stacked vertically, forming the object through material deposition layer-by-layer fashion. The FDM process incorporates pre-defined raster gaps within the material structure, which allows for the creation of a porous 3D object with a controlled and predictable microstructure [216]. This method customizes bone scaffolds by creating controlled cross-hatch patterns determined by printing parameters such as temperature, infill angle, and layer thickness. These patterns can be adjusted easily using common filaments, resulting in pharmacological products with excellent properties and minimal post-processing needs [217]. Kalita et al. produced 3D polymer-ceramic composites using a high-shear blending technique, incorporating polypropylene polymer (PP) and tricalcium phosphate (TCP) ceramic. The porosity of the composite was adjusted during fabrication. The PP-TCP composite fibers were created through a single screw extruder and fused deposition, resulting in porous structures. Compression tests revealed that increasing pore volume decreased the composite's strength. The porous scaffolds were further characterized for their potential use as bone grafts. FDM has advantages as it

enables the creation of tailor-made scaffolds with regulated porosity using biocompatible materials. FDM provides fast fabrication, precise control over scaffold architecture, and facilitates cell adhesion and tissue regeneration [218].

7.3.3. Precision extrusion deposition (PDE).

The PED scaffolding procedure comprises several steps: design, material selection, setup, layer-by-layer deposition, cooling and solidification, repetition, and post-processing. CAD software is used to design the scaffold, and a suitable material, such as biodegradable polymers like PCL, is chosen (Figure 7). According to the design, the 3D printer is prepared with an extrusion system to deposit the material layer by layer. Cooling and solidification ensure the scaffold's structural integrity, and the process is repeated until the scaffold is fully formed. Additional post-processing steps, including support removal and surface refinement, may be carried out. Overall, this procedure enables the precise fabrication of intricate scaffolds. Precise scaffold architectures facilitate cell growth and tissue development [219]. The pharmaceutical field finds the Precision Extrusion Deposition (PED) method highly applicable, especially when utilizing biodegradable polyester such as polycaprolactone (PCL). PCL, known for its low melting point, is widely employed and has received approval from the Food and Drug Administration. It has unique applications, notably in producing long-term implants and controlled drug delivery systems. These features make it a valuable technique in the pharmaceutical industry, contributing to advancements in medical treatments [220]. The PED scaffolding technique is highly beneficial in tissue engineering as it allows for precise control and accuracy during the fabricating of intricate scaffold structures, enabling customization and patient-specific designs. PED is compatible with biocompatible materials like PCL, which support tissue regeneration. Furthermore, it facilitates controlled drug delivery within the scaffolds. These advantages collectively make PED an invaluable tool for developing functional tissue constructs in regenerative medicine [221].

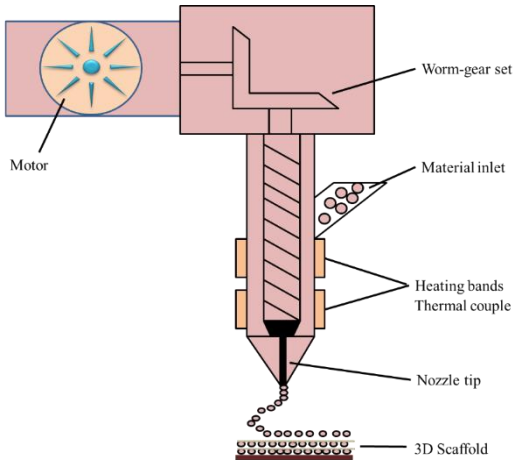

Figure 8. Precision extrusion deposition technique.

7.3.4. 3D Bioplotting.

3D bioplotting scaffolding is a technique used in tissue engineering to build intricate three-dimensional structures. It involves precise deposition of bioinks, consisting of living cells and biocompatible materials, to create tissue-like constructs. Utilizing computer-controlled robotic systems, the bioink is deposited layer by layer, enabling the formation of complex architectures resembling natural tissues. This method provides control over cell distribution

and organization within the scaffold, making it valuable for creating functional tissue constructs for transplantation [222]. This method involves designing the scaffold using computer-aided design (CAD) software and preparing a bioink containing living cells and biomaterials. The bioink is then accurately deposited layer by layer using techniques like extrusion or inkjet printing. Each layer is solidified to maintain the scaffold's structure, and post-printing steps, including incubation, may be needed for cell growth support. This technique has immense potential in regenerative medicine for generating tissue-like structures used in transplantation [223].

8. Conclusion and future aspects

Various conditions, such as trauma, tumor resection, and skeletal abnormalities, often necessitate tissue regeneration for the human body. Tissue engineering, an emerging field, aims to develop new therapies that can benefit individuals who have lost organs due to accidents or congenital reasons. Although techniques like autografts and allografts provide options for patients, they come with limitations, such as donor site morbidity and restricted blood supply. Additionally, allografts can be prone to rejection due to the recipient's immune response. To address these challenges, scientists are exploring the use of stem cells implanted at the site of injury, with the aim of growing complete organs. However, finding an effective method to implant cells into the body is a significant hurdle.

One potential solution to this problem is the use of scaffolding techniques. Scaffolds are three-dimensional structures designed to mimic the tissue structure at the affected site. They offer support for cells to attach and facilitate their growth to form a complete organ. Scaffolds can be combined with growth factors to enhance cell growth. In bone tissue regeneration, several characteristic parameters must be considered when designing an ideal scaffold. These include pore size, porosity, mechanical strength, bioactivity, biodegradability, and biocompatibility.

Researchers can develop novel scaffolds suitable for stem cell implantation by utilizing the aforementioned tools and techniques. These advancements hold promise in tissue engineering and may offer new avenues for effective tissue regeneration.

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Conflicts of Interest

The authors declare no conflict of interest.

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