4 Biomechanics of Bone Grafts and Bone Substitutes

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Several comprehensive reviews have been published on bone graft substitutes and provide valuable background information on the vast array of available materials and products [1–3] Rather than provide yet another product or materials review to add to this comprehensive collection, the focus here is to catalog the attributes of these materials in a context that may help guide the surgeon's selection of the bone graft substitutes for particular clinical applications.

Achieving the best possible clinical outcome while satisfying the patient's expectations of return to functionality should be the principal determinants in choosing which of the myriad of bone graft substitutes is the best option for any clinical application. It is known that the structural requirements should be considered in the choice of the appropriate bone graft [4]. Many times the choice of bone graft is relegated to availability in the surgical setting, commercial representation or historic clinical experiences of the clinicians. The choice of a bone graft may also be dependent on a delicate balance between biology and biomechanical stability [5]. Patient age, health status, and activity level, coupled with clinical presentation, compliance, rehabilitation options, and economics are all considerations that should be weighed in the choice to achieve the desired clinical outcome. Most bone grafts and bone substitutes initially provide very little clinically relevant structural stability and ultimately rely on biology to restore structural stability and function.

Deciding which bone graft material to select can be a confusing and daunting process. How does one differentiate between the products? Are product claims supported by reliable science and clinical experience? How should variables such as composition, handling, mechanical and biological properties, patient clinical presentation, intended outcomes and price are all factors that weigh in decision-making?

The objective in this chapter is to provide some of the information that will be useful for the clinician in making that decision. Several publications provide general reviews of the bone grafting options that are available to the clinician [6, 7]. In the current paper, particular emphasis will be placed on the mechanical properties along with material and biological properties of the bone graft with respect to short- versus long-term outcomes and patient satisfaction. With this objective we will discuss bone grafting options from the following clinical perspectives and considerations, see Fig. 4.1.

Bone grafts are used to repair and rebuild missing, damaged, or diseased bones in a human body where the clinical situation where the bone may not heal by itself or healing might be

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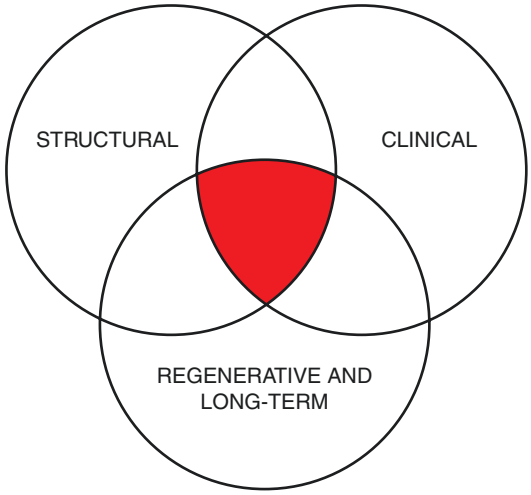

Fig. 4.1 Bone Grafting Considerations

compromised. It has been reported that there are over two million bone grafting procedures performed annually [8]. The considerations for the selection of the optimal bone graft involve biomechanical, biomaterial, and biological considerations. Among the many options available to the surgeon it is safe to say that the ideal bone graft that performs as well as the patient's own bone (i.e., autograft) has yet to be developed, but many of the available bone grafting options do have desirable and often suitable properties for a particular situation.

4.1 Bone Basics: Basic Properties and Concepts

Human bone consists of 80% cortical bone and 20% cancellous bone. Cortical or compact bone is 70% inorganic material (principally hydroxyapatite), 22% organic material (e.g., collagen, non-collagenous proteins, cells, hyaluronic acid), and the rest is water [9]. Cancellous or trabecular bone has the same constituents as cortical bone but has a lower calcium content, tissue density and ash fraction [10]. Cancellous bone also has higher water content (27% compared to 23% for cortical bone) [10]. Cortical bone is dense, strong, and difficult to fracture and thus provides most of the structural support to the body, while cancellous bone is extremely porous as it is more involved in bone remodeling. The turnover rate of trabecular bone is 25% per year versus only 3% for cortical bone [10]. With respect to mechanical properties, the compressive strength of human cortical bone ranges between 90 and 230 MPa and the tensile strength ranges between 90 and 190 MPa [11]. The compressive strength of human cancellous bone ranges from 2 MPa for osteopenic cancellous bone to 45 MPa for dense cancellous bone [12]. The Young's modulus of cancellous bone is 10.4–14.8 GPa, while cortical bone measures 18.4–20.7 GPa [9]. These physical differences also manifest themselves in biological remodeling and the susceptibility to pathologies such as osteoporosis. For cortical bone to be remodeled, osteoclasts and osteoblasts are required so the greater surface area of cancellous bone allows for more rapid revascularization and remodeling.

Bone regeneration has been defined to involve three pillars: osteogenesis, osteoinduction, and osteoconduction [13]. Osteogenesis is the synthesis of new bone by cells derived from either the graft or the host. Osteoinduction is the process where mesenchymal stem cells (MSCs) are recruited and induced to differentiate into functionally competent osteoblasts and chondroblasts. Osteoconduction is the ability process where biological interactions along the graft result in fusion of the graft with the host's bone. The overall functionality of a bone graft is dependent on the graft's ability to perform these three processes.

The timeline for normal bone regeneration and repair involves three distinct phases: inflammatory, proliferative and then remodeling [14]. The inflammatory stage (weeks 1–3) occurs after the insult or injury (hours to days, postoperatively) and involves the recruitment and proliferation of inflammatory cells and growth factors and differentiation into repair cells and the formation of callus. The proliferative phase (weeks to months, post operatively) causes the callus to organize together with a periosteal response which replaces the callus with immature woven bone predominated with vascular ingrowths and collagen

matrices. The remodeling phase (months to years, postoperatively) is predominated by the restoration of bone to its baseline strength if mechanical loaded over time. The mineralized callus is replaced with mature mineralized bone and remodeling changes the area to its original size and shape. It is this final phase of bone repair that returns the bone to its previous biomechanical state [14]. Through this complex sequence of healing events bone exhibits one of its unique properties: it is the only tissue in the body that has the ability to undergo perfect, restorative repair.

4.2 Regulation of Bone Graft Substitutes

Allograft bone products are regulated as human tissue if they comply with the jurisdictional requirements. For example, in the United States those requirements are codified in 21 CFR 1271 and USPHSA Section 361, and in the EU similar requirements are codified in a series of Commission Directives (EU), e.g., 2004/23/EC and 2015/565, 566/EC. It is noteworthy that allogeneic cell tissue-based graft substitutes, which contain processed bone and bone marrow cells derived from the same donor, are often referred to as the third generation bone graft products and are approved as stand-alone graft materials under USPHS 361. Bone graft materials like synthetic CaP bone substitutes and xenograft, that do not meet these narrowly defined requirements, are regulated in the United States and in other countries as either medical devices or biologics.

The aforementioned principles and concepts provide the basis for the design and rationale of many bone grafting options and materials (Table 4.1).

Table 4.1 Bone grafting options advantages and disadvantages [6]

Bone graft (BG)	Advantages	Disadvantages
Autologous	• High osteoconductivity	Need of an additional surgery
	• Highest degree of biological safety	
	• No risk of immune reaction	
Xenografts	• Architecture and geometric structure resemble bone	• Possible disease transmission and potential unwanted immune reactions
	• Well documented	• Lacks viable cells and biological components
	• Predictable clinical outcome	• Resorption rate is highly variable
	• Slow bio-absorbability preserves augmented bone volume	• Reduced future availability due European regulatory changes?
Natural biomaterials	Similarity to native extracellular matrix	Mechanical properties poor -biodegradability less controllable
Synthetic polymers	• Tuneable physicochemical properties	• Low cell attachment
	• Tuneable degradability	• Timing of absorption (alteration of mechanical properties)
		• Release of acidic degradation products
Synthetic bioceramics	• High biocompatibility	• High brittleness
	• Osteoinductive properties	• Low ductility
	• Chemical similarity with bone	• Not predictable absorption
	• Stimulation of osteoblast growth	
Composite xenohybrid substitutes	• High similarity with human cancellous bone	• Cleaning and sterilization process partially alters biological performances
	• Higher bioactivity	• Limited clinical data
	• Tailored degradation rates	
	• Incorporation of active biomolecules	

4.3 Autograft: The "Gold Standard" Bone Graft

Autograft, the patient's own bone, has long been considered the standard to which all other graft materials are compared. Bone is one of the tissues with the innate ability to regenerate in adult humans. With respect to bone grafting, autograft or harvesting bone tissue from one anatomical site to another site on a recipient, is considered the "gold standard." Autograft earns this designation since it possesses all the properties essential for bone formation: osteogenicity, osteoinductivity, and osteoconductivity. By containing these three elements, this enables autograft to be replaced more rapidly by host bone wherever it is implanted and provides the advantages of being histocompatible, non-immunogenic and minimizing any infective-related risks [3, 8]. Introducing some combination of these biological properties has been an aspirational goal for allograft, synthetic, and any other bone grafting materials.

Structural considerations—Autogenous bone grafts may be used in structural indications primarily if they have been sourced from an area of cortical bone. Cortical grafts are used for their structural capacity, such as compression and torsion, and are frequently used in conjunction with some form of hardware to provide additional stability or structure (i.e., plates and screws, spinal implant). Cortical bone may be used as an onlay or as an inlay graft. Onlay grafts are used frequently due to the ease of handling and placing of the graft and where there is a structural need to increase the volume of bone at the repair site. The ability to position this three-dimensional graft plays a role in graft success, incorporation and ability to sustain structural forces, i.e., spinal fusion [1]. Inlay grafts are mainly used to fill a bone defect within the anatomical skeleton. Cancellous autograft does not provide immediate structural support but is the most widely utilized form of autogenous bone graft, often harvested from the surgical site and referred to as "local bone," or from the iliac crest Because these grafts lack mechanical strength they may be used to augment bone healing and fill voids in conjunction with a more structural implant, i.e., titanium or PEEK (polyetheretherketone). Mechanical properties of cancellous bone can be enhanced using impaction but the volume available is usually inadequate for most clinical applications [15]. It should also be noted that mechanical properties of autogenous bone may vary widely and are determined by the harvest site and the patient age [16].

Clinical considerations—Autogenous bone that is harvested from a second surgical site generally results in significant harvest site morbidity. Autograft can be taken surgically removed from the iliac crest, distal femur, fibula, proximal or distal tibia, proximal humerus, distal radius, chin, ribs, mandible and some parts of the skull, all resulting in morbidity, higher complications rate, scarring and additional operative time [1, 6]. Many articles have reported the drawbacks of autograft are related to the harvesting process and time, donor site complications including infection and pain, increased blood loss, and limited volume of material [3]. Local bone can be from intramedullary reamings from the femur or tibia, or bone remnants salvaged from decortication and drilling during spinal fusion procedures. The quality of the autograft may also be related to the patient and its source site due to factors such as graft components, volume and complications [5]. It has been reported that major and minor complication rates from bone harvesting are 8.6% and 20.6%, respectively [14]. The limited autograft availability, volume and configuration, may impact utility in larger defects. Autografts may be harvested from cortical or cancellous bone and the successful choice may be related to the survival and proliferation of the osteogenic cells, conditions at the recipient site, type of graft chosen, handling of the graft, and shaping of the graft during the operative procedure [1]. If rapid osteogenesis is desired then cancellous bone is preferred. Cancellous grafts are commonly used

in fracture nonunion, dental defects, maxillofacial defects, spinal fusion, and other small bone defects [1].

Regenerative and long-term clinical considerations—The structural and density of cortical bone naturally limits the number of osteoprogenitor cells that reside in the tissue. Remodeling of cortical bone is mainly mediated by osteoclasts and the revascularization and remodeling processes are hampered by the dense architecture of cortical bone [3]. As a result cortical bone grafts will undergo a longer-term incorporation where there is osteoclastic surface resorption and appositional bone growth. This appositional bone growth over a necrotic core is the dominant means of incorporation. As a result the remodeling process for a cortical bone graft may take years depending upon the graft size and volume and the implantation site [3]. Cancellous bone is osteogenic due to its larger surface area and osteoblasts rapidly incorporate new bone and revascularization happens relatively quickly [13]. Cancellous bone's lack of early mechanical strength may be outweighed by its ability to rapidly produce new bone and develop mechanical strength. Therein lies one of the conundrums in bone grafting: which is more important—immediate mechanical strength or long-term restoration of a more natural bone structure?

Additional Autogenous Regenerative Considerations—Bone graft materials are often supplemented with bone marrow aspirates (BMAs) and platelet-rich plasma (PRP). The use of these materials is intended to enhance regenerative properties and thus clinical outcomes.

Platelets are a rich source of endogenous growth factors (GF), and platelet-rich plasma (PRP), also referred to as autologous growth factor (AGF) concentrate, is a readily available source of the GFs from the patient. PRP has been used in sports medicine and orthopedics based upon reports of encouraging tissue healing, and in tissues with low healing potential and treated in other specialties such as dermatology, ophthalmology, plastic and maxillofacial surgery, neurosurgery, urology, and cardiovascular surgery. The interest in this biological procedure as an augment to bone implants with bone and soft tissue has resulted in musculoskeletal treatments using PRP on cartilage, bone, muscle, tendon, and ligament regeneration. In a review article which looked at the role of PRP as an augmentation procedure, the conclusion was that knowledge on this topic was still preliminary and prospective randomized clinical trials were needed to support the potential of this approach to improve outcomes in implant integration [17]. In a review of bone grafts for spine fusion, the studies referenced did not demonstrate improved fusion or fusion rates even when PRP was used to enhance autograft [18].

Bone marrow is a rich source of nucleated cells, including MSCs, and bone marrow aspirate (BMA) has been utilized in orthobiologic repairs as a means of providing the cellular elements for tissue regeneration. Bone marrow can be harvested from various sites in the body including the posterior and anterior iliac crests, distal femur, proximal tibia, and distal humerus. As a matter of common practice, BMA is often concentrated in the operating room using specialized centrifugation and concentrating devices to produce bone marrow aspirate concentrate (BMAC) which can increase the nucleated cell (MSC) concentration by up to ten-fold over BMA. This concentrated cell preparation is then applied onto a scaffold or carrier and used to treat cartilaginous lesions, bone defects, and tendinous injuries [19]. It is estimated that 5–10% of all fractures result in delayed union or nonunions and this complication can result in significant additional cost per patient [19]. One of the focused applications of BMA is in recalcitrant bone nonunions where 94% successful arthrodesis was achieved when BMA was combined with allograft compared to conventional autologous cancellous bone graft alone. In patients with compromised healing capacity, the use of BMAC in ankle fractures in subjects with diabetes demonstrated greater union rates versus patients receiving

autologous bone graft [20]. Patients with diabetes treated with BMA reported a union rate of 82.1% with minimal complications compared to the 62.3% union rate with major complications in patients receiving iliac bone graft alone. BMAC has also shown promise in osteochondral lesions due to its potential benefits in healing hyaline cartilage, but also in increasing integrative potential with autologous osteochondral transplantation [21]. Some evidence also indicates that BMAC alone may result in improved defect filling, border repair integration and surface tissue repair [22].

A more recent autologous cell enhancement are adipose-derived stem cells. Adipose-derived stem cell (ASC) treatments focus on isolating the ASCs from adipose tissue. Adipose tissue is harvested percutaneously and mechanically processed to remove lipids and disrupts adipose tissue clusters while maintaining the stromal vascular fractions that are rich in adipose-derived stem cells (ASC). By volume, the number of MSCs may be greater in adipose tissue than in BMAC [23]. The current literature on ASCs is limited regarding preparation, formulation and clinical therapeutic potential as numerous studies are ongoing. ASCs are regulated as a biologic and are not approved for any clinical indication.

4.3.1 Allograft: "the best alternative" to Autograft

Allogeneic bone grafts refer to bony tissue that is harvested from one individual and transplanted to a genetically different individual of the same species [2] [24]. Allografts, which can include tissue from both living human donors and cadavers, represent the second most common bone grafting material used worldwide [6]. Since autogenous bone grafting involves the risk of complications and the amount of graft that can be harvested is limited, allograft is accepted as the next best alternative.

Allografts do have the potential risks of eliciting an immunoreaction, transmitting infection or communicable disease, and higher failure rates over long-term use [3]. The potential for an immune response to allograft bone may be mitigated by narrowing histocompatibility differences [24]. Historically, one of the concerns with allogeneic bone has been the risk of disease transmission due to bacterial and viral contamination. Fresh, unprocessed human allograft is seldom used because of this potential for triggering a clinically significant immune response combined with the risk of disease transmission. Particularly concerning are diseases which are difficult or impossible to detect. For these reasons, most allograft bone used today are processed in established tissue banks where Federal regulations, industry standards and state-of-the-art processing and testing technologies have all but eliminated the risk of infection from allograft tissues. For example, the potential for HBV or HCV transmission has been reduced to one occurrence every 500 years [25, 26]. Processing bone has consequences as, in producing an arguably safer graft, elements essential to bone formation may be removed from the tissue. For example, decellularization removes donor histoincompatibility but removes osteogenic cells required for bone formation. Processing to remove pathogens may also reduce osteoconductive and osteoinductive potential [7]. Allograft safety is further insured by sterilization which can also significantly impact the structure and biologic properties of the allograft. It is intuitive that the goal in processing of allograft is to strike a balance between eliminating the risk of disease transmission while preserving critical properties necessary for the performance of the allograft. Overall, allograft bone is generally considered osteoconductive and weakly osteoinductive [8]. The integration process of allogeneic bone is similar to what is undergone by nonvascularized autogenous bone graft, where the volume of graft influences the time of incorporation [7].

Structural considerations—Allogeneic bone is available in many forms including morselized cortical, cancellous, and corticocancellous, cortical and cancellous grafts, osteochondral, whole

bone segments, demineralized bone matrix and more recently introduced cellular allografts. Cancellous and cortical allografts are generally available through musculoskeletal tissue banks. The morselized or cube shaped cancellous bone grafts have little mechanical strength, are not suitable for use in applications requiring load bearing and are primarily used to fill voids. Cortical allografts can provide structural support and confer rigid mechanical properties and are widely utilized in applications where immediate load-bearing resistance is required [27]. An advantage of cortical bone is its natural elasticity and ease of incorporation at the graft-host interface. Furthermore, cortical allografts can be machined and customized to enhance its ease of use and meet the demands of specific applications. For example, machined cortical allografts are commonly used as structural supports in spinal fusion as "biological cages" in forms such as cervical spacers and as femoral ring allografts [28]. There is a note of caution as the mechanical performance of structural allografts may be a disadvantage, impacted by the effects of tissue processing or preservation (i.e., freeze-drying) and the less predictable effects on strength thru fatigue and postoperative remodeling on strength [27]. The freeze-drying process can reduce the mechanical strength of bone tissue by 20% [16]. Graft fractures occur and are often related to the anatomical site of implantation. Another factor that may influence the structural integrity or uniformity in tissue is processing methods.

Clinical considerations—Cancellous bone allografts are one of the most commonly used types of allografts. Since most cancellous grafts possess little mechanical strength, they are mainly used in applications such as graft extenders in spinal fusion procedures and void fillers for partial bone defects, including large depressed articular fractures, rather than segmental bone defects. Commonly freeze-dried cancellous allograft is used to pack defects in revision arthroplasty or after curettage of benign lesions [29]. As cancellous allograft is devitalized bone, it is used primarily as an osteoconductive substrate or autograft bulking agent since it lacks key elements necessary for bone formation. Used alone, cancellous allograft may result in poor clinical outcomes including failed or delayed arthrodesis or fracture. Nonetheless, used properly, allografts have a high success rate and have demonstrated similar fusion rates to autografts in spinal procedures [13].

Cortical allografts, like autograft, can be used as an onlay graft. They have been used to treat pathologies such as fibrous dysplasia, giant cell tumor, surface-based lesions resections, segmental defects after trauma or resections for sarcoma, and replacing bone lost in revision total joint arthroplasty [29]. Cortical allografts are used in spinal augmentation (spacers and wedges in various forms and designs) for filling large skeletal defects where immediate load-bearing resistance is needed. Whether the graft is frozen or freeze-dried, the cortical allograft undergoes incorporation via creeping substitution, a process initiated by osteoclastic resorption followed by sporadic formation of new appositional bone via osteoconduction [3]. The volume of allograft used affects the remodeling and conversion into host bone. Even after 5 years post implantation, large allografts may show only 20% "internal repair" Has occurred [30]. The persistence of an unincorporated and necrotic core can develop microfractures, decrease bone mineral density, and result in reduced mechanical strength and failure many years after implantation [30]. In opening wedge osteotomies, allograft performed well in mean time to union with a low loss of correction, but union time versus autograft was longer with a higher delayed or nonunion rate [31]. Allograft bone in primary arthrodesis and osteotomy procedures in foot and ankle surgery compares favorably with autograft in terms of fusion rates and clinical outcomes with fewer complications [32]. In anterior cervical discectomy and fusion, cervical spacer allografts have the highest fusion rate for the relatively low cost to other bone grafting options with equivalent clinical outcomes [33]. In revision anterior cruciate

ligament (ACL) procedures, the bone tunnel frequently requires grafting so allograft unicortical dowels have been employed to provide either a single or two-stage technique [34, 35].

Regenerative and long-term clinical considerations—Compared to autografts, a slower sequence of events happens in the remodeling process. In some cases, the allograft may be delayed by a host inflammatory response which causes fibrous tissue formation around the graft which entraps the allograft and results in incomplete resorption for many years post implantation. Fresh frozen and freeze-dried bone allografts induce more prompt graft vascularization, incorporation and bone regeneration than fresh allograft [1]. The process of freeze-drying bone offers safety advantages but it renders the tissue substantially weaker. Its use as a morselized graft in impaction bone grafting in hip surgery has demonstrated 86–90% graft survival rates 7–8 years post implantation [30].

4.3.2 Demineralized Bone Matrix (DBM)

Demineralized Bone Matrix (DBM) is an osteoconductive and osteoinductive bone graft substitute composed of allograft bone with the inorganic materials removed [36]. Produced from ground human cortical, corticocancellous or cancellous bone this highly processed allograft tissue has 40–100% of the mineral removed from the organic bone matrix by exposure to mild acid, a process that leaves the collagens, non-collagenous proteins, and growth factors naturally present in bone largely intact [3]. Demineralization renders the bone osteoinductive by "unmasking" the inductive proteins present in the bone extracellular matrix. This residual matrix is frequently combined with a carrier to improve handling and performance properties. As a result, the DBMs available for clinical use come in a variety of forms ranging from moldable and injectable putties, pastes, pastes with chips, strips, and sponges.

The collagenous and non-collagenous proteins preserved throughout the demineralization process create an osteoconductive scaffold. Furthermore, the DBMs are osteoinductive by virtue of the remaining growth factors, which are directly correlated with the preparation method and can include bone morphogenetic protein (BMP), fibroblast growth factor, transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF) [13, 36]. Natural donor-to-donor variability, as well as the differences in tissue processing methods, results in an intrinsic range of osteoinductive potential in commercial DBMs as evidenced by testing in validated osteoinductivity assays [37].

Structural considerations—DBMs lack mechanical strength and are used primarily for filling bone defects or as autograft extenders. The carriers used with particulate DBMs minimize DBM migration and can provide resistance to displacement during lavage or motion. In an attempt to provide some structural integrity, incorporate additional osteoconductive scaffold, slow remodeling rate and maintain or increase the overall bone volume, some DBM preparations incorporate cancellous allograft chips or cortical fibers. Demineralized "sponges" produced from blocks and strips of cancellous bone are able to maintain their inherent shape and volume at the site of implantation and are frequently combined with bone marrow aspirate (BMA) to provide a more biologically complete graft.

Clinical considerations—DBM products have a long history of safe and effective clinical use and their benefit to patients are widely recognized. However, the lack of industry standards for the production and performance of DBM products has resulted in vast inconsistencies in these products. There are several attributes to consider when selecting a DBM product. DBMs vary with respect to the donor sources, how they are extracted, processed, formulated, and packaged. This variability has important clinical consequences and offers assorted advantages or disadvantages. Clinicians are encouraged to understand the processing and testing of the DBM products through a review of materials provided by the manufacturer or the comprehensive literature available on the subject (Table 4.2) [37].

Table 4.2 Considerations in the selection of DBM products

Consideration	Issues
Safety	• Clinical experience
	• Tissue processor
	• Sterility
	• Regulatory status
Composition	• DBM concentration
	• Carrier
	• Processing methods
Biological properties	• Osteoinductivity testing
	• Lot-to-lot variation
Forms	• Putty
	• Paste
	• Gel
	• Strips
Handling properties	• Graft extender
	• Injectable
	• Moldable
	• Limited migration

The biologic properties of DBM graft materials that should be considered include: (1) risk of disease transmission or immune-mediated rejection; (2) remodeling and incorporation into the host tissue; (3) promotion of surface-level bone growth or osteoconductivity; (4) osteoinductive activity; and (5) foster controlled new bone growth [37]. DBM physical properties to consider may include: (1) increased total volume of the graft when used as a graft extender; (2) effectiveness as a scaffold with good mechanical strength; and (3) handling characteristics which facilitates graft manipulation and placement and; (4) resistance to migration or displacement by irrigation and movement [37]. The carriers used with DBMs and the DBM:carrier ratios greatly affect the biologic potential, clinical handling and utility in various indications [38]. Some forms of DBM are more ideal for mixing with autogenous bone or BMA, strips or sponges may be used where some support or scaffolding are required, and some may be more amenable for defect filling or injected thru a syringe. Familiarity with the various forms of DBM (putties, pastes, injectable putties, pastes with chips, strips, and sponges) will aid in the proper selection to optimize the desired clinical outcome.

Regenerative and long-term considerations—There are no industry standards for demineralization processes employed by various producers and different processes may result in variable essential components in the DBM depending on the processes used. Inconsistency is compounded by the donor-to-donor variability discussed previously. Some of the processes used may even result in partial or complete inactivation of the BMPs resulting in reduced product efficacy [39]. One of the reasons for the variability of DBM products is many DBMs are regulated in the U.S. as tissue products, where the focus is on tissue safety and traceability versus processing, formulation, and indications for use.

DBMs have also been used in revision ACL procedures to address tunnel defects or tunnel widening in two-stage procedures [40]. However, much of the effort to improve the regenerative properties of DBM has been focused on spinal applications where these products have gained wide acceptance. Numerous clinical reports in spinal applications describe DBM efficacy as an alternative to autograft [13]. There have been reports of graft collapse due to the inferior structural composition in lumbar fusion [13] and a similar report regarding graft collapse in cervical fusion [28] illustrating the need for additional long-term clinical studies.

Animals have proven useful to define best practices in the use of DBMs in spinal fusion. In a posterolateral fusion model in athymic rats, DBMs were able to successfully demonstrate fusion at a higher rate compared to other allograft alternatives but also lot variability was also evident [41]. Animal studies have also demonstrated a range of effectiveness in the different commercial DBM products [39].

The effort to improve DBM performance is proceeding on several fronts. The addition of different materials to DBM, i.e., nanofiber-based collagen scaffolds, are under investigation to enhance the biological or mechanical performance of the DBM [42]. Future products may incorporate carriers that optimize the environment to recruit cells, encourage angiogenesis, facilitate early healing and produce new healthy bone [37].

To reduce the graft rerupture rate and improve tendon-to-bone healing in ACL

reconstruction procedures, a recently developed technique combined a proprietary DBM formulation, BMA, and autologous bone collected during tunnel drilling to produce a graft mixture to fill the tunnels prior to the tendon graft passage [43].

4.4 Cellular Allografts: An Autograft Alternative

Recently, cellular allografts or cellular bone matrices (CBMs) have been developed to provide mesenchymal or osteoprogenitor cells for osteogenic grafting without the need for an autograft harvest. These products are designed to provide the scaffold, the signals, and the cells. CBMs are made using proprietary techniques which are aimed to preserve mesenchymal stem cells (MSCs). Because they contain live cells, the source and living status of the donor, the screening process, and the review of donor medical and social history is more stringent than traditional allografts [44]. Screening also includes serological and microbiological testing. The MSCs may be sourced from cancellous bone or adipose tissue. Within 72 h of death, a cadaver's donor bone is harvested and after initial evaluation, processing begins with isolation of cancellous bone chips and the milling and demineralization of cortical bone. The cancellous bone is treated to minimize immunologic issues, cryopreserved and then mixed with demineralized bone or cancellous bone ships. Other processors use cadaveric adipose tissue as the source of the human MSCs which are then mixed with demineralized bone and then cryopreserved. Total nucleated cellular concentration ranges from 250,000 to 3,000,000 cells/cc. where the number of actual mesenchymal stem cells may be a small fraction of the total. In the U.S., most commercially available CBMs fall under the HCT/P guidelines and are regulated as tissue, but some have encountered regulatory challenges due to unsubstantiated claims regarding composition and clinical efficacy.

Structural considerations—CBMs lack mechanical strength and are used in void or implant filling. Handling is similar to that seen with particulate bone grafts combined with BMA.

Clinical considerations—CBMs are regarded as premium products that are challenging and costly to produce, but nonetheless an attempt to provide the triad of components required for the ideal bone healing product. In a comparative test in posterolateral fusion in athymic rats, CBMs did not fuse as well as other grafting options [41]. Concern has been raised whether the athymic model is appropriate for testing a viable cell product. The products in the marketplace have a great variability in cellular concentration and the ideal cell concentration and type of cells may not be present [20]. This cell concentration issue can also be manifested in the donor age at the time of graft harvest as there is an age-related decline in the number of cells. The source of CBM, whether from bone or fat, may affect their ability to undergo osteoblastic differentiation in the human in vivo environment [44]. Other considerations include the number of viable cells that survive storage, thawing, transplantation, presentation at the site and recipient immunogenic response.

Regenerative and long-term considerations—CBMs conceptually have much promise and have captured much interest. In challenging foot and ankle arthrodesis and revision nonunion procedures, a specific CBM formulation demonstrated a high union rate [45]. The key to differentiating its regenerative capacity is the viability of the cell component, concentration, viability, and ability to differentiate. So, donor sourcing and selection can be an important factor. As a tissue product, any additional processing needed to ensure adequate cell concentrations would create regulatory challenges. Additionally, the manufacturing of these products requires some unique logistical challenges for tissue processors so broad availability may be limited. Additional studies will identify the efficacy of this product.

4.5 Xenografts: An Unlimited Biologic Alternative

Xenografts or heterologous grafts are bone grafts sourced from non-human sources. Xenografts are harvested from one individual and transplanted into another individual of a different species [1]. Xenografts are sourced from coral, porcine, or bovine sources. The main advantage of xenografts is the theoretical unlimited supply if they can be processed to be safe for human transplantation. Concerns with immunogenicity and disease transmission, including prions, are the primary objections to the use of xenograft tissue. To denature the proteins responsible for immunogenicity and remove lipids, some use chemical methods to remove these elements prior to terminal sterilization [30]. Other processes use high temperature thermal cycling to deproteinize the bone tissue. These processes destroy the arrangement of collagen fibrils and crosslinking [16]. These processes also effect osteogenic and osteoinductive properties since the remaining tissue is primarily a calcified hydroxyapatite scaffold and collagen. Deproteinization using milder reagents can preserve the inherent collagen architecture and can influence bone regeneration. Other processes just seek to remove cells and lipids which can elicit an immune reaction but maintain the collagen integrity and mechanical properties.

Structural considerations—Most xenografts, like allografts, are available as powders or particulates and some structural forms have also been developed. Though the mechanical properties of the xenograft bone may be similar or superior to allograft bone, the advantages are the almost unlimited availability and a mechanically consistent product. The source of the xenograft tissue is usually controlled and so a higher probability of being reproducible.

Clinical considerations—Xenograft cancellous or cortical bone incorporates in a similar manner to allograft. The clinical use of xenografts has demonstrated success as a graft extender when mixed with autogenous tissue. There have been reports of early graft resorption, loosening, and foreign body reactions, as well as satisfactory clinical reports when the xenograft is mixed with bone marrow [1, 30]. In some studies the xenograft trabecular graft was second best to the autogenous cancellous bone [1].

Regenerative and long-term considerations—To increase the biological activity of xenograft bone, the potential of xenogeneic DBM has been investigated with mixed early results. With respect to processing, multiple physical, chemical, and enzymatic methods have been used to remove antigens, but preserve the extracellular matrix and mechanical and functional characteristics. These acellularization methods may result in reducing or eliminating immunogenicity of xenografts which may enhance graft incorporation [1]. Xenografts are globally regulated as medical devices so commercialization requires demonstration of product safety and clinical efficacy. Additionally, commercial availability of xenograft bone is limited globally.

4.5.1 Synthetic Bone Graft Substitutes

Most synthetic bone graft substitutes are approved as autograft extenders, requiring that they be combined with the patient's own bone or bone marrow principally because they lack viable osteogenic cells which embody one of the triad of essential elements present in autograft.

Approval for commercial sale requires that the supplier indicate the composition and intended use of each product which should serve as a guide to the end user.

4.5.2 Calcium Sulfate

Calcium sulfate (plaster of Paris) is considered a first generation osteoconductive bone graft substitute and is still in use as a bone void filler or autograft extender. It is generally used as a nonsetting pelletized material that lacks the mechanical properties to provide sustained structural support [46]. The primary applications for calcium sulfate bone graft substitutes have been as a nonstructural bone void filler or for antibiotic delivery [47].

4.5.3 Calcium Phosphates

With a long history of safe and effective use, calcium phosphates (CaPs) are the largest category of synthetic bone graft materials. Differences in chemical composition, crystallinity, processing, shape, and porosity produce a spectrum of CaP graft materials with variable physical properties, and dissolution or degradation profiles. CaPs include β-tricalcium phosphate (β-TCP), hydroxyapatite (HA), combinations of β-TCP and HA, CaP collagen composites, ion-substituted CaPs, CaPs combined with other calcium salts, and CaP Cements. Recent reviews provide relevant and comprehensive information on these materials and useful background for the topics presented here [48, 49].

Often referred to as osteoconductive, synthetic bone graft substitutes, including CaPs, are medical devices approved as bone void fillers with the following use restrictions: intended for use as a *"resorbable bone void filler for voids or gaps that ARE NOT (*emphasis added) *intrinsic to the bone structure and…should not be used to treat large defects that in the surgeon's opinion would fail to heal spontaneously."* Other restrictions may include that the bone graft substitute is for use only as a graft extender to be combined with autograft or bone marrow aspirate (Table 4.3) [33, 50].

Table 4.3 Characteristics of bone graft options (Reproduced with permission from [29])

Graft, substitute, or augment	Comments	Advantages and disadvantages	Common applications	Product examples
Cancellous autograft	Gold standard No disease transmission	Limited availability Donor-site morbidity No structural support	Curettage and cancellous grafting	n/a
Cortical autograft (fibula, etc.)	Rapid union, osteogenic	Technically demanding if vascularized Donor-site morbidity	Segmental diaphyseal defects	n/a
Cancellous allograft	Fresh frozen has some growth factors preserved Freeze dried lowest likelihood of disease transmission	10–15% infection rate Limited shelf life (~1 year at −20 ° C)	Curettage and cancellous grafting	MTF™ cancellous chips
Cortical allograft	Structural support Osteoarticular with ligaments and tendons	10–15% graft failure 10% nonunion Immunogenic	Intercalary osteoarticular strut	MTF™ osteoarticular distal femur graft
Calcium Sulfate	"Plaster of Paris"	Rapid resorption (4–12 weeks) Inconsistent setting wound drainage	Can be mixed with antibiotics	Osteoset™ (Wright medical, TN)
Calcium phosphate	High compressive strength (4-10× cancellous bone)	Slow resorption (95% resorbed in 26 to 86 weeks)	Periarticular voids	Norian SRS™ (Synthes, PA) Hydroset™ (Stryker, MA)
Demineralized bone matrix (DBM)	Variable osteoinductivity based on formulation	No structural support possible reaction to carrier (e.g., glycerol)	Commonly mixed with allograft void filler	Grafton DBM™ (Osteotech, NJ)
Bone morphogenetic proteins (BMP)	rhBMP-2 and rhBMP-7 approved for humanitarian device exception	No osteoconductive or structural support Requires collagen or bone mineral substrate osteolysis, ectopic bone	No role in oncologic setting due to risk of increased oncogenesis	Infuse™ (Medtronic, TN) Op-1™ (Stryker, MA)
Polymethyl methacrylate (PMMA)	Unlimited supply, low cost	Excellent in compression Poor mechanical properties in shear/ tension	Currettage and till periarticular voids	Simplex™ (Stryker, MA) Palacos™ (Heraeus, Germany)

This section of the review will focus on the characteristics and use of the subset of bone graft substitutes that provide stabilization and mechanical support at the site of application. Biological characteristics and biomechanical properties, material handling and physical form, along with patient requirements, are the primary factors in determining which bone graft substitute is most appropriate for the intended application.

4.6 Items to Consider

4.6.1 Porosity

The degree of material porosity plays a critical role in the biology and clinical performance of a bone graft substitute. Porosity creates the local mechanical and biological environment while providing a necessary route for neovascularization, and migration, proliferation and phenotypic expression of regenerative mesenchymal stem cells, pre-osteoblasts and osteoblasts [11, 51]. Importantly, within comparable classes of biomaterials, porosity generally correlates with mechanical strength and resorption rates [11]. For these reasons, porosity should be one of the most important material characteristics in determining the suitability of a bone graft substitute for a clinical indication.

It is sufficient to consider pore size into two general groups: micropores (<5 μm pore size) and macropores (>100 μm pore size) [52, 53]. Whereas microporosity is considered necessary for predictable bioresorption [54], it is generally accepted that macropores >100 μm are required for osteoconductivity [48, 49] the accepted "optimal" macroporosity of a bone substitute that allows for ingrowth of bone is between 150 and 500 μm [55]. An osteogenic response, including BMP-induced osteogenesis, is reportedly better when the pore size is >300 μm [52, 56, 57].

Within a scaffold, in vivo bone formation involves creating an environment that brings together the essential elements for bone formation. Where microporosity and surface roughness enhances attachment, proliferation and differentiation of anchorage dependent bone forming cells [49] and has proven benefit as a surface treatment to improve implant fixation [58, 59], higher porosity is conducive to osteogenesis [51]. Larger pores allow vascular ingrowth and support cellular activities that enhance bone ingrowth and complete integration and potential remodeling of the graft materials after surgery.

Other factors, such as the rate of degradation and the mechanical performance of the scaffold, both of which are profoundly affected by material porosity, should be taken into account when suitability is assessed. Scaffolds fabricated from ceramics with a high degradation rates should not have high porosity (>90%) as material must persist at the site long enough to conduct new bone formation otherwise the reparative process could be compromised [52].

Where the rate and extent of bone ingrowth clearly correlates with the percent porosity of a bone graft substitute, there is no consensus regarding which type of porosity provides the optimal environment for bone formation. The rate and quality of bone integration have been related to a dependence on pore size, porosity volume fraction, and interconnectivity, both as a function of structural permeability and mechanics [51].

4.6.2 Interconnectivity

Another important factor that determines the effectiveness of porosity, and therefore the effectiveness and ultimate fate of the graft material, is the way the pores connect with each other and provide a pathway for fluids, vasculature and cells to infiltrate the innermost aspect of the material. The pores may be either interconnected or "dead-ends," features that can be reproducibly introduced into the material by design and process during manufacturing. In general, bone graft materials, and specifically calcium phosphates

with interconnected pores have a distinct advantage over biomaterials containing dead-end pores. Interconnectivity allows for ingrowth of new bone which in turn provides better long-term stability at the graft-hoist interface [53], provide for consistent incorporation of the bulk graft material and more uniform remodeling.

4.6.3 Mechanical Stability, Structure, and Biology

Mechanical stability in the microenvironment of bone grafting is essential, though often not afforded the consideration given the trinity of osteoconductivity, osteoinduction, and osteogenesis [60]. The unmet challenge is to bring all four elements together in a single material, achieving clinically meaningful and sustainable mechanical properties in a material that has the pore size, structure and cellularity to support bone formation. Optimization of performance must also include consideration of the morphology of a porous structure on the material and in situ mechanical properties [11]. These properties vary greatly among the various biomaterials and depend on their porosity, micro, and macro architecture. Significant parameters that differentiate the indications of the different scaffolds and biomaterials are the quality and density of the host bone bed and the local biomechanical demands of the graft site. Moreover, bone graft biomechanics evolve parallel to the progress of the graft incorporation and remodeling. All these issues have formed the bases for intense research efforts to improve initial mechanical properties of the available biomaterials as well as to guarantee the presence of a mechanically reliable construct throughout all the remodeling phase of fracture healing. However, there is an upper limit in porosity and pore size set by constraints associated with mechanical properties. An increase in the void volume results in a reduction in mechanical strength of the scaffold, which can be critical for regeneration in load-bearing bones. For example, an increase of the total porous volume from 10 to 20% results in a fourfold decrease in material mechanical strength [53]. Whether it is possible to increase pore size while at the same time maintaining requisite mechanical requirements of the material depends on many variables, including the intrinsic material properties, processing, and ultimately the practical suitability for the intended use. The operative or nonoperative techniques of fracture stabilization and fixation, and the chemical, structural, and mechanical properties of the graft material all interact and affect the bone repair process. The mechanical environment where a graft material is expected to serve as a substrate supporting bone formation has equal significance to the biologic properties of the graft itself in making decisions regarding which synthetic bone graft substitutes are appropriate for specific clinical applications.

4.6.4 Biomechanics of Synthetic Bone Graft Substitutes

The property that is most often used to characterize the mechanical behavior of bone substitutes is their compressive strength. Bone graft substitutes that are used a graft extenders or void fillers that by regulatory approval are described as a "r*esorbable bone void filler for voids or gaps that ARE NOT (*emphasis added) *intrinsic to the bone structure*" usually lack in vivo mechanical strength. These materials are suitable for using as a stand-alone graft (per the Instructions for Use) for smaller, stable voids and they will eventually resorb, remodel, or become incorporated into the host bone.

The biomechanical properties of the CaP bone graft substitutes are highly variable [61]. Unless in a cementitious or highly sintered forms they are brittle, often friable with little compressive or tensile strength. They do not provide significant biomechanical support.

TCPs are less brittle than HA but resorb quickly, rapidly losing what little mechanical strength they may have when implanted. Although increased porosity and pore size facilitate bone ingrowth, porosity compromises the structural integrity of CaPs resulting in an even further reduction in mechanical properties. When used in a site that is mechanically unstable or is otherwise compromised, these nonstructural CaP void fillers generally require some form internal or external stabilization to achieve successful arthrodesis.

4.6.5 Synthetic Bone Graft Substitutes with Mechanical Strength

The structural, physiological, and biomechanical properties of calcium sulfate, calcium phosphate and methacrylate cements should guide decisions regarding their safe and effective use. These materials may provide immediate, short-term, and often long-term mechanical stability but those essential characteristics come at the expense of other properties, notably lack of porosity and ability to remodel into bone in an appropriate time frame. Importantly, when comparing bone graft substitutes, including cements, similar chemistry or material composition does not necessarily indicate that materials have identical or even similar properties.

4.6.6 Calcium Sulfate Cements

Injectable calcium sulfate (CaS) cement has been used successfully to treat a variety of clinical indications, including tibial plateau fractures [62], benign bone lesions [63], plated proximal humeral fractures [64]. CaS cements are biocompatible, biodegradable, osteoconductive and integrates well with osseous tissues. The cement cures isothermally in less than 5 min and achieve a compressive strength of approximately 40 MPa, comparable to cancellous bone. An average pore size of approximately 60 μm (range 10–250 μm) has been reported for CaS cement [65]. However, CaS cement is brittle and is absorbed more quickly (6–12 weeks) than other cements. Degradation is by dissolution and reabsorption and occurs independent of bone formation, raising concerns that absorption of the graft and subsequent loss of mechanical strength could occur before bone healing is complete, increasing the risk that healing will fail or result in a pseudoarthrosis. These concerns naturally limit most clinical use of CaS cements to filling voids where structural support is not needed.

4.6.7 Calcium Phosphate (CaP) Cements

The two principal types of calcium phosphate (CaP) cements differ based on the end product of the setting reaction: apatite [$Ca_5(PO_4)_3OH$] or brushite [$CaHPO_4.2H_2O$]. Both are regarded as osteoconductive and have the highest compressive strength (10–30 MPa) of the resorbable synthetic bone graft substitutes [65]. Apatitic CaP cements have received the most attention as they represent the chemically and mechanically stable crystalline form of CaP phase found in bone. Also, apatitic cements set at neutral pH whereas brushite cements are stable below pH 4.2 and form a metastable calcium phosphate phase at physiological pH [66]. Brushite cements resorb much more quickly than apatite cements [67].

CaP cements are generally nonporous or slightly porous upon setting. With only a few exceptions CaP cements typically have <4% total porosity with pore size ranging between 40 and 100 μm [65]. Applying the conventional wisdom that pores >100 μm are necessary for osteoconduction, CaP cement pore size and overall porosity would be considered suboptimal but still qualify as nominally osteoconductive.

CaP cements, especially apatitic cements, can remain in place for years. Lacking macroporosity they undergo surface resorption or degradation from the outside in. Incorporation of faster resorbing calcium salts (e.g., calcium sulfate or β-TCP) into apatitic cements accelerate the cement resorption by creating porosity as these more quickly resorbing materials are removed, leaving behind voids in the remaining apatitic CaP. These cements achieve their highest mechanical strength upon implantation and complete setting and lose strength as they become porous. Likewise, CaP cements that have the highest porosity on setting have a faster resorption rate and inversely have the lowest initial compressive strength, typically <1 MPa.

CaP cements have been used in several clinical indications, including fractures of the tibial plateau [68], calcaneus [69], distal radius [70], femoral neck [71], and humerus [72].

Non apatitic cements, due to primarily to their faster resorption, have clinical applications where the loss of mechanical strength over time is acceptable. Notably they have been used to stabilize traumatic fractures of the spine [73, 74].

4.6.8 Methacrylate Cements

A discussion of the mechanical properties of bone graft substitutes would not be complete without including methacrylate cements. Polymethyl methacrylate (PMMA) and bisphenol A-glycidyl methacrylate (Bis-GMA) cements are widely used in orthopedics and dentistry as they provide immediate mechanical stabilization and pain reduction. They represent a unique class of biocompatible, non-resorbable materials that do not remodel into bone, and in the case of PMMA used primarily as anchoring cement in joint arthroplasty [75]. Both PMMA and bis-GMA are used in clinical applications that overlap with CaP cements, notably vertebroplasty and balloon kyphoplasty for treating pathological fractures of the vertebral body [76, 77].

Bis-GMA cement blended with bioactive glass 45S5 [78, 79] has a compressive strength comparable to dense cancellous bone [65]. This blended material has enhanced hydrophilicity compared to PMMA or unsubstituted bis-GMA and exhibits strong bonding to bone. In addition to use in vertebroplasty [77] blended bis-GMA has been used to augment pin fixation in distal radius fractures [80].

4.7 Summary

Bone grafting is one of the most common surgical methods used to augment bone regeneration. Bone grafts and bone graft substitutes continue to evolve with the emergence of orthobiologics which continues to produce new therapies in bone healing [36]. The goal to be “as good as” autografts and provide the triad of osteoconduction, osteoinduction, and osteogenesis is still elusive in commercially available products. The various categories of options reviewed in this paper all have varying degrees of these properties (Table 4.4).

The “diamond concept” for a successful bone repair response, gives equal importance to mechanical stability and the biological environment. Overall, the diamond concept encompasses a broader appreciation of the factors such as the presence of osteoinductive mediators, osteogenic cells, an osteoconductive matrix (scaffold), optimum mechanical environment, adequate vascularity, and any existing comorbidities of the patient [60, 81]. Developments continue to describe the use of additional materials (ions), bioactive molecules, and cells which are described in numerous review articles [3, 36, 82]. and biologic approaches. All of these future developments will need to be supported by high-quality clinical trials before they become the standard of care treatments and totally replace autograft (Fig. 4.2).

Table 4.4 Key bone healing properties of bone grafting options (adapted from [36])

Bone grafting options and their specific osteoconductive, osteoinductive, and osteogenic properties					
	Osteoconductive	Osteoinductive	Osteogenic	Advantages	Disadvantages
Cortical autograft	+	+	+	• Bone healing	• Limited quantity
				• All components of triad	• Quality donor dependent
				• Immunocompatible	• Harvest site morbidity
				• No infection	
Cancellous autograft	+++	+++	+++	• Bone healing	• Limited quantity
				• All components of triad	• Quality donor dependent
				• Immunocompatible	• Harvest site morbidity
				• No infection	
Cortical allograft	+	±	-	• No donor site morbidity	• Infection transmission
				• Ability to mix with bioactive agents	• Reduced biological or biomechanical properties
					• Sterilization may be required
Cancellous allograft	+	±	-	• No donor site morbidity	• Infection transmission
				• Ability to mix with bioactive agents	• Reduced biological or biomechanical properties
					• Sterilization may be required
Demineralized bone matrix	+	++	-	• Some bone healing	• Variability from lot-to-lot
				• Availability	• Bone healing variability due to processing
				• Ease of use	
Calcium ceramics	+	-	-	• Availability	• No biologic activity
				• Ability to mix with bioactive agents	• Low mechanical strength
				• Long shelf life	
				• No infection risk	
Bone marrow aspirate	-	+	+++	• Biologic activity	• Requires a scaffold
				• Minimal donor site morbidity	
Bone morphogenetic protein	-	+++	-	• Bone healing	• Potential for complications
				• Availability	
Platelet-rich plasma	-	+	+	• Biologic activity	• Lack of efficacy in bone
				• Immunocompatible	

+, activity; −, no activity; ±, activity depends on preparation process

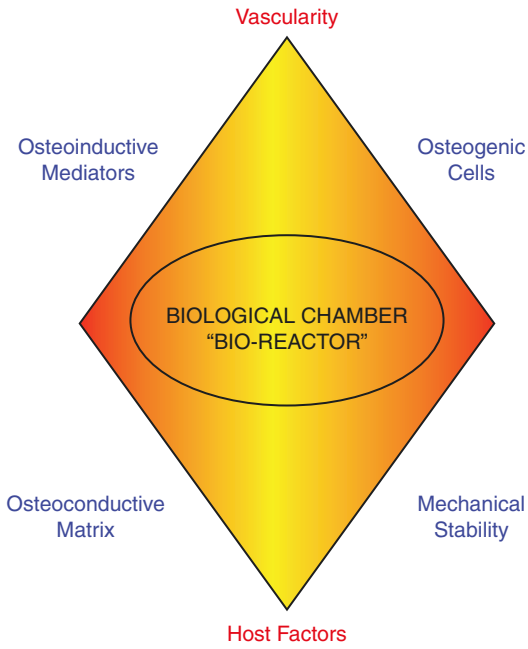

Fig. 4.2 Diamond concept of bone healing [81]

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