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Chapter

Application of Bone Substitutes and Its Future Prospective in Regenerative Medicine

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Abstract

Bone is a hard and dense connective tissue that supports and maintains the body structure and functions. Several factors like aging, drugs, hormonal changes, and physical activities lead to several kinds of bone injuries/fracture. To address these problems, autologous bone graft is considered an ideal material. However, limited availability and complications related to its harvesting process like donor site morbidity and pain limit the use of autologous bone graft in bone regeneration. With increasing advances in technology, several bone substitute materials such as synthetics, bioceramics, and polymers are emerging as a substitute of auto- or allogenous bone for the treatment of bone defect. These bone substitute materials should be biocompatible, bioresorbable, osteoconductive, osteoinductive, and support the ingrowth of new bone. In this chapter, we summarize the currently available bone graft and bone substitute materials including biological and bio-inorganic factors. An overview of the associated advantages, challenges, and future perspectives to clinical implication is also discussed.

Keywords: autograft, allograft, growth factors, bone graft substitute, tissue engineering

1. Introduction

Bone is a part of vertebrate skeleton. It plays a multitude of important roles in the body like imparting structural support, protection of organs, acting as a site for production of blood cells, and also as repertoire of minerals. Bones comprise differentiated cell types, blood vessels, protein, minerals, and vitamins that facilitate their growth and repair system [1]. Bones have an inner and outer layer. The hard-outer layer of bone which is called "cortical bone" is usually tough and strong, whereas the inner spongy part is called "trabecular bone" and is lighter and less dense. Each of these parts comprises different cell types, nonmineral proteinaceous matrix (osteoid), and matrix-deposited inorganic minerals. Another important concept in bone biology and understanding its transformational changes is that of modeling and remodeling. The scenario where the sites of bone formation and resorption are different surfaces of the bone is called bone modeling. This is responsible for increased length and girth of long bone, leading to skeletal development and changes. Bone remodeling on the other hand is important for maintenance of bone mass in adults by replacement of old bone tissue with new ones [2]. Several factors which affect bone, muscles, and joints are responsible for causing diseases

like osteoarthritis (degenerative joint disease), rheumatoid arthritis (autoimmune disease), fibromyalgia (chronic condition of pain in bones, muscles, and tender areas with fatigue) and bone fractures. Aging plays an important role in manifestation of bone-related diseases along with other sub-factors like lifestyle, level of activity, family history, level of physical activity, drug usage, etc. However, in certain clinical situations, the natural bone repair may be too slow or inadequate; therefore, an alternative bone grafting strategy is required to address this problem.

2. Bone graft

In 1861, a surgeon from Lyon, France named Leopold Ollier was the first to describe the term bone graft (French: "greffe osseuse") in his document "Traité de la régénération des os" [3]. It was considered a surgical procedure to promote bone healing for several reasons, like injury and disease utilizing a bone transplant.

Bone graft is the alternative choice to address the problem associated with bone disease. Bone grafts are basically bone-like materials that come from living donor, post mortem donors, or artificially constructed, which are used for healing, strengthening, or improvement of bone function in disease or injury.

2.1 Properties of ideal bone graft

The ideal bone substitute material should exhibit several important properties [4, 5]. This includes:

i. *Biocompatibility*—The graft should not evoke an immune response against the implanted tissue.

ii. *Durability*—The graft should be able to maintain its shape and volume over time without loss of its structural properties.

iii. *Vascularity and angiogenesis*—Porosity is important to maintain the proper transport of nutrients and oxygen for survival of the cells and tissues. A porous structure of at least 100 μm diameter is recommended. It has also been shown that supports with multiple apertures are preferred over one large pore. Also, a polymeric, ceramic, or composite material is preferred over a metallic one as the latter might not fuse completely and dissociate after implantation. Although most scaffold (structural support) materials do not induce angiogenesis, it is an indispensable requirement to meet high blood demands in bone tissue. The initial inflammatory response activates neovascularization, yet it takes several steps to form a vascular network. The established role of proinflammatory response is their catabolic effect on bone and premature failure of bone implants. However, recently in opposition to the conventional unregulated, destructive effect of inflammatory cytokines, its regulated role aiding towards fracture healing has been suggested [6–8]. The acute inflammation initiates the recruitment of MSCs, fibroblasts, and osteoprogenitor cells to promote bone regeneration [9]. The early phase of bone injury includes the inflammation phase where IL-1, prostaglandins, TNF-α, and other proinflammatory molecules recruit inflammatory cells and induce angiogenesis. IL-6 triggers angiogenesis by stimulating release of VEGF [8]. A recent study by Anghelescu et al. shows tissue formation exceeding the catabolic action of inflammation and it might be limited to specific allografts only.

iv. *Bio-absorbability*—Another important feature of a substitute material is that it should get absorbed and provide the space for new bone formation. However, the duration of availability largely depends upon the site of surgery. The spinal fusion requires the material to be degraded after 9 months or longer while in skull or maxillofacial bone the required time period is around 3–6 months.

v. *Cost efficiency and availability*—The material should be reasonable to be purchased and used.

Besides the above-mentioned characteristics, the ideal bone substitutes must strengthen bone healing by following the mechanism which involves osteogenesis, osteoinduction, and osteoconduction [10], which are mentioned here:

vi. *Osteogenesis* (acts as the origin or source)—The process where cellular elements from the host or graft donor provide the material to synthesize new bone at the graft site is called osteogenesis. Various autografts and stem cell transplants fall under this category.

vii. *Osteoinduction* (acts as initiator)—The process resulting in differentiation of recruited mesenchymal stem cells into chondroblast and osteoblast, which forms the new bone, is called osteoinduction. It is regulated essentially by various factors like bone morphogenetic proteins (BMP)-2, -4, -7, platelet-derived growth factor (PDGF), parathyroid hormone (PTH), etc.

viii. *Osteoconduction* (provides direction)—A degradable support or scaffold material which provides the surface for the production of new bone. It also exhibits osteoconductive properties. These may include metals and synthetic polymers, and most frequently used osteoconductive materials include calcium phosphate ceramics such as hydroxyapatite, calcium sulfate, and bioactive glass-ceramics.

Most widely used bone grafts (**Figure 1**) are mentioned below in detail.

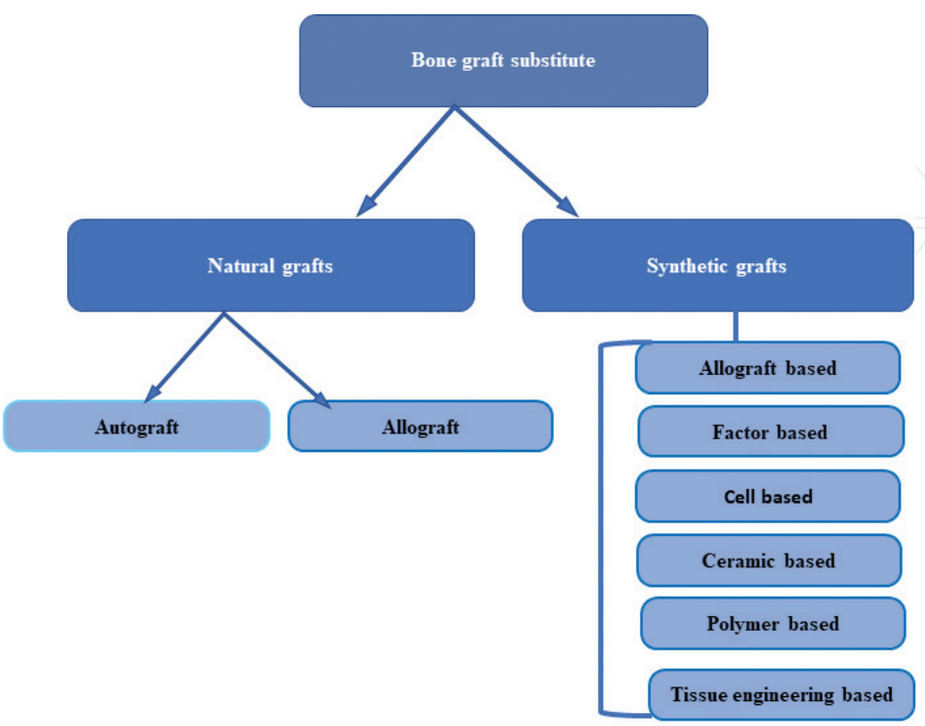

Figure 1.
Classification of bone grafting substitutes.

3. Autograft

The autologous bone grafting as the name suggests 'auto' (self) involves taking up tissue from one anatomical location and transplanting it to another in the same patient. The method dates back to 1821 when Walther repaired the holes in a patient's skull using the original bone plug [11, 12]. The method is still considered to be a 'gold standard 'as there is no immunogenic response against patient's own tissue [13]. Thus, no graft rejection or histocompatibility issues are conferred. Moreover, it manifests the properties of an ideal bone graft, that is, osteogenesis, osteoinduction, and osteoconduction. However, it is not a straightforward path to follow and comes with a set of limitations associated with it. This includes an additional operative pain at donor site, increased blood loss, and also possible injury to nearby blood vessels. Besides, the inappropriate availability of tissue amount especially from infants and older patients results in added trouble [14, 15].

3.1 Autograft-based substitute

It is considered as a gold standard to treat bone defects due to their established osteoconductive and osteoinductive properties with reduced histocompatibility issue. Although it also has inherent drawbacks associated with issues of high postoperative pain, donor site morbidity, muscle weakness, infection, high cost, and longer recovery periods limit its application.

Some of the common advantages and disadvantages associated with autograft are listed in **Table 1**.

3.2 Types of autografting commonly used are

3.2.1 Cancellous autograft

This is the most common procedure among autografts and has shown success for various purposes majorly nonunions. It starts with hematoma formation resulting in recruitment of mesenchymal stem cells (MSCs), while simultaneously, necrotic graft is eliminated by macrophages [16, 17]. Neovascularization also takes place along with this, and finally, osteoid produced by osteoblasts lining the dead trabeculae forms new bone through mineralization, which takes a period ranging from 6 to 12 months post-operation [18]. The most common donor site is the iliac crest due to large surface of trabecular architecture and availability of growth factors [19, 20]. Recent report showed superior osseous bridging after bilateral tibial tuberosity advancement (TTA) over no graft in dogs [21]. Earlier, bone union was observed in graft harvested from scaphoid nonunion distal radius or iliac crest and headless compression screw to treat scaphoid nonunion [22]. Although being the gold standard, the limitation of the amount and donor site morbidity hinders its use.

Advantages	Limitations
Osteogenic	Donor site pain
Osteoconductive	Increased blood loss
Osteoinductive	Inappropriate amount of tissue availability
No graft rejection	Increased risk of nerve injury

Table 1.
Common advantages and disadvantages associated with an autograft.

3.2.2 Cortical autograft

It shows creeping substitution, that is, deposition of new bone along with slow resorption of the graft. The process provides distinguished structural support. This graft also faces limited supply of osteoblasts and revascularization is impeded too.

3.2.3 Vascularised autograft

This type of graft promotes fracture healing and minimizes loss of bone strength after post-implantation. However, this graft also faces technical challenges for the preservation of graft's osteocytes and osteoprogenitor cells.

4. Allograft

The second category of natural bone grafts is allograft, where tissue from another individual (donor) has to be taken for grafting. The term allograft ('allo' (other)) means harvesting tissue from different individuals to transplant in the subject. Bone harvested from cadavers in different bone banks is the major source of different allografts. Apart from the above-mentioned category, freeze dried (lyophilized) and frozen bones are also used in spine graft surgeries. Allografts are considered more advantageous as they are considered less stressful to the patient. However, they are also plagued with problems of availability and immune rejection. The first report of allograft usage can be dated back to 1800s, when Macewen reported the grafting of tibial fragments to a child [23]. Experimentation reported by Bauer in the beginning of twentieth century marks the starting of the bone bank concept, where he stored bone tissues for weeks in refrigerated condition and then used them for implantation in dogs. It was also established that chilling and boiling of bone tissues before using them as allografts led to destruction of their endogenous proteins and other factors resulting in poor and slow recovery [24]. The necessities posed by the devastation of World War II resulted in advancement and growth in the bone banking approach, with refinement of methods like autoclaving, freeze-drying, irradiating, demineralizing, and chemical treatment of the bone [25]. The advancement made during the war period continued with focus on civilian need and further refinement of the banking approach [23]. Pros and cons of allografts can be summarized in **Table 2**.

Advantages	**Disadvantages**
Less chance of donor morbidity	Chances of disease transmission
No size limitation	Possibility of host immune response
Less surgical intervention	High cost
Cosmetically better results	Delay in incorporation
Reduce period for rehabilitation	Local bone resorption

Table 2.
Common advantages and disadvantages associated with an allograft.

4.1 Types of allografting commonly used are

4.1.1 Cancellous bone graft

This is primarily an osteoconductive graft, devoid of growth factors (not osteoinductive), and like cancellous autograft, it also provides less mechanical support [26]. It is prepared in small cubicle form, thus sometimes called 'croutons' and is

now marketed in varied sizes as chips or crushed form. It involves an initial cascade of inflammatory events leading to formation of a fibrous tissue around the graft, preventing complete integration of the graft. The survival and efficiency depend a lot on processing and storage conditions of the grafts. Freeze drying is the most common method of preservation of cancellous graft, which may result in destruction of osteoprogenitor cells and osteoinductive factors [27, 28].

4.1.2 Cortical bone graft

This shows a creeping substitution like its autograft counterpart, thus leading to bone healing in spite of an initial inflammatory response. It also provides structural support and thus can be utilized for load-bearing areas [10].

Both allograft and autograft have their own advantages and disadvantages. However, research is being carried out to search the materials which can be used as a substitute to replace the function of auto- and allograft. These substitutes possess the properties of one's own bone, which stimulate bone healing and provide a strong and biologically compatible environment for the growth of new bone.

5. Bone graft substitute

The bone graft substitute is basically a synthetic inorganic or biologically organic combination, which guides to stimulate bone healing and fill bone defects. A good bone grafting substitute should have low immunogenicity and higher biocompatibility, and at the same time, it should be able to mold itself according to grafting needs. Bone grafts find application and are primarily used for linkage and splintage and help in promoting ontogenesis. In the case of linkage, bone grafts are utilized for filling cavities or defects in bone, replacement of crushed bone, and arthrodesis. While splintage-related grafting protocols are utilized for nonunion bone deformities and arthrodesis [29]. An understanding of bone formation, process of osteogenesis, osteoinduction, and osteoconduction is a prerequisite for appreciating the biology of graft substitution. The type of graft used and its physicochemical properties play an important role in its success. On a global scale, around 2.2 million graft substitutions are performed annually with 9 out of 10 falling under the category of allograft or autografts. This bone grafting procedure typically follows the multistep cascade:

- Accumulation of inflammatory cells takes place due to induction by exogenous graft material, which is followed by chemotaxis reaction by mesenchymal stem cells. After that, the host cells differentiate into chondroblasts and osteoblasts with the stimulation of several osteoinductive factors [30].

- The osteoinductive matrix serves as a scaffold for supporting progenitor cells and osteoblasts. It also provides a porous structure, which helps in migration of new cells.

- The osteoblast cells start the formation of new bone under suitable conditions.

- Lastly, the osteoinductive proteins start stimulating the differentiation of osteoblasts. This leads to initialization of bone graft revascularization and necrotic graft resorption. As a result, bone production followed by bone remodeling started from the osteoblasts on the graft's three-dimensional structure [31]. An outline of bone graft substitute process is shown in **Figure 2**.

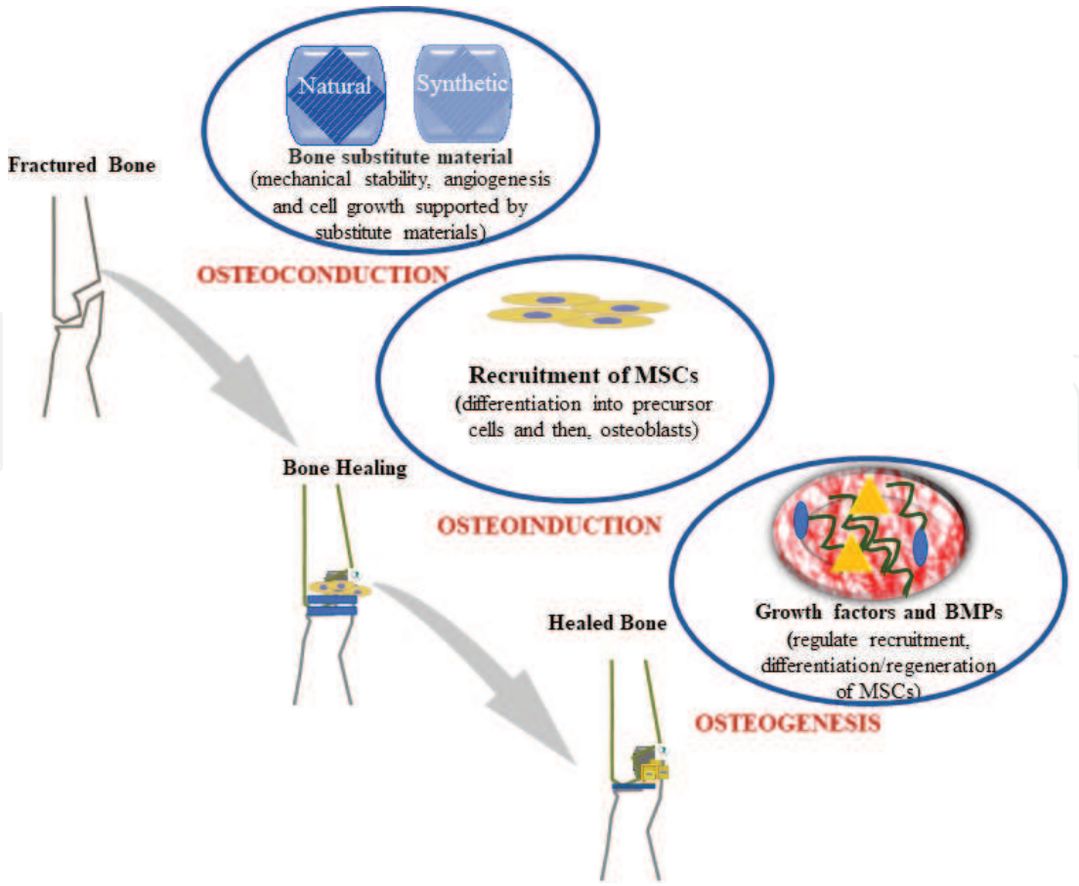

Figure 2.
Schematic representation shows the process of bone graft substitues.

Broadly, the substitute materials can be categorized as natural and synthetic grafts. Under the natural grafting substitutes fall autografts and allografts.

5.1 Natural substitute material

5.1.1 Factor-based substitutes

Factor-based bone grafts include natural and recombinant growth factors and hormones, which are generally used alone and/or together with other materials. The bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), parathyroid hormone (PTH), transforming growth factor-beta proteins (TGF-β), and many more come under this category. This group too does not come without associated disadvantages, such as protein instability, risk of uncontrolled proliferation or cancer, and high cost.

5.1.1.1 Bone morphogenic proteins

Nearly 30 human proteins fall under the BMP name and this group comprises the largest division of transforming growth factor (TGF)-β family of ligands. Since the last 40 years, the significance of BMP signaling in skeletal development has received immense approval [32]. It was first identified as a substance in extracellular matrix of bone, which stimulates new bone formation by Marshall R. Urist, and later named BMPs. In the United States, in 2002, the Food and Drug Administration (FDA) approved the first bone graft substitute, which was the recombinant human (rh) BMP-2 for single-level anterior lumbar interbody fusion (ALIF) within a titanium cage of implant grade. Subsequently, the increased use of BMP-2 for various spine

fusion injuries as an alternative to autograft acted to be a savior as bone substitute. Despite that, starting from 2006, independent research groups started reporting complications associated with the use of rhBMP-2. The side effects ranged from seroma formation [33, 34], ectopic bone growth [35–37], osteolysis [38, 39], and increased risk of cancer [40, 41]. This led to a systematic review and meta-analysis, which was reported in Annals of Internal Medicine in June 2013. It was conducted as joint work of Medtronic and Yale University Open Data Access Project (YODA), and reported biased results in earlier industry-funded publications [42, 43]. Now, the use of rhBMP-2 (INFUSE) is limited to oblique lateral interbody fusion (OLIF) and anterior lumbar interbody fusion (ALIF) upon nonavailability of autograft. Also, BMP-7 or human osteogenic peptide-1 (OP-1) was given an FDA Humanitarian Device Exemption in 2004 [44, 45], but later studies [46] ultimately led to the FDA rejection of Pre-Market Approval of OP-1 in April 2009.

5.1.1.2 Platelet-derived growth factor (PDGF)

Platelets, monocytes, and endothelial cells are responsible for production of PDGF. They recruit various inflammatory cells at the fracture site resulting in increased cellular proliferation and collagen deposition. The potential use of PDGF has been suggested in the treatment of osteoporosis and bone healing. It is thought that recombinant PDGF-BB form assists to release the pericytes from the abluminal side to release free and activated MSCs [47, 48].

5.1.1.3 Parathyroid hormone

It is produced by parathyroid glands and is involved in calcium and bone metabolism. It is suspected that it might accelerate fracture healing and might reduce overall fracture risk [49, 50]. According to reports, PTH can exert anabolic as well as catabolic effect on bone metabolism. This feature depends extensively on the duration of administration wherein continuous administration results in bone resorption, while intermittent administration leads to increased bone deposition and induction of IGF-1 [51, 52]. Thus, it is speculated as a potential agent for osteoporosis treatments.

5.1.1.4 Insulin-like growth factor (iLGF)

The recombinant human insulin-like growth factor-1 (rhIGF-1) is also called mecasermin (International Nonproprietary Name (INN)). Children who are very short of their age are the target group of the product [53] and are FDA approved for the mentioned condition called severe primary Insulin IGF-1 deficiency under the trade name of INCRELEX. The FDA approval (in Aug, 2005) was based on five clinical trials of the drug (subcutaneously injected), of which four were open-label studies and one was double-blinded and placebo-controlled. The integrated results, followed up each year for 8 years, showed a considerable increase in height velocity (HV) with reference to pre-treatment [54]. HV is calculated between consecutive annual study visits as the difference in height, divided by the difference in age [55].

5.2 Synthetic grafts

Synthetic materials that are used for grafting should also possess the properties of biocompatibility, bioresorbability, osteoconductivity, osteoinductivity, cost-effectiveness, and the ease of use to make an ideal grafting material. It should also possess the similar features of cortical or cancellous bone which include toughness,

modulus of elasticity, and compressive strength. With all these specificities and different mechanism of actions, a large number of synthetic bone alternatives have emerged which can be used for orthopedic applications.

5.2.1 Ceramic-based bone grafts

Ceramic-based grafts are ionically bonded inorganic preparations, which can be described as a family of materials with a wide variety of composition, porosity, manufacturing, and structure. Calcium sulfate, hydroxyapatite, tricalcium phosphate, bioactive glasses (silicon dioxide, calcium oxide, phosphorous pentoxide, and sodium oxide), and synthetic hydroxyapatite come under this category. Not only this group of materials varies in terms of material properties, but also differs in biodegradability, mechanistic strength, and binding. Glass ionomers have been used to seal defects in the skull, sinus augmentations, and otorhinolaryngologic surgeons for auditory ossicular reconstructions [56]. They are also used for the application of orbital implants, ossicular replacements, and prosthetic joint linings [57]. Calcium phosphate does not show any osteogenic properties; however, this material is used with the combination of hydroxyapatite and tricalcium phosphate and calcium carbonate and monocalcium phosphate monohydrate. This mixture is used as an injectable into the bone defect site, to harden. This product undergoes long-term remodeling, and the graft is eventually replaced with the in-growth of new bone [58, 59]. Hydroxyapatite-based materials are used for coat implants because of their great osteo-integrative capabilities [60, 61].

Over all, ceramic-based bone grafts are specifically found useful in iliac crest bone grafting and as a bone graft extender in lumbar spine fusion procedures [62]. The special feature of ceramic-based graft is their porosity, which is helpful in adhesion of mesenchymal cells, and later gets differentiated into osteoblasts. Further, this group of grafts is superior as the benefits of its different constituents can be availed simultaneously leading to better bone regeneration [63]. Some of the commercially available synthetic bone graft substitutes are mentioned in **Table 3**.

5.3 Polymer-based bone grafts

Polymer-based grafts can be broadly grouped as synthetic or natural polymer-based grafts and can further be classified as biodegradable and nonbiodegradable substitutes. This category of grafting materials is specifically utilized for dental implants, as they are found helpful in restoring of edentulous site in the lost tooth. In the case of long bone-related congenital defects, or cases requiring bone segment replacements after invasion of malignant tissues, polymer-based grafts (vascular fibulas) are found helpful in restoring skeletal integrity. Other polymers like chitosan, collagen, gelatin, and poly lactic acid (PLA) are reported to exhibit improved bone regeneration capabilities both *in vitro* and *in vivo* when combined with hyaluronic acid [64]. Another advantage shown by these materials is that of better bone-matrix interference since they also act as bio-mimetic, thus helping in deposition, precipitation, and enhancing formation of calcium phosphate [65]. The type of commercially available polymer-based bone graft substitute is mentioned in **Table 4**.

5.4 Tissue engineering and cell-based bone graft

The interdisciplinary field of tissue engineering seeks to combine the bone graft substitutes to stimulatory effects of growth factors, (bone morphogenetic proteins and osteogenic proteins) to provide structural support and promote more rapid bone growth and healing. The prime aim of the bone tissue engineering approach is to

Commercial product (name)	Substitute materials	Properties	Applications
Osteograf	Ceramic	Osteoconductive, limited osteoinductive when mixed with bone marrow	Bone void filler
NovaBone	Bioactive glass	Osteoconductive, limited osteoinductive when mixed with bone marrow	Filling surgical or traumatic bone gaps
Osteosat	Surgical grade calcium phosphate	Osteoconductive and bioresorbable	Hip and knee joint repair
Calceon 6	Calcium sulfate	Osteoconductive and bioresorbable	Bone void filler; provides strength
Norian	Monocalcium phosphate, tricalcium phosphate, and calcium carbonate	Good compressive strength	Skull bone defect; injectable paste, craniofacial reconstructions
Hard tissue-replacement (HTR)	Poly methyl methacrylate (PMMA)	Good strength, durable, and surface osteoconductive	Craniofacial reconstruction
Alpha BSM	Calcium phosphate cement	Good compressive strength	Dental application for bone and cartilage defects
Mimix	Synthetic hydroxyapatite tetra-tricalcium phosphate	Good compressive strength	Cranial defects
ELIZ (Kyeron)	Composed of (40%) β-tricalcium phosphate and of (60%) hydroxyapatite	Ultrahigh porosity, biocompatible, and osteoconductive	It has been successfully implanted in more than 1200 patients without any side-effects.
OSIQ (Kyeron)	Fully synthetic ultrapure nano-hydroxyapatite	Ready to use, injectable, and biodegradable	Filling or reconstruction of small and medium bone defects
AXOZ QS (Kyeron)	Resorbable phosphocalcic compounds and a polymer	Injectable and fully resorbs	Supports bone growth
COLLAPAT II (Symatese)	Composed of a collagen structure in which ceramised hydroxyapatite granules are dispersed	Strong hemostatic power, completely resorbable in a few weeks, and osteoconductive	Induces bone substance replacement in maxillofacial surgery and odontostomatology
CopiOs (Zimmer Biomet) Bone Void Filler	Calcium phosphate, dibasic (DICAL), and highly purified Type I bovine collagen	DICAL provides significantly more calcium and phosphate ions at equilibrium than either β-TCP or HA	CopiOs paste acts as an osteoconductive scaffold for the growth of new bone

Table 3.
List of some commercially available synthetic materials and their applications.

guide and enhance osteogenic differentiation of stem cells into 3D constructs, so that later it can be engineered successfully into applicable bone constructs. The limitation in availability of both allografts and autografts can be addressed to a great extent by tissue engineered bones or healing of fracture critical defects. The tissue engineering approach for bone grafts differ from other approaches, in the fact that in earlier case,

Commercial product (name)	Substitute material	Properties	Applications
Cortoss	Polymer system with reinforcing particle bioactive glass	Forms biological interface	Augmentation of screws in osteoporotic bone (hip, spine, etc.)
Open porosity polylactic acid polymer (OPLA)	Polylactic acid	Osteoconductive and bioresorbable	Articular cartilage regeneration
Collagraft	Mixture of tricalcium phosphate, bovine collagen, and hydroxyapatite	Bioresorbable and osteoconductive	Use for the treatment of long bone fracture and void filling
DynaGraft	Demineralized bone matrix	Heat sensitive copolymer, injectable gel, limited osteo-induction	Dental bone graft substitute
MedPor	Porous polyethylene	Higher porosity	Orbital reconstruction and facial contouring
Collapro/matrix	Human collagen in lyophilized strip	Lack of immunogenic property	Use in development
Healos	Hyaluronic acid-coated collagen sponge	Osseo-inductive property	Replacement of autograft/autograft extender for spinal fusion
Immix	PGA/PLA polymer to be produced in chip, flex forms	Provides structural support	Bone graft extender
OsteoScaf (Bonetec)	Macroporous poly(lactide-co-glycolide)/calcium phosphate (PLGA/ CaP) foam matrices	Fully resorbable, osteoconductive, and mechanically robust	Heal tissue defects

Table 4.
List of some commercially available polymer-based graft materials and their applications.

integration of engineered bone with patients takes place, thus providing specific and more resilient treatment for condition [66]. Further tissue engineering is more convenient in the case of bone regeneration therapies, as osteogenic differentiation can be achieved through multiple stem cell types [67]. The type of scaffolds and cells used for tissue engineering graft is discussed in more detail.

5.4.1 Scaffolds

One important requirement for bone tissue engineering is the scaffold, which helps in the migration, proliferation, and differentiation of osteogenic cells promoting new bone formation and regeneration [68]. In this regard, it is required that the scaffold must be stable, biocompatible, and biodegradable and should be porous and permeable for cell seeding, nutrient transport, tissue ingrowth, and vascularization. It should also be osteoconductive, osteoinductive, and osseo-integrative in nature. In clinical settings, a wide range of synthetic and natural scaffolds have been explored for bone repair and bone tissue engineering. Broadly, these materials can be categorized as composites, ceramics, and polymers, with each having specific properties and limitations. Type I collagen polymer matrix serves as a good natural material but suffers from low mechanical modulus.

Market name	Allograft type	Form of/additive with	Carrier used
Grafton	DBM	Gel, putty, and flexible sheets	Glycerol carrier
Opteform	DBM	Cortical bone chips	Gelatin carrier
Osteofil	DBM	Injectable bone paste	Collagen-based hydrogel matrix
Dynagraft	DBM	Syringe	Pleuronic reverse phase copolymer
Orthoblast	DBM	Cancellous bone	Bioresorbable reverse phase copolymer

Table 5.
Marketed DBM-based bone graft substitutes.

One important group of scaffold, which has been extensively used for tissue engineering, is demineralized bone matrix (DBM). DBM is typically produced from allograft bone by the acid extraction method (known as decalcification). Based on manufacturing techniques, DBM may be presented in different forms like sponges, freeze-dried powder, gel, paste, injectable putty, or strips. It is basically a decalcified allograft retaining collagen and noncollagenous proteins [69]. It is osteoconductive as well as osteoinductive but has no osteogenic property due to its processing. Recently, it is being used in conjunction with cancellous/cortical bone chips. The unlimited availability and reduced immune response owing to acidic demineralization give it superiority while it is generally used as a bone graft additive in spine fusions [70]. The types of DBM bone graft substitute which is commercially available are mentioned in **Table 5**.

5.4.2 Cell type involved in bone tissue engineering

A variety of *in vitro* culture protocols are used in bone tissue engineering, which requires use of growth factors and cytokines along with specialized dynamic bioreactors. It also requires a large scale of bone extracellular matrix-producing cells, primarily the MSCs. In this regard, the adult bone marrow stem cells (BMSCs) are the cells of choice for tissue engineering. The quantity of the isolated stem cells varies between patients and is indirectly proportional to patient's age [71, 72]. Also, the isolated cells should have high biosynthetic activity, expression of osteogenic markers, and right cell phenotype. Another source for cell-based bone tissue engineering is the adipose tissue which consists of adipose stem cells (ASCs). They undergo differentiation into several cell lineages such as osteogenic, chondrogenic, and endothelial. Comparative studies of ASCs and BMSCs have shown that they share their surface antigen expression pattern involving CD44, CD71, CD90, and CD105 [73]. However, there are differences too, such as the BMSCs mark the absence of hematopoietic and endothelial lineage markers [74–76]. Researchers are currently investigating the ability of other multipotent cells such as periosteum, umbilical cord, cord blood, and fetal tissues to be used for bone tissue engineering [77].

5.4.3 In-vitro culturing

In this approach, bone marrow is harvested from the patient, followed by *in vitro* culturing and seeding them on scaffolds prior to implantation into the same patient for tissue regeneration. The bone repair process required many cell types. These cell types are involved in many inflammatory and angiogenic responses and play an important role in development of the bone formation mechanisms. They release cytokines and various growth factors like PDGF, BMPs, VEGF, and interleukins

that attract and simulate the mesenchymal stem cells (MSCs), which are directly involved in bone repair. However, cell sourcing for bone regeneration is still a critical issue for cell-based therapies that need to be addressed. Also, for the sake of achieving homogenous growth in 3D environment, a new bioreactor cultivation system with mass transport capabilities has been explored [78].

5.4.4 In-vivo studies on bone graft applications

Multiple reports have been published showing not only the application of bone tissue engineering in the case of ectopic bone formation (nonbone environment) [79] but also in orthotropic bone formation (bone environment) [80]. In 1991, Caplan first reported the usage of autologous stem cells for fast and specific skeletal tissue repair. Mesenchymal stem cells (MSCs) or mesenchymal colony-forming cells (CFU-F) were identified as cell type present in the bone marrow, which have the capability to differentiate into different cell types of bone [81]. In 2004, first time successful transplantation in humans was reported, where lamellar bone was regenerated and repaired by infusing periosteum-derived osteoblasts for 90 days [82]. Thus, bone marrow has been recognized as a source of MSCs and other osteogenic cells, with relative ease of cell collection using the aspiration method [83]. Ever since the identification and recognition of MSCs for repair and engineering of mesenchymal tissues like cartilage and bone, new cell types and putative strategies have been explored vastly. Among newly developed cell types for bone tissue engineering, deciduous dental pulp-derived stem cells and adipose derived cells were the prominent one showing osteogenic potential [84]. Successful tissue engineering-based bone regeneration depends on multiple factors like the number of healthy osteogenic cells transplanted, the use of proper scaffold for seeding the cells at the site of regeneration, enough vascularization of repair site, and osteogenic differentiation factors. The report provided by Coventry and Tapper [62] has proved successful regeneration of ectopic bones in rodent models, fulfilling all the above-mentioned requirements using ceramic based scaffolds [85]. Further bone marrow aspirate and plasmid-rich plasma approaches have been explored for facilitating effective tissue engineering-based grafts.

In addition to these materials, research is still continuing to modify the products for the production of graft materials, which possess all the properties of ideal bone graft. A multidisciplinary approach will be required to improve implanted cell survival on biomaterial substrate with addition of cytokines and other growth factors for prompt new bone ingrowths. This will yield a bony union that resembles natural form and structure. However, the unavailability of widely accepted specific guidelines, standardizing minimum standard for bone grafts, is one of the limiting factors. Also, the size of *in vitro* grown tissue grafts is often found to be small, in the case of critical size defects.

6. Conclusion and future perspective

The challenge of bone loss and other musculoskeletal complications during surgeries is well recognized, and to address the same issue, different grafting substitutes have emerged. These bone regeneration grafts can be broadly categorized as natural and synthetic grafting materials. Autograft and allograft comprise natural grafting substitutes and between these, allografts are gaining ground and more acceptability with time. There have been revolutionary changes during the past decade, which bring allograft as a first alternative after autograft, which were subsequently interchanged and/or replaced by demineralized bone matrix in

certain circumstances [83]. The selection of ideal bone graft substitute materials is a difficult task and often lacks substantial scientific backing. However, it is a very fast and broad field of investigation. Current trends remarkably increase the use of synthetic bone graft as alternatives. The introduction of cost-effective, biologically improved synthetic materials that owe the property of ideal bone graft addresses the present clinical needs for the optimal treatment. It is not mandatory to use these synthetic materials alone for reconstructive procedures. However, when it used in the right situations and in combination with autologous, allograft, or other synthetics, in combination with growth factors, they give potentially more desirable results.

With the advancement of biomechanical research, an interdisciplinary field of tissue engineering has emerged. Limitations associated with the autologous bone grafting method can be addressed to a great extent with the help of bone tissue engineering. However, selection of optimized combinations of cells, synthetic materials (scaffolds) and factors will remain a challenging and complex process. One of them is the selection of materials and cells. This may include the materials, which possess appropriate mechanical properties, degradation rates, and chemical functionality; likewise, for stem cells, it should be isolated from patient-specific cells from the appropriate lineage and directed down on a scaffold construct to heal the proper bone tissues. Cheaper and safer alternatives will probably emerge with a better understanding of the inherent ability of material to induce bone formation after bone graft implantation.

Thus, these developments must also be nurtured and monitored by the group of clinicians and researchers with the knowledge of medical necessity, basic biological principles, and commercial practicality. This may be used for the production of versatile, and easy to implement, allowing for bioengineered bone grafts to more quickly make the leap from bench to bedside. This will help to improve the quality of life of pediatric and adult patients suffering from bone disease and/or disorder. This may be helpful for bridging the gap between bone tissue engineering research and its clinical implications.

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