Review article:  
Bone transplantation and immune response

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ABSTRACT

Bone is the second most common transplant tissue after blood, with the iliac crest autologous graft being most used. Bone transplantation induces osteogenesis to repair bone defects. Despite being the most efficient, autogenous bone requires an additional incision and its supply may be inadequate. Deep-frozen allogeneic bone can be an alternative, but is at risk of microbiological contamination, transmission of unrecognised germs, delayed incorporation, and cellular and humoral immune reactions. Synthetic graft substitutes combine scaffolding properties with biological elements to stimulate cell proliferation and differentiation and eventually osteogenesis. However, they generally lack osteoinductive or osteogenic properties and have various effects on bone healing. We present an overview of bone grafts and graft substitutes in clinical use, and the immune responses to allogeneic bone.

Key words: bone substitutes; bone transplantation; immune system; major histocompatibility complex

BASIC SCIENCE

Bone cell types

Bone formation (osteogenesis) entails cellular activity by osteoblasts, osteocytes and osteoclasts. Osteoblasts possess signalling molecules and act as mechanosensory cells. Mechanical stress enhances osteoblastic activity.1,2 Osteoclasts resorb bones and are multinucleated and formed by the fusion of cells of the monocyte/macrophage lineage. Osteocytes are mature bone cells accounting for over 90% of adult bone cells.

Bone structure

The matrix (made by osteoblasts) is the major constituent of bone that surrounds the bone cells. Bone matrix has inorganic and organic constituents. The inorganic components are mainly crystalline mineral salts and calcium in the form of hydroxyapatite,2,3 initially laid down as unmineralised osteoid. Mineralisation then occurs through the action of osteoblasts secreting vesicles containing alkaline phosphatase. This enzyme cleaves the phosphate
groups from monophosphate ester substrates, which act as foci for calcium and phosphate deposition. The vesicles then rupture and act as a nucleus for crystals to grow on. Transforming growth factor β (TGF-β), vitamin D metabolites, and parathormone (PTH) are important mediators of calcium regulation. The organic constituent is mainly composed of type-I collagen, which is synthesised intracellularly to tropocollagen and then exported out of the cell. Tropocollagen then associates into fibrils. Other components include glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, and cell attachment factor.3

Bone and immune system

Bone provides solid support for body structure and is an organ that acts as a reservoir for critical ions such as calcium and phosphate. Immune cells (T cells, B cells, and natural killer [NK] cells) are produced in the bone marrow. NK and B cells are matured in the bone marrow. Memory-B and -T cells migrate back into the bone marrow. The interaction between immune cells and bone is classified as osteoimmunology.5

Major histocompatibility complex

Major histocompatibility complex (MHC) is a genetic region important for the immune system and a key mediator for allogenic transplantation. MHC molecules are called human leukocyte antigens in humans, RT1 in rats, and H2 in mice (Fig. 1). The MHC gene complex is divided into 3 sub-regions, the class I, II, and III. Only class I and II are involved in allotransplantation. MHC molecules are heterodimeric transmembrane glycoproteins that belong to the immunoglobulin superfamily. They are able to present endogenous and foreign peptides on the surface of antigen-presenting cells (APC) and communicate with T cells. Acute rejection is generally mediated by T cell responses to donor MHC antigens that differ from those in the recipient (MHC-mismatch). Chronic rejection is due to a chronic alloreactive immune response. Rejection may also be initiated by non-MHC genetic factors such as minor histocompatibility antigens, which are peptides derived from proteins encoded by polymorphic genes outside the MHC locus and inherited independently from MHC molecules.4

Bone transplantation

Transplantation of organ or tissue within oneself is an autograft, from others is an allograft, from a genetically identical recipient (such as an identical twin) is an isograft, from another species is a xenograft, and from one animal to another within the same inbred strain is a syngeneic graft.6 Allograft rejection (e.g. as occurs in 60 to 75% of first kidney transplants and 50 to 60% of liver transplants) is a major concern. Bone transplantation stimulates skeletal repair and regeneration. Bone grafts have biological and mechanical functions. The biological activity of a graft includes osteogenesis (inherent activity), osteoinduction (activity of surrounding host tissues), and osteoconduction (ingrowth of host tissues). The mechanical environment affects bone remodelling. Skeletal graft incorporation is a multifaceted process in which multiple variables determine success or failure. The sequence includes haemorrhage, inflammation, revascularisation, substitution, and remodelling.7,8 Successful graft incorporation is defined as the ability of the graft and surrounding tissue to function and maintain mechanical integrity.4 Autologous bone is the most efficient material and usually harvested from the iliac crest, but has disadvantages (donor-site morbidity, inadequate supply, size and shape problems). Allografts from cadavers are increasingly common, but risk disease transmission and immune reactions.9,10 Autologous bone is also superior to allogeneic bone.9,11,12

Immunoreactions to bone grafts

Frozen or cryo bone allo-transplantation is less likely to result in complications and rejection. Even deep-frozen allogeneic bone (containing no living cells) can cause complications in 25 to 35% of patients.4 Bone can be stored frozen for indefinite periods. Sterilisation and disinfection can reduce the immunologic defence reactions and the risk of infection without damaging the bone matrix. Nonetheless, the causes of rejection remain poorly understood.

NK cells do not participate in the rejection of solid organ transplants. Cardiac and skin allograft transplanted into mice with intact NK cells but absent T and/or B cells do not get rejected and survive.

![Figure 1](image_url) The major histocompatibility complex gene region of the rat (RT1), mouse (H2), and human (human leukocyte antigens, HLA)
indefinitely. However, NK cells are able to recognise and reject intact allogeneic MHC class I molecules on foreign cells by their receptors. Unlike T cells, which can recognise foreign or mismatch MHC molecules directly or indirectly, NK cells predominate to recognise foreign molecules directly. In bone cryopreservation, intact cells are unlikely to survive and thus NK cells do not have a role in cryoallograft rejection.9,12

T cells express receptors on the surface that are able to recognise either the whole MHC molecules (direct recognition) or MHC molecules presenting non-self peptide on their peptide-binding group (indirect recognition). MHC is highly polymorphic and varies between individuals, and therefore the host T cells that usually recognise the foreign MHC lead to variably powerful immune responses. Identical twins and cloned tissue are MHC-matched, and therefore not subject to T cell-mediated rejection. On their peptide-binding grooves, MHC class I and II present peptides of 8 to 9 and 15 to 24 amino acids in length, respectively. Foreign peptides presented on the MHC-I and -II molecules can activate cytotoxic T cells (CD8+ T cells) and T-helper cells (CD4+ T cells), respectively. They have no cytotoxic activity and cannot kill infected host cells. They activate and direct other immune cells such as macrophages via interferon-γ production, or other cytokines, and dramatically inhibit bone generation (Fig. 2).13

**BONE GRAFT PROCESSING**

To reduce immune sensitisation and disease transmission, superfluous proteins, cells, and tissue should be removed as much as possible. The most common methods for preservation are freezing to below -70°C (which has minimal effect on bone properties) and freeze-drying (which has substantial effect). Autogenous, cancellous bone is robustly osteogenic as it contains living osteogenic cells that can induce differentiation of host mesenchymal cells into osteoblasts and its structure can serve as an osteoinductive support for the ingrowth of sprouting capillaries, perivascular tissue, and osteoprogenitor cells from the recipient bed. However, it does not provide structural support. Autogenous, cortical bone provides structural support and is somewhat osteogenic, osteoinductive, and osteoconductive. However, revascularisation is slow and primarily the result of peripheral osteoclast resorption and vascular invasion of haversian canals. Therefore, the graft becomes weaker than normal bone, and large portions of dead cortical autograft may remain for long periods.

Deep frozen bone retains its material properties and can be used after thawing. Freeze-drying alters its material properties, and thus even after rehydration it is friable and remains weak in tension and bending.14 Cortical allografts provide structural support and are osteoconductive to a limited degree, but are not osteogenic or osteoinductive. Vascularisation is lacking and such grafts sustain fatigue micro-damage by cyclic loading over time. As necrotic bone cannot repair itself in response to damage, up to 50% of these grafts may fracture.

Morcellised and cancellous allogeneic bone grafts provide limited mechanical support in compression and are osteogenic only. However, they have an open, porous lattice-like structure that provides the same stages of incorporation as in cancellous autografts.

**EXPERIMENTS IN RATS**

A rat model of tibial allograft transplantation was developed to investigate biomechanical and histological changes of fresh versus prefrozen graft and the corresponding humoral immune responses. Necrosis of the fresh allogeneic grafts was confirmed by mechanical testing and radiological signs of resorption, and
an alloantibody response was detected. Although frozen bone allografts impede the antibody response against MHC antigens and improve incorporation, they nevertheless perform more poorly than frozen syngeneic grafts.9

Even though the bone marrow was drained after 2 hours of freezing at -80°C, it is possible that MHC peptide proteins from the donor cells (such as osteocytes deep inside the bone) survive the freezing and can be targeted by APCs after transplantation. APCs present these peptides on their MHC-II proteins to CD4+ cells and lead to a gradual, long-term immune response. No measurable antibody response was detected after transplantation of frozen allogeneic bone. If immune responses against MHC antigens contribute to the poorer healing of frozen allogeneic (compared with syngeneic bone), it is likely the mediated cells rather than humoral responses are responsible.

**SUBSTITUTES TO BONE GRAFTING**

Dematerialised bone matrix
This is produced through decalcification of cortical bone. It retains the trabecular collagenous structure of the original tissue and can serve as a biologic scaffold without structural strength.15 It possesses osteoconductive properties, but its clinical results are not uniformly good,16 owing to the biological properties of the graft, the host environment, and the methods for graft preparation.

Bone morphogenetic proteins
Bone morphogenetic proteins (BMP) have tremendous potential in bone formation at fracture and non-union sites, and are effective alternatives or enhancers of autologous bone grafts for bone regeneration in animal studies.17 Two recombinant BMPs—rhBMP-2 and rhBMP-7 (also known as osteogenic protein-1) are available for clinical use. Each has been evaluated in randomised controlled trials involving trauma patients.18 The action of BMP is mediated through receptor kinases and transcription factors called Smads that regulate the expression of target genes. More clinical and long-term experience is necessary to confirm that treatment with BMPs is beneficial.

Osteoconductive bone graft substitutes
Synthetic graft substitutes, or matrices, are formed from a variety of materials (including natural and synthetic polymers, ceramics, and composites) designed to mimic the 3-dimensional characteristics of bone tissue.19 Matrices also act as delivery vehicles for growth factors, antibiotics, and chemotherapeutic agents, depending on the nature of the injury to be repaired. This intersection of matrices, cells, and therapeutic molecules is known as tissue engineering. The composition of these materials can be adjusted depending on the specific application of the matrix. They are osteoconductive but not osteoinductive and vary in structural support. They are usually supplied as ceramics or injectable materials that harden in situ.

Scaffold ceramics are mostly made of calcium phosphate.20 They are osteoconductive but not osteoinductive, unless growth factors or BMPs are added to create a composite graft. They are brittle and have little tensile strength.21 They provide an osteoconductive matrix for host osteogenic cells to create bone under the influence of host osteoinductive factors. After incorporation with the bone, they gradually acquire mechanical strength similar to cancellous bone.22 Injectable calcium phosphate combines cement properties with those of a void-filler.23 The initial compressive strength when hardened is similar to that of cancellous bone. Eventually it undergoes long-term remodelling and is replaced by new bone.

**CLINICAL APPLICATION**

Traumatic complications
Bone loss and delayed or non-unions are difficult to treat. Autogenous corticocancellous bone grafting provides the most effective standard of care. Implantation of osteogenic protein-1 in combination with a collagen matrix results in the repair of critical size defects in long bones.24-26 After debridement and stabilisation, intramedullary application of BMPs in combination with bone grafts or bone substitutes has given good results.27 BMPs appear effective for the treatment of traumatic complications although the number of patients is small.

Spinal fusion procedures
Autogenous bone graft from the iliac crest remains the gold standard because it has all 3 properties essential for adequate fusion. Demineralised bone matrix products and synthetic bone grafts (ceramics) were developed to minimise the invasive harvesting of
autografts\textsuperscript{28} and to provide scaffolds similar to those of autologous bone.\textsuperscript{29-31} They are effective in filling bone defects, but synthetic bone grafts do not have osteoinductive properties. Thus, they require the host bone to be well vascularised to enable availability of appropriate cellular and osteogenic factors. For anterior lumbar fusions, the use of rhBMP-2 and a collagen sponge versus autografts resulted in equivalent fusion rates and clinical outcomes.\textsuperscript{32} A human pilot study using rhBMP-2 with biphasic calcium phosphate also demonstrated similar results for posterolateral fusions, with significantly reduced surgical time and blood loss.\textsuperscript{33}

**Revision of endoprostheses**

In order to select the appropriate bone graft material, bone loss around prostheses is classified as cavitary, segmental, contained, and uncontained.\textsuperscript{34,35} In type-I revisions, no graft is required. In type-II revisions, bone graft is required for filling cavitary defects or reconstructing a segmental bone loss. In type-III revisions, a large quantity of bone graft is required for implant stability. Cavitary or contained bone defects (type II) can usually be treated with morcellised autogenous or allogeneic cancellous bone. Mixing demineralised bone matrix with the morcellised material may enhance the grafting. Significant segmental bone deficiency (types II and III) requires structural or bulk allografts for reconstruction. Corticocancellous allografts can also be used to reconstruct large cavitary defects. Segmental cortical defects of the femur are usually reconstructed with cortical allogeneic onlay grafts. The cortical grafts must be securely fixed to the host and cover at least half the surface of the recipient bone. Major cavitary defects (types II or III) require an impaction allografting technique using morcellised grafts to restore bone stock,\textsuperscript{36,37} but there are no long-term studies to support this strategy.\textsuperscript{38}

**FUTURE**

Bone preservation techniques\textsuperscript{39} (including freezing, freeze-drying, thimerosal, alcohol, demineralisation, boiling, and autoclaving) have an impact on the host's immune response to the graft. Improved preservation techniques for donor bones are needed, especially to exclude any immunogenic peptide longer than 5 amino acids that can evoke immune responses in the host. Proteases such as trypsin and pepsin are useful to degrade foreign peptides;\textsuperscript{40} but may destroy useful proteins such as collagen. Trypsin and pepsin need acidic conditions (pH 2–3) to be fully active and this may be harmful for bone mass. Extensive panel testing and critical analysis of the experimental system are needed to determine the right conditions (pH, duration of exposure, choice of protease and buffer) for successful destruction of allopeptides during bone grafting. Collaboration between immunologists, biochemists, and material scientists is necessary for the selection and modification of biological and biomechanical parameters so as to optimise the outcome of allografting.

**REFERENCES**