Use of Allografts in Knee Reconstruction: I. Basic Science Aspects and Current Status

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Abstract

Allografts were first used in reconstructive surgery of the knee early in this century. Their widespread use and acceptance paralleled the development of modern tissue banks and our increased understanding of the immune system. Advantages of allogeneic tissue use include less surgical morbidity, shorter surgical time, smaller incisions, and the wider selection of graft sizes and types of tissue. Disadvantages include the risk of disease transmission, a slower biologic remodeling process, and the potential for a subclinical immune response. Allografts can be obtained in several forms, including fresh, fresh-frozen, freeze-dried, and cryopreserved, each with its own advantages and disadvantages. Graft sterility is most commonly ensured by aseptic techniques of harvest and procurement. Other methods, such as irradiation and chemical sterilization, have the potential to damage the collagen structure of the graft and must be used with care. Surgeons who use allografts should make sure that the tissue bank supplying their graft adheres to any applicable guidelines of the Food and Drug Administration and the American Association of Tissue Banks, and uses top-quality testing procedures. In addition, the physician should thoroughly understand the structural and biologic influence of the preservation technique used for that tissue.

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The choice to use allogeneic tissue in knee reconstruction is influenced by many factors. Autogenous tissue is superior when considering disease transmission, tissue compatibility, and rate of healing. The use of an allograft eliminates the damage and associated morbidity entailed in harvesting an autograft. The use of autogenous tissue may also be constrained by limits of size, shape, and availability. Meniscal replacement depends entirely on allogeneic tissue because no autogenous tissue is available, and attempts to regenerate menisci to date have not produced a satisfactory replacement.

Until recently, the only successful articular cartilage replacement required an allogeneic graft. Advances in articular cartilage regeneration through implantation of live chondrocytes grown in vitro[1] and the transplantation of plug grafts from one area of a joint to another area have produced new options for treating articular defects. Ligament surgery presents a practical choice of graft source, since all tissues typically harvested for use as autografts are also available as allografts. Selecting which graft type to use in reconstructing the knee should be a decision reached after all advantages and disadvantages have been considered by both the patient and the surgeon.

In this article, we will review the technical aspects of allograft usage—procurement, sterilization, storage, and physiology. In the accompanying article, we will specifically discuss current usage of articular cartilage, ligament, and meniscal allografts.

History

MacEwen's use of a donor bone graft in 1880 was the first reported case of a musculoskeletal allograft.[2] Lexer[3] reported 23 cases of articular cartilage transplantation between 1908 and 1925 and rated 50% as

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successful. Both Noyes et al[4] and Shino et al[5] began using allografts in ligament reconstruction in 1981 and have subsequently reported good results. Milachowski et al[6] was the first to transplant a human meniscus in 1984.

The work of these early investigators, along with the evolving science of transplant immunology, led to the development of modern tissue banks. These banks provide a supply of high-quality allogeneic tissue to orthopaedic surgeons who perform knee reconstructions. Today there are 53 tissue banks in the United States that are accredited by the American Association of Tissue Banks (AATB). Guidelines and standards for processing tissue have been developed that ensure quality and sterility.[7] These guidelines are periodically reviewed and revised by the AATB.

Procurement

The AATB first printed its *Standards for Tissue Banking* in 1984. Since then, this publication has been revised and updated six times, most recently in 1996. It contains the minimum guidelines that all accredited tissue banks adhere to in procuring and processing tissue.

A potential cadaveric donor must first pass through a detailed medical, social, and sexual history questionnaire completed by the next of kin or life partner. Any history of exposure to communicable diseases, reports of unprotected sexual contacts, drug use, neurologic diseases, autoimmune diseases, collagen disorders, or metabolic diseases is also documented. Any positive finding will disqualify the individual as a donor.

A physical examination to detect hepatosplenomegaly, lymphadenopathy, cutaneous lesions, or other signs suggestive of infectious disease is completed. If an autopsy was performed, the results are included.

Laboratory tests required by the Food and Drug Administration (FDA) and the AATB are performed on serum from the donor. They include aerobic and anaerobic blood cultures, cultures from the tissue harvested, antibodies to human immunodeficiency virus (HIV) types 1 and 2, hepatitis B surface antigen, hepatitis C antibodies, syphilis antibodies, and human T-cell lymphotropic virus antibodies.

Despite these extensive efforts to identify grafts that may contain unwanted pathogens, there is still a window of vulnerability between infection and the production of detectable antibodies by the donor. In the case of HIV, this window averages 25 days but can be as long as 6 months. To decrease this window, up to 50% of tissue banks use polymerized chain reaction (PCR) testing to directly detect viral antigens. Both HIV-1 and HIV-2 can be detected by PCR. The test is very sensitive; as few as 5 to 20 viral DNA copies per sample can be detected. Utilization of PCR testing decreases the window of vulnerability to approximately 19 days (confidence level, 95%) and costs approximately $120 per donor tested.

Tissue harvest for musculoskeletal grafts takes place within a few hours of the death of the donor, usually after organ procurement teams have completed their tasks. Harvesting can be aseptic (sterility of the graft is maintained throughout harvest and processing) or can be simply clean (absolute sterile technique is not employed, and utilization of secondary sterilization is required). Once the grafts have been harvested, they are cooled and rapidly transferred to the tissue bank, where they are inspected and washed to remove unwanted blood and marrow elements. The final decision on acceptance of a graft is the responsibility of the medical director of the tissue bank, after a thorough review of all the collected data.

Sterilization

All allografts should come with the highest possible assurance that they are free of pathogens. Unfortunately, most of the methods by which we sterilize materials used in surgery are unsuitable for use on human tissue. Heat and high doses of gamma radiation (>3.0 Mrad) are effective but weaken the collagen structure,[8] obviously an undesirable side effect. The use of chemical agents such as ethylene oxide, while effective in removing unwanted microorganisms, leaves behind a chemical residue that may cause chronic synovitis and graft failure.[9]

The most common method of ensuring graft sterility is to adhere to sterile techniques during harvest, transport, and processing. The tissues are soaked in antibiotic solution at 4°C for at least 1 hour, and multiple cultures are obtained during processing. Low-dose gamma radiation (2.0 to 3.0 Mrad) may be used as an adjunct. The goal is to kill pathogens without damage to the collagen structure in the graft.

An alternative method to ensure graft sterility is a clean but not aseptic harvest and inspection, followed by sterilization of the graft by gamma irradiation or chemical agents. Effective sterilization can be attained with the use of chemical agents such as ethylene oxide, but all of the chemical residue must be removed.

Gamma radiation is an effective method of sterilization, but doses in excess of 3.0 Mrad are necessary to kill viruses. The difficulties associated with sterilization of a cleanly procured graft have led to the development of a technique for

aseptic harvest and processing augmented by antibiotic soaks, multiple cultures, and low-dose gamma irradiation (<3.0 Mrad), which has become the most commonly used process for producing a sterile graft.

A potential complication of allogeneic tissue use is the transmission of HIV or hepatitis to the recipient. Buck et al[10] calculated the risk of HIV transmission in properly screened and tested donors to be 1:1,600,000 and stated that adequate serologic testing and histopathologic examinations are most important in securing safe, sterile grafts. Procurement methods should not be counted on to destroy viruses within the graft, as HIV has been cultured from bone after cryopreservation as well as freeze-drying.

Despite the risk of HIV infection from allogeneic grafts, there has been only one reported case in which HIV was proved to be transferred from an infected donor.[11,12] The contaminated tissues were transplanted in 1985 (before mandatory standards) into three recipients, who converted to HIV-positive status after transplantation. The three grafts had been simply cleaned and frozen; if today's screening methods had been available, the presence of HIV would most likely have been detected. Recipients of freeze-dried grafts from this donor did not become infected, suggesting (but not proving) that freeze-drying may kill HIV.

Storage

Storage of allogeneic tissue depends on the type of graft, whether viability of cells is important, and whether the collagen matrix of the graft can withstand harsh methods of preservation. Many unsuccessful methods of preserving grafts have been tried, including irradiation, boiling, treatment with chemicals, deproteinization, decalcification, and refrigeration.[2] Currently, the only acceptable methods of allograft preservation are cooling and fresh transplantation within 24 hours, freeze-drying, and storage at –80°C or liquid nitrogen storage at –196°C with or without cryopreservation. Preservation methods for ligaments differ significantly from those for articular cartilage and menisci.

Most articular cartilage allografts have been transplanted fresh, which preserves both normal cells and matrix. These grafts contain marrow elements within the bone, which increases both the antigen exposure to the recipient and the possibility of viral disease transmission. Because of the short storage time, they must be used on a semiemergent basis; therefore, obtaining the correct size of graft can be difficult. Viable chondrocytes can be maintained in lactated Ringer's solution cooled to 2°C to 4°C for 7 days; however, after 24 hours there is a decrease in the percentage of viable cells. The success of grafts implanted after 24 hours decreases, with an increase in graying and delamination of the articular cartilage and fragmentation of the underlying bone.

Cryopreservation, a process of controlled-rate freezing with extraction of cellular water by use of dimethylsulfoxide and glycerol, is used for preserving menisci and ligaments. The process of cryopreservation, originally developed to preserve sperm and embryos, prevents cell death by altering water crystallization within cells during freezing. With this process, up to 80% of cells survive. This preservation of cells is considered beneficial for a successful meniscal allograft. Grafts are initially cooled to 0°C and processed within 48 hours of donor death. They are then incubated in an antibiotic solution for 24 hours at 37°C, subjected to controlled-rate freezing to –135°C, and packed in a cryoprotectant solution.

Cryopreservation of ligaments, while an effective method, has not proved superior to deep-freezing, and the added expense must be questioned. Cryopreserved grafts can be stored at –196°C for as long as 10 years, an important factor in solving timing and sizing problems. Cryopreservation of articular cartilage has not proved as satisfactory as the use of fresh grafts, due to damage to the cartilage matrix during freezing.

Deep-freezing is the simplest and most widely used method of ligament allograft storage. After recovery, the graft is frozen for 2 to 4 weeks pending the results of serologic studies, after which it is thawed and processed. After a 1-hour antibiotic soak at room temperature, it is packaged without solution and frozen to –80°C. It can then be stored for 3 to 5 years. All cells are destroyed within the tissue, but no deleterious clinical effect has been noted due to the acellularity of ligament tissue (unlike menisci and articular cartilage). The process may even enhance success by removing potential antigens located on the cells.

Freeze-drying is used for ligament allografts. After recovery, the graft remains frozen for 2 to 4 weeks pending the results of serologic studies. It is then thawed and processed. A 1-hour antibiotic soak at room temperature is followed by refreezing and lyophilization to a residual moisture less than 5%. The graft can then be packaged and stored at room temperature for 3 to 5 years. Rehydration of freeze-dried ligament grafts with attached bone plugs requires a minimum of 30 minutes before implantation. The color, appearance, and strength of the graft are usually altered. None of these factors has proved deleterious in clinical studies involving the use of freeze-dried ligament allografts.

Physiology

Allogeneic tissue functions as a scaffold, providing a structure that is rapidly incorporated by the host. The process involves stages similar to those seen in avascular necrosis. Initially, there is cell death (in fresh or cryopreserved grafts), which is followed by revascularization, cell repopulation, and finally remodeling. The initial stages progress very rapidly. Jackson et al[13] demonstrated the complete replacement of donor cells by host cells in the goat anterior cruciate ligament by 4 weeks after transplantation.

The remodeling phase of an allograft is lengthy; an allograft may take one and a half times as long as an autograft to complete remodeling and regain comparable strength.[14] This longer maturation process may be due to tissue-antigen mismatch presented to the host and a resulting subclinical immune response.

Antigens present on the cells in bone and cartilage have proved capable of producing a typical immune reaction, and the finding that there is no direct evidence of clinical rejection of these grafts has been the subject of intense investigation. The absence of a humoral or cellular immune response has led to the postulation that allografts are protected by a "blocking factor."[15] However, the facts that thorough washing removes most of the marrow elements of the graft and that chondrocytes and fibrochondrocytes are deeply embedded in an avascular matrix may also explain the lack of host response. Careful analysis of synovial fluid after allograft implantation has shown a slight increase in immunomarkers, but a clinically significant reaction does not appear to occur.

Summary

The use of allogeneic tissue has broadened the alternatives that surgeons can use to treat knee disorders. In some cases, the use of allograft tissue may be the preferred, or indeed the only, way to reconstruct the defect. The risks and benefits of allografts have been defined, so that both patient and surgeon can make well-informed decisions.

Modern tissue banks have developed safe harvest and storage methods that ensure an adequate, safe supply of grafts. Any surgeon using allografts should make a point of being familiar with the exact techniques and standards used by the bank supplying the grafts. Only tissue from banks that adhere to the standards of the FDA, the AATB, or both, and that use high-quality test kits and reference laboratories should be used. Utilization of additional tests, such as PCR testing, while not required by the FDA or the AATB, adds to the safety of the grafts. Surgeons should feel comfortable that everything reasonable has been done to ensure that the grafts they use are of the highest quality available.

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