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Basic Science Update

Allograft Transplantation in the Knee: Tissue Regulation, Procurement, Processing, and Sterilization

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Use of musculoskeletal allografts has become increasingly popular, with widespread use among knee surgeons. The advantages and disadvantages of their use have been documented. In the knee, allografts are used for ligament reconstruction, meniscal transplantation, and articular surface reconstruction. The purpose of this review is to present issues surrounding the allograft industry, including regulation of tissues and tissue banks and procurement, processing, sterilization, and storage of allograft tissue. Tissue bank regulation is ultimately under the jurisdiction and authority of the Food and Drug Administration; some individual states regulate tissue banks. The American Association of Tissue Banks is a scientific organization that encourages education, research, and voluntary accreditation of tissue banks. It promotes safety and standards for retrieval, processing, storage, and distribution of transplantable human tissue. Allograft tissues are generally harvested and processed aseptically, which may not prevent contamination. Tissue sterilization is difficult and controversial. Tissue banks historically have used one of two methods of sterilization, ethylene oxide or gamma radiation. Both methods have risks and benefits. Newer methods of sterilization are being developed. Allograft tissue that is not transplanted fresh can be freeze-dried or deep frozen for storage. Ultimately, allograft transplantation in the knee facilitates knee form and function and enhances the patient's quality of life. Orthopaedic surgeons who use allograft tissue must understand the tissue banking process to provide safe and effective tissues to their patients.

The first report of an allograft transplantation in a human appeared in 1881.[40] Years later, reports appeared in the literature about early methods of storage of allograft material.[23,32] Inclan[32] reported on the first dedicated bone bank in 1942. In the early 1950s, bone banks began to appear and the concept of large, regional tissue banks became popular.[15,31] A tissue bank is defined as an organization that provides donor screening, recovery, processing, and storage or distribution of allograft tissue. Tissue banks can be dedicated eye banks, sperm banks, cardiovascular banks, embryo banks, or skin banks. There are general or multitissue banks that supply musculoskeletal tissues and there are dedicated musculoskeletal tissue banks. A total of 154 tissue banks were identified in a January 2001 report issued by the Office of the Inspector General Department of Health and Human Services.[42] In 1999, more than 20,000 donors provided cadaveric tissue, an increase from approximately 6000 donors in 1994. This report also estimated that tissue banks distributed more than 750,000 musculoskeletal allografts for transplantation in 1999.

Musculoskeletal allograft use has become increasingly popular, with studies demonstrating that musculoskeletal allograft tissues show comparable results to autograft tissue for ACL reconstruction.[25,38,51,53] A survey of 36 tissue banks showed that there was a 46% increase in connective tissue allografts distributed in the United States from 1990 to 1992.[66] Connective tissues commonly distributed by the tissue banks were bone-patellar tendon-bone (95%), Achilles tendon (90%), fascia lata (86%), and meniscus (33%). The bone-patellar tendon-bone, Achilles tendon,

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fascia lata, and hamstring tendon allografts are used for ACL reconstruction, and specific shortages have been noted for the commonly used bone-patellar tendon-bone allograft. Meniscal allografts have become more common in recent years for meniscal transplantation. More recently, osteochondral allografts have been made available by tissue banks for transplantation into large focal articular surface defects in the knee.

There are many advantages in using allograft tissue for the knee. These include no donor site morbidity, shorter operative time, availability of larger grafts, smaller surgical incisions, and a claim of a lower incidence of arthrofibrosis.[25] There are also potential disadvantages, which include the risk of viral and bacterial disease transmission, potential for histocompatibility rejection, and longer incorporation period, which can require longer protection from potentially injurious forces.[1]

The purpose of this review is to present the major issues surrounding the allograft industry to the orthopaedic surgeon. These areas of knee allograft transplantation include regulation of allograft tissues and tissue banks and procurement, processing, disinfection, and storage of allograft tissue.

TISSUE BANK REGULATION

Many tissue banks are members of various trade organizations or are certified or licensed by quality standards organizations. One of the more popular scientific organizations is the American Association of Tissue Banks (AATB), a not-for-profit organization that was founded in 1976. It was formed to establish and promulgate voluntary standards to encourage the provision of transplantable cells and tissues of uniform high quality. The AATB strives to prevent disease transmission and to assure optimum clinical performance of transplanted tissues. The organization promotes education and research for this field of medicine among its member tissue banks.

In 1986, the AATB began offering inspection and accreditation of tissue banks to its members. In 2002, the AATB accredited 72 tissue banks, 11 of which are reproductive tissue banks. The remaining 61 banks involve musculoskeletal, cardiovascular, and skin allograft tissue. Accreditation is based on compliance with the AATB voluntary standards relating to the retrieval, processing, storage, and distribution of transplantable human musculoskeletal, skin, reproductive, or cardiac tissues.[2] Musculoskeletal AATB tissue banks vary in number between 26 and 48, depending on their specific accreditation in these areas. Accreditation lasts 3 years and renewal of accreditation is granted on reinspection.

The AATB first published its "Standards for Tissue Banking" in 1984 to help ensure that the conduct of tissue banks met their safety and ethical standards.[27] These standards outline the minimum guidelines for the procuring and processing of tissue as practiced at member banks. These standards are reviewed periodically and have been revised and updated several times by the AATB Standards Committee to incorporate current member practice. Tissue banking procedures must be followed by member banks regarding record keeping, quality assurance, donor suitability determination, and safety. In 1988, the AATB initiated the formation of the Tissue Network, a network composed of AATB-accredited facilities to ensure that an adequate supply of tissues was available for requesting physicians.

Tissue banks are not required to become accredited by the AATB. A report by the Inspector General of the Department of Health and Human Services in January 2001 reported that 58 tissue banks were accredited by the AATB, and at least 90 tissue banks were identified that were not accredited by the AATB.[42] Additionally, the AATB has no policing or governing authority over any nonaccredited tissue bank. The AATB is a voluntary organization that is not the final authority in control of tissue banking quality. The Food and Drug Administration (FDA) has the power to shut down a tissue bank, fine or imprison its owner/operators, and can force a recall and destruction of all its tissues. The American Academy of Orthopaedic Surgeons (AAOS) believes that musculoskeletal allografts represent a therapeutic alternative for eligible patients. These tissues should be acquired from facilities that demonstrate compliance with AATB regulations and use well-accepted banking methods and good tissue practices. The AAOS urges all tissue banks to follow rigorous national guidelines and standards.[1] Even so, all tissue banks are required to follow mandatory federal and applicable state regulations for the safe conduct of tissue banking, and many adhere to AATB standards for tissue banking, despite not being accredited.

In 1987, the United States government formed the Center for Biologics Evaluation and Research (CBER) after reorganization of the Center for Drug Evaluation and Research (CDER). The CBER is the division of the FDA that regulates biological products. Although the FDA has no legal authority for granting approval or licensing of tissue products as it does for biological pharmaceutical or medical device products, the agency has issued regulations and guidelines for tissue manufacturers. In 1993, the CBER published an Interim Rule, *Human Tissue Intended for Transplantation*.[30] This rule required that all tissue intended for transplantation be tested for human immunovirus (HIV) and hepatitis B and C "for the prevention of spread of communicable diseases."

Since 1993, the FDA has promulgated additional regulations to address the inherent risks associated with human tissue. *Human Tissue Intended for Transplantation: Final Rule,* 21 CFR Part 1270, was published in the *Federal Register* of July 29, 1997.[29] While it does not strengthen the screening provisions of the Interim Rule, it contains provisions for the inspection of the manufacturing facility as well as details on the retention, recall, and destruction of human tissue. The Final Rule also elaborates on the requirements for the use of an algorithm when determining plasma dilution and identifies documents to be included in the summary of records (the maintenance of records used in determining the suitability of the tissue for transplantation and shipment).

The FDA's *Guidance for Industry: Screening and Testing of Donors of Human Tissue Intended for Transplanta-*

tion, dated July 1997, provides recommendations regarding the type of questions that should be included in the donor medical history interview.[21] It also provides guidance for conducting a physical examination to determine whether the donor exhibits signs or symptoms of active HIV, hepatitis infection, or high-risk behavior. Required screening tests, viral marker test performance, and plasma dilution and testing algorithm are included in this guidance document.

The FDA's *Proposed Rule on Suitability Determination for Donors of Human Cellular and Tissue-Based Products*, published in the *Federal Register* of September 30, 1999, would require medical history screening and donor blood testing for various known pathogens to prevent the unwitting use of contaminated tissues with potential to transmit infectious diseases.[60]

In the *Federal Register* of January 8, 2001, the FDA published 21 CFR Part 1271: *Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products; Inspection and Enforcement: Proposed Rule,* superceding the Proposed Rule of 1999.[14] The purpose of this body of regulation is to help ensure that donors of human cellular and tissue-based products are free of communicable diseases, that the cells and tissues are not contaminated during manufacturing, and that cells maintain their integrity and function. This regulation is expected to be finalized at the beginning of 2004.

On January 19, 2001, 21 CFR 1271 *Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing: Final Rule*, was published in the *Federal Register.* This rule requires manufacturers of tissue-based products to register with the FDA and list their products.[28] As of October 2002, this regulation had resulted in the registration of 537 tissue establishments.

On March 8, 2002, the FDA issued a document entitled *Guidance for Industry: Validation of Procedures for Processing of Human Tissues Intended for Transplantation* for immediate implementation in accordance with 21 CFR 10.115(g).[22] No prior comment was sought because the FDA had determined that prior public participation was not appropriate in light of recent reports regarding contamination of human tissue intended for transplantation.

Even though the FDA has published several guidelines for tissue banks, the publication *Oversight of Tissue Banking* from the Office of the Inspector General in January 2001 recognized the deficiencies and the importance in regulation and inspection of the tissue banks.[42] Since 1993, the FDA has conducted 200 inspections and recalled more than 15,000 tissue products.[20,42] Of 154 tissue banks that the Department of Health and Human Services was able to identify in January 2001, there were 36 tissue banks that had never been inspected by the FDA.[42] Of 118 tissue banks that FDA had inspected, 68 were inspected only once. Because of limited resources, the agency has had to establish a priority list for follow-up inspections and has identified reinspection as a primary priority.[42]

Regulation of tissue banks is also administered by some states. New York and Florida are the only two states to license and inspect tissue banks. In addition to screening and testing, these states require tissue banks to report adverse effects. Furthermore, they address areas such as tissue procurement processes, tracking practices, emergency procedures, equipment standards, conflict of interest, labeling standards, laboratory testing, and disposition of unused tissue. A few other states require tissue banks to be licensed by the state, but do not perform inspections. These states include California, Georgia, Maryland, and Illinois.[42]

Some of the more industrious tissue banks have subjected themselves to the intense scrutiny of a certification inspection by the International Organization for Standardization. Although this certification is voluntary in the US, many other countries require it on the part of manufacturers of a broad range of products, including human allografts. The International Organization for Standardization certification is recognized globally as a tissue bank's commitment to product safety and organizational quality.

TISSUE PROCUREMENT

A major procurement source of tissue has historically been directly through organ procurement organizations (OPOs), which were developed to meet the need for transplantable organs. In 1986, the Health Care Financing Administration was empowered to certify OPOs to serve as central coordinating agencies for recovery and distribution of donor organs in designated areas. That same year, the Consolidated Omnibus Budget Reconciliation Act was passed requiring hospitals to have written policies and procedures for the identification of potential donors and a system to inform potential donor families, notify Health Care Financing Administration-certified OPOs of all potential donors, and be affiliated with the Organ Procurement Transplant Network. All OPOs are members of the Organ Procurement Transplant Network, which is run by the United Network for Organ Sharing under contract with the US Department of Health and Human Services and the Health Resource Services Administration.

The OPOs work as a nationwide network. Each is responsible for the recovery and distribution of organs in its federally designated service area and then for sharing donated organs based on need. The OPOs evaluate the potential donors, discuss donation with family members, and arrange for the surgical removal of donated organs. They are also responsible for preserving organs and arranging for their distribution according to national organ-sharing policies. Currently, there are approximately 59 organ-procurement organizations operating within the US and Puerto Rico. The OPOs enhance tissue procurement efforts because the law also requires that OPOs maintain contracts with tissue banks for donor referral. This assists tissue banks in locating sources of allograft tissues. Once a potential donor is located, tissue banks can begin the procurement process. The procurement process involves a trained donor coordinator obtaining consent from the patient or family members to procure organs or specific tissues. The coordinator must inform family members how every tissue that is being donated may be used and must request consent for research separately.

Federal law requires that tissue donors be screened for relevant high-risk behavior for potential exposure to HIV, hepatitis B, and hepatitis C. The FDA recommended that this information be obtained by conducting an interview with the patient, if living, or a knowledgeable person who can provide a medical history and by performing a physical assessment of the donor's body to discern any signs or symptoms related to the viruses mentioned.[21] Tissues from donors determined to be free of risk factors for these viruses may proceed to tissue banks with detailed documentation of patient history and procurement documentation. Blood samples taken either at the time of recovery or from a banked hospital sample from the current admission are collected and sent to a laboratory that has been certified under the Clinical Laboratory Improvement Amendments of 1988. The FDA-licensed tests for use on cadaveric blood samples, where applicable, are used.

Tissues may be procured and processed in an aseptic environment such as an operating room. In a survey of tissue banks by Vangsness et al.,[66] 33% of procurement took place in the operating room exclusively, 39% in the hospital morgue, 22% in coroner's facility, and 14% at a tissue bank facility. The time limit for retrieval of soft tissues after death of the donor varied among the tissue banks; 86% of the tissue banks surveyed had a 24-hour limit for refrigerated cadaveric donors. For donors procured after room-temperature storage, 82% of the tissue banks had a 12-hour limit for retrieval. Some banks have extended their retrieval period to 48 hours for refrigerated cadaveric donors. Once tissues are recovered, they are transported and stored under conditions designed to maintain tissue integrity until they are further processed for transplantation. Documentation of every stage of the process is required.

ASEPTIC TISSUE PROCESSING

Just as there is no absolute sterile surgery, there is no absolute sterile recovery of musculoskeletal allograft tissue. As surgeons, we are attempting to transplant tissue without infecting the patient—a situation called asepsis. Aseptic processing is the most common method of allograft preparation in the US today. The term "aseptic processing" refers to methods used by a manufacturer to avoid adding contamination to a product. Aseptic processing per se does not remove contamination. Tissues may be contaminated before they reach the tissue-processing facility. This contamination may be the result of an occult infection in the donor, postmortem invasion of the tissue by bacteria from the gastrointestinal tract, or potential contamination introduced during the recovery process. Aseptic processing neither removes contamination nor does it remove all blood, lipids, and other cellular elements from the tissue. Remaining blood and lipids may harbor pathogens.

It has been calculated that the risk of implanting an allograft tissue from an undetected HIV carrier can be reduced to 1 in less than 1,000,000 by observing aseptic processing and strict adherence to donor screening and testing.[9] Although this risk is quite low, there have been two documented cases of HIV transmission due to musculoskeletal allograft tissue transplantation.[64] There have also been cases of allograft-related transmission of hepatitis C[12] and human T-cell leukemia virus in 1991,[52] and hepatitis B in 1951.[55] These transmissions occurred before FDA requirements for donor screening for bloodborne viruses and bacteria (that is, medical and social history, and serologic testing) and availability of validated serologic tests. Recently, bacterial transmission has been documented.[65]

To reduce the chance of infectious disease transmission, some tissue processors use antimicrobial solutions. These solutions may consist of antiseptics (for example, iodophors and alcohol) or antibiotics (for example, polymixin and gentamicin), or both. These solutions, however, may not penetrate the tissue and thus may only be effective against surface contaminants. Infectious agents deep within the tissue matrices (for example, from contamination after postmortem tissue invasion by bowel flora) may remain.

TISSUE STERILIZATION

Sterilization has been defined as the process or act of inactivating or killing all forms of life, especially microorganisms.[7] According to the Association for the Advancement of Medical Instrumentation, a standards-setting organization for the medical industry, sterility assurance level (SAL) is defined as the probability of an item being nonsterile after it has been exposed to a validated sterilization process.[58] Implantable medical devices are sterilized to achieve a SAL of 10^{-6}, meaning that there is less than a 1 in 1,000,000 possibility of a contaminating organism surviving the treatment. Sterilized tissues do not transmit infectious agents. However, sterilization of musculoskeletal tissue has associated challenges. First, the biomechanical properties of tissue can be adversely affected by heat and irradiation.[6,39,49,57] Second, not all sterilants (especially gases and liquids) have adequate tissue penetration. Without good tissue penetration, sterility cannot be assured. Third, in contrast to synthetic materials such as metals or plastics, musculoskeletal tissues may be contaminated with large numbers of organisms (that is, have a high bioburden). The higher the number of microorganisms, the longer or more concentrated the treatment must be to achieve sterilization. Fourth, tissue is an organic material that can serve to protect microorganisms and may cause sterilization-process failure.

As of March 11, 2002, the Centers for Disease Control and Prevention (CDC) had received 26 reports of bacterial infections associated with musculoskeletal tissue allografts after a nationwide investigation prompted by a patient death in November 2001 from an infected allograft.[65] These reports underscore the need to develop and implement sterilization technologies that can be used effectively with human tissue grafts. As noted by the CDC, "When possible, a method that can kill bacterial spores should be used to process tissue. Existing sterilization technologies used for tissue allografts, such as gamma

irradiation or new technologies effective against bacterial spores, should be considered. Unless a sporicidal method is used, aseptically processed tissue should not be considered sterile, and health-care providers should be informed of the possible risk for bacterial infection."[65]

The efficacy of sterilization processes can be demonstrated by using biologic indicators containing highly resistant bacterial spores. The percentage of reduction (log reduction or D value) provides a measure of efficacy of a sterilization process. An optimal sterilization process should adequately address all of the challenges noted and not impair the biomechanical integrity of the tissue. If tissue banks use sterilization, they typically employ one of two methods of sterilization: ethylene oxide or gamma irradiation.

Ethylene Oxide

For more than 40 years, ethylene oxide (EO) has been used for the sterilization of heat- and moisture-sensitive medical devices,[43] and it has been an effective sterilant of bacterial[47] and viral particles.[34,45] Ethylene oxide is applied in a gaseous state (boiling temperature, 10.7°C) in mixture with inert diluents such as CO_2 or Freon (dichlorodifluoromethane, Dupont Corporation, Wilmington, Delaware) to avoid accidents during processing because of its inflammability. This is followed by removal of EO or its replacement with an inert gas like CO_2.[16]

Reports in the literature have noted toxic effects of EO as an industrial fumigant,[26,46] and the removal of residual gases from bone grafts has been a major concern for EO-sterilized allografts. In 1978, concern over the toxicity of EO and its byproducts led the FDA to establish recommendations of minimum levels allowed for residual concentrations of EO, ethylene chlorohydrin, and ethylene glycol for sterilized products. Epidemiologic evaluations of EO-exposed workers have shown that there is an increased risk of malignancy with extended or intermittent exposure and an abnormally high rate of spontaneous abortions in pregnant female workers who are exposed to the gas.

Cloward[11] reported using 187 EO-sterilized bone grafts in 58 patients for spinal fusion in 1980. When observing patient radiographs from 4 months to 1 year, he noted the rate of fusion to be comparable with other bone grafts with no complications. Prolo et al.[48] published perhaps one of the more extensive reviews of EO in 1980, and they found it to be a safe and inexpensive sterilant of bone (primarily cranial bone) and soft tissue (fascia lata and dura). They stressed the importance of adhering to a strict protocol that reduces the concentrations of residual gases. They recommended irrigation with deionized water before sterilization to rid the bone of marrow and freeze-drying them for more than 72 hours. The authors concluded that EO was ideally suited for sterilization of tissues. In some cases, aided with aeration at room temperature, the formation of the reaction products ethylene chlorohydrin and ethylene glycol were reduced to acceptable levels.

A recent study by Kaku et al.,[37] involving sterilization of bone chips with EO, found that several preoperative aeration steps and storage time for more than 2 weeks at room temperature, as well as intraoperative rinsing of grafts with a 500-ml salt solution for 10 minutes, reduced EO residuals in grafts. Arizono et al.[3] examined the concentration of EO after sterilization in bone chips and recognized the importance of defatting and freeze-drying to reduce the residual EO concentrations. The higher the residual EO concentration, the lower the fibroblastic culture activity. In a study evaluating the osteoconductive properties of EO on bone, Thoren and Aspenberg[63] used a rat bone model and found that EO-treated bone allografts impaired bone ingrowth, even though the residual concentrations were lower than the FDA-approved guidelines. The study stated that EO might have a deleterious effect on graft incorporation, when using the bone model investigated by the authors.

One concern about EO is its ability to penetrate large cortical bone. Prolo et al.[48] reported that one test sample of four femoral diaphyses showed delayed growth of *Bacillus subtilis,* attesting to partial but incomplete penetration of bone by EO. Kakiuchi et al.[36] evaluated EO penetration into bone by using a defatting procedure with a mixture of chloroform and methanol at room temperature. They were able to demonstrate EO penetration into the core of femoral heads. Additionally, Kakiuchi and Ono[35] conducted a retrospective clinical study using EO-sterilized, defatted, and lyophilized bone grafts. Of 215 thick cortical bone grafts, 4 (1.9%) developed infection.

Although adverse reactions to the use of EO-sterilized bone grafts have not been reported, there are two studies that suggest intraarticular reactions after the use of ligament grafts sterilized by EO. The study by Jackson et al.[33] raises the question of whether EO causes intraarticular synovial and immune reactions. Of 109 patients who underwent ACL reconstruction with a freeze-dried, EO-sterilized, bone-patellar tendon-bone allograft, 7 patients (6.4%) developed a persistent intraarticular reaction. The reaction was characterized by persistent synovial effusion with collagenous particulates and cellular inflammatory response. There was no direct evidence of toxicity with EO or its byproducts, but removal of the EO-sterilized soft tissue grafts resulted in resolution of the reactions. Using gas chromatography, they examined one of the seven grafts that was removed and measured high levels of ethylene chlorohydrin. It was unclear whether the synovitis reaction was due to the EO-sterilized grafts. The human leukocyte antigen conversion was noted in three of the seven allograft recipients.

In a study by Roberts et al.,[50] 8 of 36 patients (22%) who received freeze-dried EO-sterilized bone-patellar tendon-bone allografts had complete dissolution of the graft on repeat surgery. The exact cause of graft dissolution was unclear, but the authors agreed with Jackson et al. that the most probable cause of graft failure was EO and its byproducts. Roberts et al. did not measure ethylene residues at the time of graft insertion or clinical follow-up. Bechtold et al.[5] examined the biomechanical properties of bone-patellar tendon-bone allografts sterilized with EO and found no significant differences for ultimate stress to failure and modulus elasticity. In addition to intraarticu-

lar reactions from bone-patellar tendon-bone grafts, Silvaggio et al.[56] reported significant levels of IL-1 generated from EO-treated bone-patellar tendon-bone particles compared with controls in an in vitro culture study. There was concern that such wear particles would increase tissue inflammation.

The reports from studies that do not favor EO as a method for sterilization have led to a trend for tissue banks to limit the use of EO for sterilization. A survey by the AATB of tissue banks during 1987 and 1988 reported that EO was used in two-thirds of the banks, and irradiation for sterilization was used in one-third,[41] compared with an increase in ionizing irradiation that was noted in 1992, where irradiation was used twice as often as EO.[59] Therefore, although EO continues to be used by hospitals and industry, many tissue banks now rely on other methods for sterilization, such as gamma irradiation.

Gamma Irradiation

The virucidal and bactericidal effects of gamma irradiation are created by two mechanisms.[24] The primary mechanism is direct alteration of nucleic acids leading to genome dysfunction and destruction. A secondary mechanism is the generation of free radicals, primarily from liquid water. This secondary effect is not realized, however, when an item has been lyophilized or is frozen at the time of irradiation, as are most tissue products.[6] Because of the differences in efficacy of gamma irradiation in the presence and absence of free water, items presented for gamma irradiation sterilization in the frozen or freeze-dried state require significantly higher doses to achieve the same effect as would be realized if the item were in the liquid, hydrated state.

At this time, it is not known how many tissue banks use gamma irradiation and the irradiation levels used. A survey of 36 tissue banks by Vangsness et al.[66] found that a range from 1 to 3.5 Mrad (10 to 35 kGy) of gamma irradiation was used to sterilize tissues. Gamma irradiation is very effective against bacteria at doses of 1.5 to 2.5 Mrad. However, gamma irradiation is much less effective against viruses. Fideler et al.[19] determined that 4.0 Mrad was needed to inactivate HIV in bone-patellar tendon-bone allografts. Conway et al.[13] estimated that more than 3.6 Mrad may be needed to inactivate all but 1 in 1,000,000 HIV-infected cells. A 1999 study showed that 3.5 Mrad was required to inactivate the HIV from bone.[10] This study also showed that 8.9 Mrad was required to reach a sterility assurance level of 10^{-6}.

Increasing the level of irradiation can affect the biomechanical properties of allografts in the laboratory. No significant human outcome studies have been conducted. Cortical bone loses significant strength in bending and torsion if exposed to more than 3 Mrad.[8,44,54] Lower levels of gamma irradiation can affect ligaments. In a study by Fideler et al.,[18] it was determined that greater than 2 Mrad of irradiation of bone-patellar tendon-bone allografts adversely affected four of seven structural properties that were analyzed. Statistically significant reductions were found in this study for all of the structural properties for irradiation at 3.0 and 4.0 Mrad. For these reasons, orthopaedic surgeons should be aware of the dose of gamma irradiation used by tissue processors for musculoskeletal tissues.

Other Sterilization Methods

New low-temperature chemical sterilization methods with good tissue penetration have been developed. These appear to be sporicidal and do not appear to adversely affect the biomechanical properties of tissue.[61,62,67] Other sterilization methods are under development. These include supercritical CO_2 and the use of antioxidants in combination with gamma irradiation.

ALLOGRAFT STORAGE

Currently, the methods of knee allograft tissue preservation before storage are cooling and fresh transplantation within 24 days, freeze-drying, and deep freezing at –80°C or –196°C. Preservation and storage methods for the meniscal and ligament tissue can differ significantly from those for articular cartilage and bone.

Cryopreservation, a process of controlled-rate freezing with extraction of cellular water by means of dimethylsulfoxide and glycerol, is one method used for preserving menisci and ligaments at some tissue banks. Because of damage to the cartilage matrix during freezing, cryopreservation of articular cartilage has not proved as satisfactory as the use of fresh grafts. The process of cryopreservation, originally developed to preserve sperm and embryos, prevents cell death by altering crystallization within cells during freezing. A typical cryopreservation process may include the following steps: Grafts are initially cooled to 0°C and processed within 48 hours of donor death. After decontamination with antimicrobial solutions, allografts are subjected to controlled-rate freezing to –135°C and packed in a cryoprotectant solution. Cryopreserved grafts can be stored at –196°C for as long as 10 years.[53]

Deep-freezing is the simplest and most widely used method of ligament and meniscal allograft storage. After recovery, the graft may be frozen, pending the results of donor screening and testing, after which it is thawed and processed. Freezing to –80°C is typical for frozen storage. 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 because of the relative acellularity of ligament and meniscal tissue.

Freeze-drying (lyophilization) can be used for ligament and meniscal allografts. Standard graft processing is followed by freezing and lyophilization to a predetermined residual moisture level. The graft can then be vacuum-packaged and stored at room temperature for up to 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, as well as the structural integrity and material properties of collagen, are weakened with this lyophilization process.

Currently, most osteochondral allografts are trans-

planted fresh, which better preserves both cartilage cells and matrix. These grafts contain marrow elements within the bone, which increases both the antigen exposures to the recipient and the possibility of disease transmission. Because the graft must be used on a semiemergent basis, obtaining the correct size can be difficult. Viable chondrocytes can be maintained in lactated Ringer's solution cooled to 2° to 4°C for 7 to 14 days[4]; however, after 24 hours there begins to be a detectable decrease in the percentage of viable cells. Generally, fresh articular cartilage allograft is transplanted within days of harvesting, with the understanding that the longer the wait, the greater the death of cartilage cells.

CONCLUSION

Advances in medical science and cell biology have allowed transplantation of cells and tissue from one human to another. Allograft transplantation has been shown to facilitate the reproduction and restoration of knee form and function, as well as enhancing the patient's quality of life. It is essential that orthopaedic surgeons who use allograft tissue understand the tissue banking process and the origins of the allograft tissue they use. As science and technology advance, use of musculoskeletal allograft tissue transplantation in the knee will continue to improve and expand. The goal of tissue banking is to provide safe and effective tissue for orthopaedic surgeons and their patients.

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