■ ASPECTS OF CURRENT MANAGEMENT

Bone allografts

WHAT THEY CAN OFFER AND WHAT THEY CANNOT

C. Delloye,
O. Cornu,
V. Druetz,
O. Barbier

From Université
Catholique de
Louvain, Cliniques
Universitaires St-Luc,
Bruxelles, Belgium

Bone allografts can be used in any kind of surgery involving bone from minor defects to major bone loss after tumour resection. This review describes the various types of bone grafts and the current knowledge on bone allografts, from procurement and preparation to implantation. The surgical conditions for optimising the incorporation of bone are outlined, and surgeon expectations from a bone allograft discussed.

Bone allografts have long been used as a natural substitute to repair skeletal defects. They offer an attractive alternative to bone autograft because their supply is less limited, they allow structural restoration of the skeleton, and their surfaces support bone formation.

The demand for allograft has expanded rapidly, driven by the increasing number of revision arthroplasties being carried out in an ageing population, and by newer trends of minimally-invasive surgery, particularly in the spine, where the need for bone grafts or substitutes is growing fast. The quantity of allograft available has increased sharply over recent years. It is the most-used bone substitute in Europe. This mirrors the situation previously observed in the USA, where about 800 000 such grafts are used each year.^{1,2}

There are an increasing number of bone substitutes available on the market. However, bone is unique, being a composite of fibre and mineral, not just a mineral.

The increased demand for bone allografts makes their supply difficult when the femoral heads of living donors are the only source.^{3,4} Nationwide operating tissue banks, however, recover allografts from living donors and also from organ and post-mortem donors.^{5,6} Procurement from an organ donor is the most likely means of obtaining a long bone for orthopaedic oncology.⁷ Large bone segments can be further processed into smaller units for their use in other clinical indications. However, the safety of any bone allograft remains a major concern. To exclude donors, who are at risk for the transmission of disease, standards have been issued to maximise the quality and safety of the allograft by professional

organisations⁸ and, more recently, by the European Union.⁹

Cancellous bone autograft remains the optimum standard to which every substitute must be compared. Autogenous cancellous bone has a major advantage in that it supplies not only a three-dimensional bony lattice, but also the osteogenic cells that will form the new bone. The drawbacks of using autograft include pain at the donor site, the potential for local complications such as haematoma or fracture, and the limited supply.¹⁰ The high incidence of morbidity during the harvest of autogenous graft makes the acquisition of material from other sources more desirable.

This review discusses the current scientific understanding of small and large bone grafts that have been preserved and not revascularised. Various aspects of bone grafting will be discussed. A bone graft can be categorised by its individual properties. The success of the graft may depend on the quality of the bone bed from which most of the revascularisation arises. The level of safety associated with bone allografts will vary depending on the donor. In addition, the processing, preservation and sterilisation of bone may influence its biophysical properties. Different kinds of bone allograft are available to the surgeon. Corticocancellous bone allografts will be distinguished from both osteoinductive bone allografts and massive, structural allografts.

Properties of the bone graft and the host bed

A bone graft can be considered *osteogenic* if it contains living osteogenic cells.⁷ This occurs only when autogenous bone has been implanted immediately, or when a substitute has been enriched with cultured autogenous bone cells.

■ C. Delloye, MD, PhD,
Orthopaedic Surgeon,
Professor, Chairman
■ O. Cornu, MD, Orthopaedic
Surgeon
■ V. Druetz, MD, Orthopaedic
Surgeon
■ O. Barbier, MD, Orthopaedic
Surgeon
Department of Orthopaedic
Surgery
Cliniques Universitaires St-Luc,
10, Avenue Hippocrate, B1200
Bruxelles, Belgium.

Correspondence should be sent
to Professor C. Delloye; e-mail:
delloye@orto.ucl.ac.be

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Bone is considered an *osteoconductive* material⁷ when its structure can serve as a support for cells that migrate from the host and differentiate into osteogenic cells. New bone formation will then occur within the scaffolding. Osteoconduction can be assayed and measured experimentally.¹¹ This property is not bone specific, as substitutes, such as ceramics, have the same capacity.

A bone graft is *osteoinductive* when it can induce the differentiation of mesenchymal cells into osteoblasts.¹² This property is only seen *in vivo* after heterotopic implantation of bone graft into a non-osteogenic site such as a muscle.¹² Unless it contains a preserved osteoinductive factor, no bone graft material can be considered osteoinductive.

The goal of using bone allograft is to initiate a healing response from the host bed that will produce new bone at the host-graft interface and within the porous body of the graft material. Besides the properties of the graft itself, the vascularity of the bed and the mechanical stability of the graft are of vital importance. For optimal incorporation of the graft, the host bed should either already contain enough pre-osteogenic or osteogenic cells, or must be enriched by a source of these cells, such as autograft or autogenous bone marrow.¹³⁻¹⁵ The bed must be prepared to leave bleeding bone. The host-graft interface should be stable in order to allow vessels to grow into the graft. These factors are largely reliant on the surgeon and emphasise the importance of the surgical approach and the preparation of the host site.^{16,17}

Transmission of disease by bone grafts

Implanted bone allograft can transmit disease, and safety is a prime consideration.¹⁸⁻²¹ Among the potential transmissible diseases, viruses and prions are the most difficult to track. The transmission of hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) through bone grafts has been well documented.¹⁹⁻²⁶ Implants of dura mater have caused Creutzfeldt-Jakob disease, but this has not been seen with bone and related tissues. In order to reduce the risk of transmission of these agents, careful selection of donors is vital until specific diagnostic tests become available.²⁷ Although HIV is important, HCV is more prevalent and carries more risks for transmission.^{18,22-24} Bacterial contamination of the allograft can occur and may be life-threatening.²⁸

Procedures designed to ensure the supply of safe bone^{7,20} include guidelines on donor selection, tissue quarantine and tissue processing. Current standards of tissue banking incorporate safety and quality as their main features, and have been adopted in Europe.⁹ Any European tissue bank must be controlled by its national authority and all tissue must be traceable. With stringent donor selection and multi-step screening, the risk of viral transmission is remote and is even less likely after tissue processing.^{5,20} However, it should be borne in mind that tissue banks screen a limited

number of known viruses and there still remains the possibility of transmission of unknown pathogens.

Source of bone

Living donors. The most convenient source of bone is the femoral head of a patient undergoing a hip replacement. The patient is screened before operation and again after four to six months. During this period, the bone is quarantined. In the United Kingdom, 48% of potential live donors of a femoral head are rejected after medical assessment, and of those accepted, a further 22% will be rejected after medical screening.⁴ In our bank, 7032 femoral heads were collected between 1997 and 2003. The rejection rate after screening the medical history was 16%.

Multi-organ donor. Long bones are procured under sterile conditions in the operating theatre after organ explantation. This is the major source of bone for a nationwide tissue bank. The mechanical properties of the bone are excellent as the donors are young. During the last two years, we have received bone from 59 organ donors with a mean age of 44 years (17 to 68). Procurement has always been by an orthopaedic surgeon. The selected donor is screened with the various recommended tests together with other assays, using nucleic acid amplification technology, which can reduce the window period for seroconversion.²¹ In order to offer maximum biological safety, the harvested bones are quarantined until the donor can be further screened by testing the organ recipients at three months.⁷

Post-mortem donors. Testing the tissue from cadaver donors raises two concerns. A false negative result presents the possibility of transmission of an undetected viral disease, whereas a false positive may cause the donor tissue to be discarded. The use of live blood samples should be favoured when screening a donor. If this is not possible, nucleic acid amplification technology assays will always be used in the screening.²⁹ As tissue retrieval is performed in mortuaries, bacterial contamination is higher than in an operating theatre, and the subsequent rate of discard is higher. At our institution, there were only 15 selected post-mortem donors, with a mean age of 63 years (45 to 72) in the last 20 months.

Bone processing

Processing describes any activity carried out on recovered tissue. Bone can be processed under strict aseptic conditions or be sterilised at the final stage, usually with irradiation. One of the purposes of processing is to shape and size the graft material for its intended use. It is also necessary to inactivate and remove any harmful agents from the bone to reduce the risk of disease transmission, since only unprocessed, fresh-frozen allografts have been documented as sources of viral infection in recipients of bone grafts.¹⁹⁻²¹ Among the most important steps in processing bone is the elimination of bone marrow and cellular debris with fluid and detergents, which, by its clearing effect, will improve the osteoconductive capacity of the bone.¹¹ Fluid

pressurisation allows the full penetration of the inactivating or eliminating agent in a large bone.^{30,31} Ethanol, acetone and ether are often used, as they have been shown to inactivate coated viruses such as HIV and the hepatitis viruses.³² Hydrogen peroxide has long been used as a bleaching agent, and has been shown to be virucidal and bactericidal as a consequence of its capacity for forming free radicals. Osteoinductivity of the bone is preserved if exposure to hydrogen peroxide lasts for less than 60 minutes.³³ It should be emphasised that tissue processing of any kind does not allow tissue pooling from various donors, nor can it replace the careful selection of donors.

The preservation of bone and the influence of the sterilisation technique

The main ways to preserve bone are through freezing at -0°C, in liquid nitrogen at -196°C, or freeze-drying.^{7,34} Freeze-drying has a logistical advantage in that it allows further storage of the tissue at room temperature. Bacterial sterilisation is achieved at the usual dose of 25 kGy if the bone has been properly managed before final sterilisation. However, this dose is not virucidal for HIV,³⁵ whose risk prevention should rely on screening procedures and inactivating treatments.

Deep-frozen bone, whether irradiated or not, retains its original mechanical properties. Non-irradiated freeze-dried bone also retains its mechanical strength, but irradiation of dried bone will substantially reduce its mechanical capacity.³⁶ In the frozen state, damage to collagen is reduced because of the smaller amounts of free radicals generated by ionisation of frozen water, whereas at room temperature more free radicals are produced.³⁷ In freeze-dried material most of the water content has been removed, but ionisation has a direct effect of breaking the collagen chains and hence the mechanical resistance.³⁸ Freeze-dried and sterilised bone can provide some mechanical support, mainly in compression, but because it is less resistant it must be used in an area that will be mechanically protected, with or without osteosynthesis.

Frozen bone can be handled and reshaped like normal bone and is fully workable, but freeze-dried bone, unless rehydrated in saline, is brittle like ceramic, and is not fully workable.³⁹ The surgeon must be aware of the material properties of freeze-dried bone.

Types of bone allografts

Corticocancellous bone allografts. Corticocancellous bone has only an osteoconductive property but no osteogenic or osteoinductive capacity. It is prepared from femoral heads or from the extremities of long bones, and can provide some mechanical support, depending on its mode of preparation. It is the most widely used form of allograft.

Any bone allograft can be enriched with growth factors or cultured stromal stem cells in order to stimulate vascular invasion of the graft and new bone formation.⁴⁰⁻⁴³ Supplementation with these expensive biological materials

appears to promote the incorporation of the bone into the host in experimental conditions, but adverse results have also been observed.⁴⁴ More experience with these techniques is required before they can be recommended.

A modern tissue bank will have various preparations of bone available for the surgeon.

Unprocessed, frozen femoral heads procured under sterile conditions. These are the main source for many local and regional tissue banks in Europe. They are delivered unprocessed as a full head, or have been cut under sterile conditions into two or more units.

Processed corticocancellous bone. This comes either from an osteoarthritic femoral head of a living donor undergoing a hip replacement, or from an epiphysis of an organ donor. Processing will include defatting and removal of bone marrow. Delipidation has been shown to promote better osteoconduction.^{11,45} The complete removal of blood and debris allows the Rhesus factor of the donor to be ignored in a young female recipient.^{46,47} Processed bone can be machined in various forms, including morsellised disc, a dowel or a wedge. Bone which is processed under sterile conditions can be stored, frozen or freeze-dried, and if not processed in this way, it will usually be irradiated.

We recommend the use of freeze-dried bone for small defects (< 5 cm³), but prefer frozen material for large or uncontained cavities.

Corticocancellous bone morsels. These can be used either as frozen or as dried material. However, impaction into the femur is easier and faster with freeze-dried morsellised bone than with frozen bone.^{48,49}

Osteoinductive bone allografts. Demineralised bone matrix is the only allograft that has an osteoinductive capacity. The basic action of demineralisation on cortical bone was discovered by Urist in 1965¹² and led, after four decades, to the isolation of the bone morphogenetic proteins (BMP). Once demineralised, cortical bone still contains collagen, bone proteins (among which are the BMPs), glycoprotein and proteoglycans. However, for osteoinduction to occur, there must be the presence of a BMP, the carrier (most often collagen type I) and the responding (inducible) cells. The major advantage of demineralised bone matrix is that it already contains two of these in the shape of human BMP and collagen type I. Various types of ready-for-use demineralised bone preparations have been developed in which demineralised bone has been mixed with substances such as calcium sulphate, bovine or porcine collagen and bioglass. Their consistency is variable, but is always easy and convenient for use.⁵⁰ These products are a fast-growing market in the USA and are becoming increasingly popular in Europe.^{1,2}

Most commercial products have a set of experimental data that defines their main characteristics and claims. However, comparative scientific data are rarely available to support these and it remains difficult for a surgeon to select the appropriate product. There is clearly a need for a

standardised assay to assess and compare the osteoinductive property of various demineralised bone matrices.

The most appropriate use of demineralised bone matrix is with nonunion or delayed union. Relative indications are trauma and any conditions that require new bone formation. An unusual indication is in the expanding phase of a primary and biopsy-proven aneurysmal bone cyst. Demineralised bone matrix may halt the osteolytic phase of the cyst and promote healing by osteoinduction. Healing has been achieved with this method in 11 of 13 patients (85%) with a long follow-up.⁵¹

Massive structural bone allografts. Massive bone allografts have been used primarily for limb salvage in orthopaedic oncology and remain an option for reconstructing large bony defects, where they can provide immediate structural support associated, if necessary, with a prosthesis, an osteosynthesis or a vascularised fibular graft.⁵²⁻⁵⁴ They are usually procured sterile from organ donors and stored at -80°C. They are used for reconstruction after tumour resection and revision arthroplasty, and more rarely after trauma. In most cases, deep-frozen bone will be preferred to freeze-dried because of its better mechanical resistance.

There are various forms of structural bone allograft.

Osteochondral allograft. This may be used for partial joint reconstruction at the knee or ankle in children and in the upper limb in adults. Total joint reconstruction with a preserved joint allograft is not a good option in the long-term, as it produces a Charcot joint with rapid deterioration.⁵⁵

Intercalary allografts. This includes the use of a bone segment similar to the one removed.

Segmental allograft with arthrodesis. This type of reconstruction is usually carried out at the knee or the ankle.

Segmental allograft with a prosthesis. Joint reconstruction using a prosthesis and allograft bone is the most preferred combination with the lowest complication rate.

Cortical strut. This may be used to buttress weakened cortical bone or to stabilise a peri-prosthetic fracture.

Advantages and complications of massive allografts. Massive allografts offer anatomical reconstruction of the skeletal defect, biological union to host bone through callus formation, soft-tissue adherence around the grafted bone, the possibility of tendon reinsertion, and the concomitant use of a prosthesis. However, they are poorly revascularised, and nonunion or fracture will occur in 15% to 20% of cases.

Nonunion. Vander Griend⁵⁶ found an incidence of nonunion of 11% with large frozen allografts. The mode of fixation had no influence on the rate of nonunion. An initial gap of 3 mm appeared to be critical in the development of a nonunion.⁵⁶ The diaphyseal junction healed between nine and 12 months, whereas that in the metaphysis united more rapidly, usually by six months.^{56,57} The mode of osteosynthesis is still a matter of debate.

The aim of fixation should be to obtain uniform contact between host and allograft bone, with a stable interface. This is achieved more easily with plating than with nail-

ing.⁵⁶ The use of a step-cut to improve rotational stability when intramedullary fixation is used requires the ends of the bones to be well matched in size. Modification of the geometry of the step-cut has been proposed in order to increase stability.^{58,59} Although step-cutting augments the contact surface of the bone ends, it is not associated with a better rate of union.⁵⁷ Augmentation of the junction with bone autografting is not necessary to obtain bone healing, but the use of autograft will promote the formation of callus by its intrinsic osteogenetic capacity, and will help to reduce junction voids. Another potential alternative is to replace the autogenous bone by an osteoinductive substitute or by growth factors at the site of junction.⁴⁵

Fracture. Fracture occurs in about 16% of massive allografts and is usually seen two years after implantation.^{60,61} Fracture may jeopardise the outcome of a massive bone graft and its occurrence remains unpredictable. Structural fracture through the shaft of the allograft is usually irreversible because of the limited intrinsic healing potential. Spontaneous healing may be seen rarely, usually in the tibia in young adults. Applying autograft at the fracture site has not been consistently successful, with only about 30% uniting.^{54,60,61} Apart from in the tibia, replacement of a fractured allograft should be undertaken. Attempts to heal these fractures with BMP-2 or BMP-7 have failed.⁴⁶ It is generally believed that most fractures of structural allograft occur through areas where revascularisation and ingrowth of host tissue are absent.⁶¹ As with any inorganic material, a bone allograft will show fatigue, with the appearance of micro- and then macro-cracks and ensuing failure.⁶² Recently, Wheeler and Enneking⁶³ investigated massive bone allografts, which were retrieved after failure, and observed both a gradual reduction in the strength of the bone and an increase in crack density following implantation. Such potential complications should be managed either by mechanical reinforcement of the allograft by cementing the medullary canal^{54,64} or by improving revascularisation through cortical perforation.^{62,65} Perforations allow improvement of the growth of host tissue within the allograft, with an increase in the formation of new bone. More new bone growing within the dead bone would reduce the complications. As holes are stress risers with a consequent risk of fracture, perforating an allograft may present a higher risk of fracture in the short term while reducing it in the longer term, explained by the presence of creeping substitution. The minimum level of these biological activities required to achieve a capacity for self-repair of the allograft is unknown.⁶² Growth factors have shown a good response when used experimentally, but this success has not been confirmed so far with human allograft.^{46,66}

Infection. Infection of an allograft is a devastating complication. The reported incidence varies between 6% and 13%.^{39,54,67} The proximal tibia is most commonly affected. Many factors, such as blood transfusion, the location of the tumour, surgical revision and arthrodesis, have

been implicated, and the risk is cumulative.⁶⁷ It is necessary to achieve viable cover of the graft. To minimise infection, the graft can be soaked in an antibiotic solution at the time of harvest and procurement, and it has been shown that bone can be an appropriate vehicle for the local delivery of antibiotics such as vancomycin or rifampicin.^{68,69} Unpublished work from our laboratory has shown that rifampicin-impregnated bone can release antibiotic at an active concentration for at least three weeks after implantation even after freezing for six months. Biopsy of vancomycin-impregnated bone morsels used to revise hip replacement has shown normal bone formation around the graft, suggesting that vancomycin does not influence bone healing.⁷⁰

Bone substitution in massive structural allografts. An allograft will serve primarily as a spacer that allows osteoconduction of the host cells into its mass, resulting in progressive incorporation of the graft into the host bone. Incorporation is a series of events leading to gradual replacement of grafted bone by host bone through a mechanism of osteoclastic resorption followed by deposition of new bone. Stevenson et al¹⁷ defined successful incorporation as the graft uniting with the host, with the graft-host bone construct able to tolerate physiological loads without fracture or pain. However, this intricate process is very limited for time and space and there remains a final bulk of dead bone that has been poorly substituted by new bone.^{56,71} Efforts have been made to overcome this limited substitution by improving the revascularisation of the bone via perforation and the introduction of stem cells and growth factors. Despite this, no major progress in clinical series using massive allografts has been accomplished so far.⁷²

Requirement of a bone allograft

The bone must be of good quality for its intended use and must be safe for the recipient.

Demineralised bone must exhibit osteoinductive activity, with subsequent new bone formation, when being used in a nonunion, but this requirement for biological activity is not necessary when filling a bony cavity with irradiated bone.

A bone must be strong enough to allow restoration of structural defects, but not necessarily when considering femoral impaction.

The form and consistency of the allograft should allow easy handling in the operating theatre.

Characteristics of a bone allograft

Unlike an industrial product, a bone allograft is not standardised and does not have reproducible mechanical and biological characteristics.

A bone allograft is produced from processing of untreated human tissue unlike synthetic medicinal products. If it is demineralised in order to promote its osteoinductive properties, the release of growth factors from the bone will vary from donor to donor.

Unlike a prosthetic modular component, a massive bone allograft cannot be lengthened during surgery. The surgeon

must plan the length of bone resection after careful imaging studies, or must shape the implant after having excised the pathological bone.

Cumulative expectations of a bone allograft

A successful result with allograft is an interaction between three parties. The surgeon has to define his need, prepare the host bed and handle and fix the graft. The tissue bank selects and screens the donor. It then prepares and chooses the bone according to its intended use. The patient must be fit enough to allow successful healing of the graft, and must comply with the prescribed post-operative treatment.

Definition of terms

Structural allograft. An allograft that provides a mechanical function and that is able to resist or transmit loads.

Massive allograft. A structural allograft which is at least 5 cm long and includes the full circumference of the bone segment to be replaced.

Non-structural allograft. An allograft in various forms, such as paste, morsellised fragments or chips, placed in a contained defect.

Creeping substitution. The osteoclastic resorption of dead bone from the allograft and its replacement by new living bone made by osteoblasts from the host.

Osteointegration. The bony apposition of a pure titanium implant or coated implant without any fibrous interposition. This term is rarely used for describing a bone graft.

Incorporation. Substitution of the old necrotic bone by living new bone as a result of creeping substitution. A bone graft is considered to be incorporated when there is no abrupt histological change between the host bone and the graft.¹⁷ There is a substantial replacement of the dead bone by living bone. Experimentally, about half of a massive cortical bone autograft will be replaced by living bone after six months of implantation.⁷³ However, in retrieved human allografts substitution of less than 10% has been observed.^{59,71}

Fusion. An osseous bridge forms between the allograft and the host bone. This denotes union.

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