INTRODUCTION

The need for bone grafting to replace skeletal defects or augment bony reconstruction has become more prevalent recently because of enhanced capability to salvage major bone loss. There are many bone graft options available for the surgeon, including autografts or allografts, either of a cortical or cancellous structure, each of which has specific biological and mechanical properties. Some grafts are more dependent on the host bed for successful incorporation, such as freeze-dried allografts, while others, such as vascularized autografts, are capable of incorporating into the host bone under adverse physiological conditions. An understanding of the biological events and biomechanical aspects of autografts and allografts is important in understanding the processes that influence the incorporation of the bone graft into the host skeleton.

DEFINITION OF BONE GRAFT TERMS

The first level of describing bone grafts refers to the origin of the graft (1,2). A graft transplanted from one site to another within the same individual is called an autograft. Allografts are tissues transferred from two genetically different individuals of the same species. Xenografts are transplanted from one species to a member of a different species. An isograft is transferred from one monozygotic twin to the other. This is usually described for laboratory experiments when tissue is transferred from inbred, genetically identical strains of animals. The anatomical placement of the graft is an important descriptor of bone transplantation. A graft transplanted to an anatomically appropriate site is defined as orthotopic, whereas if it is transplanted to an anatomically dissimilar site, it is termed heterotopic. Additionally, the graft may be described as cortical, cancellous, corticocancellous, or osteochondral. Fresh grafts are transferred directly from the donor to the recipient site. These grafts are usually autografts because of the immunogenetic potential of fresh allografts. The graft may be vascularized with its own blood supply or it may be nonvascularized. Allografts are usually modified or preserved to reduce immunogenicity before transplantation. These modification processes include freezing, freeze-drying, irradiation, or chemomodification (1).

BONE GRAFT FUNCTION

The biological activity of bone graft is a result of two functions: osteogenesis and mechanical support (Table 1). Bone regeneration usually requires three processes: osteoinduction, osteochonduction, and osteogenic cells (3). Osteogenesis is the physiological process whereby new bone is synthesized by cells of the graft or cells of host origin. Surface cells that survive transplantation of either cortical or cancellous grafts can produce new bone (4–7). This new bone may initially be important for the development of callous during the early phase of bone graft incorporation. Cancellous bone, because
of its large surface area, has a greater potential for forming new bone than does cortical bone. Osteoinduction provides osteogenic potential by inducing the host bed to synthesize new bone. This is achieved by the recruitment of mesenchymal cells that differentiate initially into cartilage and then bone-forming cells. This much-studied process is achieved through the recruitment of graft-derived proteins that drive this physiological process. The most completely studied of these low-molecular-weight peptides are the bone morphogenetic proteins (BMPs). A number of BMPs have been identified, and some are already in clinical use. The most active BMPs in bone include BMP-2, -4, and -7 (8,9). These proteins play an important role in the differentiation of stem cells into osteoblasts and are also important in fracture healing and bone remodeling (10). The presence of BMP in bone has been demonstrated both experimentally and clinically when the matrix has been demineralized and sequentially extracted to remove any antigenic materials (3,11). This activity does not require viable graft cells because it is property of bone matrix. It is present in all bones, whether autografts, or allografts that have been preserved using a method that does not destroy the BMP, such as autoclaving. Osteoinduction is provided by all grafts as well as by biomaterials such as ceramics. This graft function provides the three-dimensional configuration for the ingrowth into the graft of host capillaries, perivascular tissue, and osteoprogenitor cells from the recipient.

Bone graft incorporation requires an interaction of osteoinduction and osteoconduction, described as creeping substitution. This ultimately leads to the replacement of the graft by host bone in a predictable pattern under the influences of load bearing (3). Bone graft incorporation is a sequence of well-balanced processes between the graft and the host bed. Under most circumstances, all of the functions described above are in play. The initial inflammatory response results in the migration of inflammatory cells and fibroblasts into the graft. The hematoma formation that occurs enhances the release of both cytokines and growth factors. Osteoinduction drives chemotaxis, mitosis, and differentiation of the host osteoprogenitor cells. By d 5, chondrocytes are usually recognizable; and osteoblasts can be seen by the 10th posttransplantation day. Host blood vessels quickly invade the graft through existing haversian and Volkmann canals and also provide the osteoclasts that resorb the surfaces of the graft. Both intramembranous and endochondral bone formation usually occurs on graft surfaces. Osteoconduction proceeds in large cortical or cancellous grafts for many years and ultimately results in the resorption of the original graft tissue and replacement with new host bone. The remodeling that results

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is a response to weight bearing. A successful outcome depends on a balance between revascularization and osteogenesis and the graft’s response to applied loads. A biological balance must be achieved between the graft and the host bed to ensure successful incorporation. Clearly, bone graft incorporation is a dynamic interplay of the biological function of the bone graft, the graft environment, and the host–graft mechanical interactions.

**BIOLOGY OF BONE GRAFTS**

Bone graft incorporation is a prolonged process that involves a sequence of complex steps involving the interrelationship of the graft and host (12–13). Autografts in general are more rapidly and completely integrated into the host than allografts. However, because of the morbidity associated with autograft harvesting and inadequate material, allografts have been utilized (14). Allografts, although potentially functional, may remain an admixture of graft and host bone for many years. Because of the prolonged remodeling activity of the graft, it is usually difficult to define the absolute end point of incorporation. However, one can consider a bone graft functional when it can withstand the normal loads of activities of daily living. Although there have been many approaches to understanding the incorporation of bone grafts such as radiographic, histological, and biomechanical methods, it is the histological process of bone graft incorporation that reflects the biological events of this process.

**BIOLOGY OF AUTOGRRAFTS**

**Cancellous Autografts**

Hematoma formation and inflammation are rapidly seen in the early phases of bone autograft incorporation. Surface osteocytes may survive and are important in synthesizing new bone during the initial phases of incorporation (15). The inflammatory infiltrate is minimal and consists mainly of small mononuclear cells. Revascularization of the graft occurs rapidly and is characterized by considerable capillary ingrowth (16). Both osteoclasts and osteoblast precursors are seen early. The graft-derived BMPs play an important and central role in inducing host mesenchymal stem cells to migrate into the graft and differentiate into osteoblasts. There is an early, dynamic equilibrium established among inflammation, revascularization, osteoinduction, and osteoconduction, that by 4 wk provides active bone resorption and formation throughout the graft. Following the ingrowth of capillaries, osteoconduction proceeds rapidly. Osteoblasts line the edges of the dead trabecular during this stage, and new bone formation takes the form of immature woven bone on the surfaces of the original graft trabeculae. Seams of osteoid tissues surround the core of necrotic bone of the graft. Ultimately, hematopoietic cells accumulate within the transplanted bone and form a viable new bone marrow. The graft is well underway to complete resorption and new bone formation by 6 mo after transplantation. The remodeling and complete replacement of the nonviable graft bone is directed by Wolff’s law. By 1 yr, the process of incorporation is usually complete and the graft is completely resorbed and replaced with new viable bone (Fig. 1).

**Cortical Autografts**

Cortical grafts differ from cancellous grafts mainly in their rate of revascularization, their mechanism of repair, and the completeness of the repair (1,13,17). The overall process of incorporation proceeds in a manner similar to that of cancellous autograft incorporation; however, because of the density of cortical bone, a cortical graft has a decreased rate of revascularization and remodeling. Vascular penetration of the graft by host tissue occurs only after resorption of the dense cortical surface by osteoclasts is initiated. The slower revascularization of these grafts in contrast to cancellous grafts usually results in the cortical bone becoming more radiolucent and significantly weaker than normal bone. This reduction in strength may last from months to years after transplantation, depending on the graft size and the implantation site. Unlike cancellous grafts, bone incorporation of cortical grafts occurs
by appositional bone growth over a necrotic core (18–22). Cortical grafts may be involved in load bearing sooner than cancellous grafts if the graft–host junction is stabilized by adequate fixation and heals rapidly to the host (20).

Initially, following transplantation there is little histological differences between autologous cortical and cancellous grafts. There is a similar rapid inflammatory response and hematoma formation. The pattern of revascularization that follows slowly behind osteoclast resorption follows preexisting haversian canals from the periphery to the interior (1,21,22). By 2 wk, widespread resorption of the grafts is well underway, and it increases during the initial 6 mo. As discussed earlier, the result of this process is a generalized decrease in the strength of cortical autografts. There is a fine balance between graft resorption by osteoclasts and new bone formation by osteoblasts. There is, however, a normal physiological uncoupling of bone resorption and bone formation as a result of the differential activity of osteoblasts and osteoclasts. Osteoclasts can resorb bone at a rate of 50 μm/d, whereas osteoblasts can synthesize new bone tissue at a rate of only 1 μm/d. This uncoupling may cause cortical bone grafts to fail, even under the best circumstances. Any additional significant uncoupling of this process will cause delay in the incorporation process. Lack of immediate blood supply in nonvascularized cortical autografts leads to the death of most of the graft’s osteocytes. This may reduce the osteoinductive potential of these grafts, although osteoconduction is present throughout the process of incorporation. Normal marrow may appear in the remodeled bone graft by 9 mo following implantation.

Because nonvascularized cortical autografts result in osteocyte death and the reduction in the efficiency of function, vascularized cortical autografts have been used to reconstruct deficient bone. Vascularized cortical autografts provide an immediate blood supply and experience only a transient ischemia. Many studies have demonstrated the superiority of vascularized grafts in the immediate, short- and long-term postoperative period (23–25). Clinical studies have demonstrated a 50% reduction in failure rate when these grafts are used to reconstruct defects larger then 6 cm in length when compared to nonvascularized autografts. Over 90% of the donor osteocytes may survive the transplantation. Rapid healing is seen at the graft–host interface, and the uncoupling of bone resorption and formation is diminished. Vascularized cortical grafts are therefore not usually weakened by resorption. In general, these grafts during the first 3 mo are stronger, stiffer, and less porous than nonvascularized autografts.

Fig. 1. Photomicrograph of a cancellous autograft 1 yr after transplantation to a canine ulnar defect, demonstrating complete replacement of the graft with viable host bone (hematoxylin and eosin ×25).
Because vascularized cortical autografts may be incorporated independently of the host bed, they may function in biologically deficient host environments. Remodeling of cortical autografts is a complex process that requires graded controlled loading. During the initial phases of incorporation, protection of the graft–host interface with the use of internal or external fixation is crucial to prevent the formation of fractures and failure of the grafts.

**BIOLOGY OF ALLOGRAFTS**

Bone allografts have been used effectively in clinical practice because of the inadequate supply and donor site morbidity associated with autogenous bone grafts. Bone allografts have been shown to be immunogenic and to demonstrate a higher failure rate when compared to autografts. The immunogenicity of these grafts appears to play an important role in the successful incorporation of bone allografts (26–32). The antigens most responsible for the recognition of the graft by the host are those of the major histocompatibility complex (MHC) (32). The MHC antigens most important in this process are those of the Class I and Class II molecules. These major alloantigens are recognized by the host responding T-lymphocytes. The cells of all musculoskeletal tissues display Class I MHC antigens, and some cells may display Class II antigens. Minor histocompatibility antigens may also be present on the surface of cells and can be important in the late rejection of bone allografts. In general, the mechanism of immune rejection of bone grafts is similar to that of parenchymal organs. These mechanisms may include cell-mediated toxicity, antibody-mediated toxicity, and antibody-dependent cell-mediated cytotoxicity. Many experimental studies have shown that bone allografts may invoke all of these responses in vivo (32). Furthermore, these studies have shown that when histocompatibility differences are reduced by either matching tissue types or modifying allografts to reduce immunogenicity, allograft acceptance is improved. However, the exact mechanism and importance of the immune response in bone allograft incorporation is unclear. These studies suggest that the immune response delays and may destroy the initial osteoinduction phase of the bone graft, and that any blood vessels present in the allograft are quickly surrounded by inflammatory cells, occluded, and result in rapid necrosis of marrow cells and osteoblasts. Additionally, animal studies have demonstrated that bone allografts do induce graft-specific antibodies (30,31,33). Mismatched fresh grafts appear to invoke humoral responses to Class I antigens more than when frozen allografts are utilized (34). Other studies have demonstrated that T-cells may be activated by MHC-mismatched allogeneic bone (31). Studies have demonstrated that bone marrow cells may be the primary means of inducing the immune response, while other studies suggest that cells within the cortex are also capable of activating allogeneic T-cells (32). Taken together, these experimental studies provide data that allografts can induce an immune reaction in the host. However, the clinical significance of these responses is still unclear. Notwithstanding this controversy, it is the capability of fresh bone allografts to evoke a rapid immune response that results in destruction of the graft that has led to the use of preserved modified allografts (3).

**Cancellous Allografts**

Preservation of cancellous allografts reduces the immune response and does improve graft acceptance. Preservation of allograft using freezing or freeze drying has been demonstrated to improve incorporation of the graft (3,35). Revascularization and remodeling of these processed cancellous allografts are delayed compared with fresh autografts, but osteoinduction and osteoconduction are generally preserved and incorporation of the graft can be complete. The overall process is slower, although a similar sequence of events occurs when compared to autografts. Initially, hemorrhage and a cellular infiltrate is seen. It usually reaches its peak during the first 2 wk. The processes of resorption of the grafts, osteoinduction and osteoconduction, proceeds until viable bone is present that can withstand load bearing. The clinical incorporation of massive frozen allografts is improved when rigid internal fixation is provided. Other preservation techniques such as decalcification and demineralization, although somewhat less effective than freezing or freeze drying grafts in regenerating bone, do
provide to some extent osteoinduction and osteoconduction. Demineralization of grafts results in the loss of their inherent mechanical strength, while morselized cancellous frozen or freeze-dried material may retain some resistance to compression. Because processed cancellous allografts remain an admixture of necrotic graft bone and viable host tissue for a prolonged period of time, their clinical use has been confined to filler material for cavitory skeletal defects.

Cortical Allografts

Because fresh cortical allografts also invoke an immune response, clinical use of cortical allografts is generally confined to processed allografts that have been either frozen or freeze-dried (3). The processing of these allografts also plays an important role in ensuring the safety of bone allografts. The chance of transmission of communicable diseases—mainly human immune virus and hepatitis B and C—has been dramatically reduced by appropriate donor screening and sterilization techniques (36). The transplantation of cortical allografts free of marrow and blood products has resulted in safe outcomes. When allografts are modified and stabilized to the host with internal fixation devices, the biological process of incorporation proceeds in orderly steps. Osteoclastic resorption is the initial event and provides the means for vascular invasion of the haversian and Volkmann’s canals by host capillaries and osteoprogenitor cells. The process is delayed compared to fresh autografts, but new appositional bone growth occurs by osteoconduction. Cortical allografts may be substantially weaker than autografts for as long as 2 yr after surgery (20,37). However, results from retrieved human bone allografts demonstrate that bony union occurs at the host graft cortical–cortical junction by means of bridging external callous that originates from the periosteum of the host bone and extends for up to 3 cm on the surface of the allograft (20,38). Junctional discontinuities are filled with fibrovascular tissue that progresses to woven bone, and eventually haversian bone under the influence of Wolff’s law is formed. Central to successful bone allograft functioning is the stability of the graft–host junction. Even in experimental models using fresh allografts, when interfaces were successfully stabilized, fresh allograft host junctions healed (31). When intimate host–graft contact was not achieved or the union was not stable, these interfaces invariably failed.

Although freeze drying reduces the inflammatory response to bone graft, it also reduces the mechanical properties of the graft and results in a significantly weaker graft (39). Sterilization of bone by irradiation of more than 30 kGy may destroy any osteoinductive function. Chemosterilized, autolyzed, antigen-extracted allogenic bone, although providing inductive capabilities, has little strength (3).

In an effort to improve incorporation of cortical allografts, new methodologies have been developed. Perforation of the graft from a biological standpoint increases the available surface area for ingrowth and ongrowth of new bone (40,41). Additionally, it provides easier access to the intramedullary canal. Several studies have demonstrated that perforated grafts indeed have more new bone ingrowth when compared to similar nonperforated grafts (40,41). These grafts are also more porous in the 6 mo following surgery because of the increased area availability to osteoclasts for bone resorption. This method may also help revascularization of the allograft by providing channels for ingrowth of host blood vessels. However, the overall repair process is not that different from that in standard cortical grafts. Perforation of the graft has raised some concern in the past about the possibility of stress rises at the perforation sites (42,43). Studies have shown, however, that the strength of the bone immediately following drilling is not diminished significantly in either compression or bending (17,40). There is a decrease in the overall strength after 4 wk, but this was associated with the increased porosity of the graft rather than drill holes. By 6 wk after transplantation, the strength of the graft returned to that of the nonperforated grafts. Recent studies have combined partial demineralization with perforation of the graft and have demonstrated a positive effect on overall osteoinduction while preserving some of the biomechanical properties of this graft (41). Overall cortical allograft incorporation is a complex process that has significant variables which influence the ultimate incorporation and function of the graft.
BIOMECHANICS OF BONE GRAFTS

Mechanical performance of a bone graft in vivo as has been discussed is a function of the intrinsic property of the graft and the properties of the graft–host interface (44). The intrinsic properties of the graft are a function of its geometry as well as its composition and includes properties such as fracture toughness, yield strength, and its elastic modulus. If the graft possesses the same mechanical properties and geometry as the host bone, it may function in a clinical setting almost immediately (45). In a situation where the mechanical properties of the graft are inferior to that of the host, additional graft material should be used or the construct may be augmented with internal fixation until remodeling occurs and the graft can provide load-bearing function. A bone graft must be biologically incorporated into the host in order to function successfully in load bearing. Incorporation of the graft is related directly to the mechanical and biological properties of the graft–host junction. A well-incorporated graft such as a cortical graft bridging a femoral defect shares some of the load of the femur and remodels to the requirement of the host. If the same graft does not heal to the host, aberrant loads may be experienced and failure of the construct usually occurs. In a large segmental defect, it may be necessary to augment the graft–host junction with either internal or external fixation in order to protect the graft while it is being remodeled. Studies have shown that a dominant parameter in determining the material properties of bone is volume fracture of tissue in any given sample (44,46). This value, also known as porosity, is related directly to the stiffness of the tissue (as a third power of porosity) and yield strength (as a second power of porosity) (46). As a result, small changes in porosity result in large changes in the material properties of bone. Cortical bone grafts initially may have as little as 5–10% porosity. The biological sequence of events proceeds and, as graft incorporation occurs, large increases in porosity may result. This significantly reduces the strength of the bone graft. This critical period may last for as long as 2 yr and ultimately, through the biological processes described earlier, result in the successful incorporation of the bone graft. However, if the process becomes uncoupled and bone resorption significantly outstrips bone accretion, and the graft–host interface is inadequate, rapid failure of the bone graft may occur.

Cortical and cancellous grafts have different biomechanical properties because of the different biology of each graft type. Porosity of cancellous grafts may be as high as 70–80%, leading to material strength roughly equivalent to 4% of that of cortical bone (44). The strength of cancellous bone grafts increases as new bone is laid down on the preexisting trabecula. However, until the new graft is successfully integrated into the host, it is critical that fixation methods sustain a significant portion of the load. In both cancellous and cortical grafts, the remodeling process is driven by functional loading under the influence of Wolff’s law. It is important to achieve a balance between appropriately protecting the graft during the remodeling phase while allowing the bone graft to experience physiological loads necessary for remodeling to occur. Internal and external systems have been developed that provide protection for the graft while allowing some loading by the patient. As has been discussed previously, bone allografts do evoke an immune response. In order to reduce the immune reaction, modification of the graft has become a common practice as a method of preservation and sterilization. These modifications, however, have a profound effect on the biomechanical properties of the graft. Freezing has been shown to have minimal effects on the biomechanical properties of the graft, while freeze drying significantly reduces both the yield strength and stiffness of the bone graft (47). Other methods have varying effects. Autoclaving has been shown to produce a dose-dependent decrease in both strength and stiffness of bone (48). Irradiation, although effective in destroying bacteria at relatively low doses (<20 kGy), does not usually destroy viruses at this dose. However, virucidal doses greater the 30 kGy significantly reduce the material properties of the bone graft. Complete demineralization of the bone graft, although significantly reducing the immunogenicity of the bone, results in loss of all of virtually its mechanical properties. The ultimate success of any bone graft, however, requires a balance between its biological functions and biomechanical properties.
CONCLUSIONS

A successful clinical outcome for a bone grafting procedure requires an understanding of the biological and mechanical environment into which the graft will be placed. Although the biological aspects of bone graft incorporation are critical in determining this outcome, the technical aspects of the surgery are as important. A clean, well-vascularized host bed is critical in providing the satisfactory host environment. Wide excision of scar tissue, treatment of infection, protection of the blood supply, and satisfactory soft tissue coverage is mandatory. The selection of appropriate graft material for the desired clinical function will also help determine the clinical outcome of bone grafting. Central, however, in the successful incorporation of the bone graft is a stable fixation and contact between the host bone and the graft. Experimental studies have demonstrated that when the host–graft interfaces are tightly apposed and fixed with internal fixation, the interfaces healed whether the grafts were autogenous, allogeneic, allogeneic, fresh, or frozen. Even under stable conditions, but without closely apposed host bone, graft tissue retrieval studies have demonstrated that interfaces did not heal and had a profound effect on the biological characteristics of the graft (38). When no apposition or stability was provided at this interface, bone grafts have uniformly failed. It is therefore important to provide intrinsic and stable graft–host fixation and satisfactory soft tissue coverage. The bone graft must also be protected from full weight bearing until remodeling enables it to function fully in a loaded environment. When the appropriate bone graft is selected and the surgical technique is synergistic, bone grafts do incorporate both biologically and functionally and provide clinically functional load bearing.

REFERENCES


