In the United States, organ and tissue procurement has been organized in such a way so as to maximize availability in an efficient fashion that is fair to both donor and recipient. There is a growing cooperation between organ procurement organizations (OPOs) and tissue recovery organizations to facilitate meeting the requirements of both organ and tissue procurement. Successful tissue preservation has encouraged the use of bone and soft tissue grafts, cardiovascular tissue, and skin in the clinical setting. Semen cryobanking has been practiced for many years, and the demand for embryo banking services has greatly increased recently as assisted reproductive procedures have become more diverse and much more widely available. As technology in this area continues to improve, hospital transfusion services may play an increasingly important role in coordinating, supporting, and directing transplantation and transplant-related interventions.

**Growth of tissue banking**

Each year, hundreds of thousands of patients benefit from donated bone, cartilage, ligament, and tendon used to reconstruct and rehabilitate joints and other bony structures; corneas to restore sight; skin to treat burns and wounds and reconstruct soft tissues; veins and great vessels to restore blood flow; heart valves to restore cardiac function; and reproductive tissue to treat infertility. In addition to whole grafts and sections, bone, in particular, can be machined into specialized allografts. It can also be ground and further treated to become demineralized bone matrix (DBM), which is available as a ground powder or as combination forms such as gels, pastes, strips, and even moldable grafts designed to avoid migration in specific applications. Myriad types of tissues are transplanted in a variety of medical and dental specialities for diverse clinical applications (Table 40.1). The most commonly transplanted allografts are bone, musculoskeletal soft tissue, and corneas.

The total number and range of tissues collected, processed, and transplanted in the United States are difficult to determine, in the absence of a national reporting system. Organ statistics, in contrast, are readily available through a number of organizations (donatelifeline.net and UNOS.org). In 1999, tissue banks accredited by the American Association of Tissue Banks (AATB) distributed approximately 750,000 allografts for transplantation. By 2003, the figure had more than doubled. By 2007, more tissue banks became accredited and the figure had nearly tripled. The quantity of musculoskeletal (bone and soft tissue) allografts distributed annually is greater than 2,000,000 units. A focused survey revealed this number had increase by at least 10% during 2012 (personal communication, Scott Brubaker, Senior Vice President, Policy, the AATB). Skin is distributed at the rate of about 30,000 square feet annually. Distribution of cryopreserved allograft heart valves is now estimated at >6000 annually. Cornea transplants numbered >72,000 in 2013.

In addition to such tissues from deceased donors, many tissues for clinical use are derived from living donors, including semen and oocytes for use in artificial insemination and assisted reproductive technology procedures as well as dermis for various soft tissue reconstruction procedures. In 2005, New York State–licensed semen banks located throughout the United States processed 27,118 ejaculates from 962 semen donors. In addition, semen from 16,347 client depositors was collected and cryopreserved for later use by the client depositor’s wife or other “intimate partner” (New York State Department of Health, unpublished data, 2005). Donor oocytes were used in approximately 13% of all assisted reproductive technology cycles carried out in the United States in 2012. Tissue from living donors is also considered by some to include human milk to nourish low-birthweight, premature infants. The Human Milk Banking Association of North America reports the existence of 16 active member banks. The US Food and Drug Administration (FDA), however, does not subject human milk to regulations that apply to human allograft tissue.

Because no comprehensive national usage figures are available, it is difficult to determine the rate of increase in the demand for tissues. However, novel uses for tissue in transplantation have emerged that had not been envisioned even a few years ago. Amniotic membrane is transplanted to correct epithelial eye and soft tissue defects (e.g., ulcers); bioengineered skin allografts are used for wound healing; and nerve tissue is transplanted to serve as a conduit for nerve regeneration in damaged limbs. In late 1998, the medical world was fascinated by the first hand transplant surgery; there have been at least 65 worldwide since. The world’s first face transplant procedure was performed in November 2005. Since then, more than 20 patients have received full or partial face transplants at institutions around the world. In the future, human pluripotent progenitor cells from embryos may be used in the treatment of a variety of diseases considered incurable at this time (Parkinson, diabetes, and spinal cord injuries as examples).
Table 40.1 Some common human allograft applications, by specialty

<table>
<thead>
<tr>
<th>Specialty</th>
<th>Procedure/Application</th>
<th>Allograft Types Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>General orthopedics</td>
<td>Trauma/fracture repair; osseous defect repair; acetabular repair; total joint revision/arthroplasty</td>
<td>Femoral head; femoral condyle; whole, proximal, or distal bone shaft (femur, tibia, humerus); hemi-pelvis; cancellous bone; corticocancellous bone; cortical strut/screw/pin; bi-cortical strip; tri-cortical wedge; whole joint (knee, ankle, shoulder, elbow); osteoarticular graft*; osteochondral graft*; DBM; osteobiologics</td>
</tr>
<tr>
<td>Sports medicine</td>
<td>Tendon, anterior cruciate ligament, posterior cruciate ligament, other knee ligament repair; meniscus repair/replacement; osteochondral defects; rotator cuff repair; ankle/tendon ligament repair; hand/wrist repair</td>
<td>Patellar ligament; Achilles tendon; tibialis tendon; semitendinosus tendon; gracilis tendon; peroneus longus tendon; fascia lata; rotator cuff; meniscus; meniscus with/without plateau; osteochondral plug*; femoral head-icamy*; acellular dermal matrix</td>
</tr>
<tr>
<td>Craniofacial/ maxillofacial</td>
<td>Cranial reconstruction; maxillary/mandibular reconstruction; facial palsy repair</td>
<td>Mandible; dura mater*; fascia lata; acellular dermal matrix; bi-cortical strip; tri-cortical wedge; DBM; osteobiologics; sclera</td>
</tr>
<tr>
<td>Dental</td>
<td>Alveolar ridge augmentation for dental implant placement; onlay grafting; sinus elevations/augmentation; socketridge preservation; intrabony defect repair</td>
<td>DBM; osteobiologics; sclera; acellular dermal matrix; particulate and structural cortical and cancellous grafts and combinations</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>Presequester corneal edema repair; Fuchs dystrophy repair; glaucoma drainage valve implantation; coneo-scleral fistula repair; keratoconus correction; phaco burn repair; orbital reconstruction following enucleation; eyelid ectropion repair; eyelid reconstruction</td>
<td>Cornea*; sclera*; pericardium</td>
</tr>
<tr>
<td>Neurosurgical</td>
<td>Cervical/lumbar interbody fusion; intermediary rod placement; durotomy replacement</td>
<td>Dura mater*; fascia lata; pericardium; cancellous bone; corticocancellous bone; cortical strut; bi-cortical strip; various machined and constructed proprietary bone forms*; amniotic membrane*; acellular dermal matrix</td>
</tr>
<tr>
<td>Burn treatment</td>
<td>Wound covering†</td>
<td>Fresh skin*; cryopreserved skin*; freeze-dried skin*; acellular dermal matrix; amniotic membrane*</td>
</tr>
<tr>
<td>General surgery</td>
<td>Urologic incontinence procedure; pelvic floor reconstruction; nephrahyphoraphy; breast reconstruction</td>
<td>Fascia lata; pericardium; lyophilized skin*; acellular dermal matrix</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Congenital anomaly repair (both valve and outflow tract major vessel repair/replacement*); cardiac valve and vessel repair/replacement; major vessel blood “shunting” procedures</td>
<td>Aortic valve*; pulmonary valve*; various conduit-use-only grafts* from the ascending aorta or thoracic aorta or from the pulmonary artery trunk or its branches</td>
</tr>
<tr>
<td>Vascular</td>
<td>Vasoc-occlusive disease (peripheral, abdominal, thoracic); cardiac artery bypass grafting; arteriovenous shunt insertion; muscle-flap or organ transplant vascular bed extensions; replacement of infected prosthetic devices</td>
<td>Greater saphenous vein*; aorto-iliac artery; iliac vein; iliac artery; femoral vein*; femoral artery*</td>
</tr>
</tbody>
</table>

* Not sterilized.
† Can be life-saving.
DBM, demineralized bone matrix.

**Tissue donation**

**Living donors**
The tissue most commonly donated by living donors is blood, the primary subject of this book, but living donors also provide other tissues. Tissue donation by a living person generally is limited to renewable tissue, such as gametes, extraembryonic tissue, and milk. Except for autografts, which can be expanded by culturing for use on burned patients, skin is usually recovered from deceased donors. Cartilage can also be cultured for autologous transplant in knee repair. Bone can be obtained from living donors in the form of a bone graft from the iliac crest (femur, tibia, humerus); hemi-pelvis; cancellous bone; corticocancellous bone; cortical strut/screw/pin; bi-cortical strip; tri-cortical wedge; whole joint (knee, ankle, shoulder, elbow); osteoarticular graft*; osteochondral graft*; DBM; osteobiologics.

**Deceased donors**
In contrast to organ donors, tissue donors (excluding living donors, of course) need no functioning circulation. Tissues such as bone, eyes, and skin can generally be collected up to 24 hours after cessation of the donor’s cardiac and respiratory functions, depending on the temperature and environment in which the donor body is stored. Tissues such as bone and skin can be donated by deceased organ donors (a standard criterion for organ donors in the United States is brain death), but more commonly, tissues are donated by other hospitalized patients who have been judged deceased by both cardiorespiratory and neurologic criteria. Tissue procurement practices have become more effective as a result of increased cooperation between tissue banks and OPOs, through routine referral requirements. Referrals to eye banks are also made through OPOs.

The availability of tissues for transplantation depends on strong public support, the presence of laws that clear legal barriers to donation and that authorize consent (authorization) before death (through such means as driver’s licenses, donor registries, and advance directives), and the availability of health professionals trained in how to approach the next of kin or other authorizing person on the subject of donation. (The next of kin is/are usually a deceased person’s closest relative[s], as determined by a hierarchy specified in state law as defined by the Uniform Anatomical Gift Act.)

In the late 1980s, most states enacted “required request” laws that sought to increase the supply of organs and tissues by requiring that hospital personnel request permission of the next of kin for organ and tissue donation at the time of a patient’s death (if the prospective donor was medically eligible). Following findings that families were more likely to consent to donation if approached by requesters who had received training in the most effective ways to present donation opportunities, some states amended these laws to require referral of all deaths to an OPO or designated tissue bank, whose specially trained requesters would then ask for consent. Federal routine referral requirements were implemented by the Health Care Financing Administration (now the Centers for Medicare and Medicaid Services) in Hospital Conditions of Participation for
Organ, Tissues, and Eye Donation, which became effective August 21, 1998.9 These rules mandate, as a condition of Medicaid and Medicare reimbursement, that all hospitals establish formal relationships with an OPO in the service area, with a tissue bank, and with an eye bank. Further, the rules require that hospital personnel notify OPOs of all deaths and imminent deaths so that potential donors are identified, and so that the next of kin will be approached about donation, when appropriate. Under routine referral, the costs associated with around-the-clock reporting of deaths and imminent deaths are proving to be high in certain areas, although the cost to OPOs is shared by Medicare and the transplanting hospitals through “standard acquisition charges” applied to individual organs transplanted. Uniform Anatomical Gift Laws were developed to standardize state requirements for first party consent, requesting personnel, and recovery. A 2006 revised version, which expanded definitions, clarified roles, and enhanced focus on personal autonomy, has been enacted in nearly every state (see http://uniformlaws.org). The National Association of Attorneys General adopted a resolution in 2010 in support of respecting and upholding the decisions made by persons who elect to be organ, eye, and tissue donors.10

**Organization of tissue banking in the United States**

Unlike the organ procurement and sharing system, tissue banking is not formally organized based on geography. There are approximately 21 independent tissue banks that process conventional musculoskeletal tissue, four that process cardiovascular tissue, and 14 that process skin. Although most are not-for-profit, a few for-profit companies process human tissue for transplantation. Processors usually independently perform donor eligibility assessment and laboratory testing, even if the tissue bank that recovered the tissue had already performed such steps. There are approximately 80 not-for-profit eye banks accredited by the Eye Bank Association of America (EBAA; see http://www.restoresight.org). Almost all cornea allografts are provided by these eye banks. Blood centers with expertise in donor recruitment, donor eligibility determination, cell cryopreservation, and compliance with standards and regulations are good candidates to undertake tissue banking services. A few blood centers have taken on the challenge of providing comprehensive tissue bank services, and they recover, process, store, and distribute tissues.

Procedures to achieve allograft safety and efficacy are guided by national professional standards set by such organizations as AATB,11 EBAA,12 and the American Society for Reproductive Medicine.13 The FDA and some states have regulatory and licensure requirements (see the “Oversight” section).

### Tissue transplant-transmissible diseases and their prevention

#### Transmissible diseases

Despite a careful donor selection process, the risk of donor-to-recipient transmission of viral, bacterial, fungal, and prion diseases cannot be eliminated (Table 40.2).14,15 In one well-publicized case, 48 organ or tissue recipients received an organ or tissue from a single donor who, although he had no apparent risk for HIV infection according to medical history, proved to have been recently infected with HIV and in the window period before HIV-1 antibody could be detected by the assays in use at the time (October 1985).14 All four organ recipients became infected with HIV, but the majority of tissue recipients did not. Whole unprocessed frozen bone did transmit HIV to three recipients, whereas bone from which the marrow had been removed did not; transplanted corneas, lyophilized soft tissue, and γ-irradiated dura mater also did not transmit the virus. This case additionally served to highlight vulnerabilities associated with inadequate disposition records, given that six recipients could not be identified from hospital records. In 2002, before routine implementation of hepatitis C virus (HCV) nucleic acid testing (NAT) for tissue donors, tissue from a man with no identifiable infectious disease risk by history or physical examination, and a negative test for anti-HCV, was found to have transmitted HCV to recipients.16 All organ recipients who could subsequently be tested were found to be infected with HCV. Among 32 tissue recipients, five probable cases occurred: one of two saphenous vein recipients, one of three tendon recipients, and three of three recipients of tendon with bone allografts. No cases occurred in recipients of skin, cornea, or irradiated bone. All eight recipients whose infection was linked to the transplant were determined to be infected with the same HCV genotype. The current risk of viral transmission is thought to be exceedingly low17,18 as a result of stringent donor selection, testing strategies, and processing methods now in use that reduce the risk for tissues not requiring viable cells.

West Nile virus transmission linked to breastfeeding of a woman’s own infant, to organ transplantation, and to blood transfusion have been reported,19 but transmission through donor tissue has not been observed. Chagas’ disease has been linked to transplantation of organs but transmission of parasitic diseases through vascular tissue allografts (considered likely to be possible) has not been reported.20,21 Although vessel grafts associated with organ transplantation, in which arteries or veins from a different donor may be used if the donor and/or recipient vessels are damaged or insufficient, are not considered tissue by the FDA, such grafts caused documented transmission of rabies to two recipients in 2004.22 Transmission of malignancy via tissue transplantation has not been reported, but is thought to be possible. Infectious diseases can also be transmitted through transplantation of tissue from living donors. Cases are well documented in the semen banking arena with

<table>
<thead>
<tr>
<th>Allografts</th>
<th>Infectious Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>Hepatitis C</td>
</tr>
<tr>
<td></td>
<td>Hepatitis, unspecified type</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus type 1</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis</td>
</tr>
<tr>
<td></td>
<td>Fungus</td>
</tr>
<tr>
<td>Cornea</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td></td>
<td>Rabies</td>
</tr>
<tr>
<td></td>
<td>Creutzfeldt–Jakob disease</td>
</tr>
<tr>
<td></td>
<td>Cytomegalovirus (?)</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Fungus</td>
</tr>
<tr>
<td>Dura mater</td>
<td>Creutzfeldt–Jakob disease</td>
</tr>
<tr>
<td>Heart valve</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>Skin</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td></td>
<td>Fungus</td>
</tr>
<tr>
<td>Pericardium</td>
<td>Cytomegalovirus (?)</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus type 1 (?)</td>
</tr>
<tr>
<td>Pancreatic islet</td>
<td>Bacteria</td>
</tr>
</tbody>
</table>

Source: Adapted from Eastlund.15
a variety of agents and diseases having been transmitted to semen recipients. Human immunodeficiency virus, type 1 (HIV-1) has been transmitted both by unrelated donors and from husband to wife. This virus has the greatest number of reports of transmission. Hepatitis B virus (HBV), gonorrhea, Ureaplasma urealyticum, Mycoplasma hominis, Trichomonas vaginalis, Chlamydia trachomatis, group B Streptococcus, and herpes simplex virus type 2 (HSV-2) have also been reported. Transmission of HCV and human T-cell lymphotropic virus, type 1 (HTLV-1) is likely, and transmission of cytomegalovirus, human papilloma virus, and syphilis is a possibility.\textsuperscript{25}

In addition, transmission has occurred with the use of bone removed during surgery, such as a femoral head collected during hip arthroplasty.\textsuperscript{15} Transmission of bacteria is a known risk of tissue transplantation. Bacteria can be present in the donor, either as a normal occurrence or as the result of a disease process or medical intervention.\textsuperscript{15} Resuscitation efforts can increase the dispersion of such organisms. In addition, agonal bacteremia is a well-known process whereby endogenous bacteria, such as normal intestinal flora, begin to disperse throughout the body after cessation of cardiopulmonary functions, as the putrefaction process begins. This process is accelerated in persons with sepsis, rhabdomyolysis, or a cocaine overdose before death.\textsuperscript{15} Bacteria can be introduced during tissue recovery; during processing, whether through cross contamination, insufficient aseptic technique, or contaminated chemicals or solutions; or even during packaging. Some tissues, including ocular tissue, skin, and semen, are inherently not sterile even if collected aseptically; fortunately, the contaminants are primarily skin contaminants with low virulence in most recipients, and are susceptible to standard antibiotics.

At particular risk for transmission of bacteria are the articular cartilage allografts used in knee surgery, because these cannot be subjected to extensive processing if their mechanical properties and chondrocyte viability are to be preserved (note, however, that the latter has not been proven to be essential). In 2001, a 23-year-old man was found to have died from Clostridium sordellii sepsis following receipt of a femoral condyle allograft. An extensive investigation identified 14 patients who had received allografts between 1998 and 2002 and who developed postoperative infections with Clostridium species.\textsuperscript{15,24} The tissues were derived from nine donors, but all were prepared by the same processor, and investigations identified several factors that could have contributed to the infections. Although the tissues were cultured, they had already been suspended in antimicrobial solutions, likely leading to false-negative results. Additionally, for two of the donors, including the donor of the tissue that was implicated in the fatal case, the interval between death and refrigeration of the body (19 hours for the donor in the fatal case) exceeded the industry's voluntary standards at the time of 15 hours maximum without refrigeration of the deceased donor. This likely permitted excessive bacterial proliferation prior to tissue recovery. In addition, tissue was processed aseptically without the use of terminal sterilization, and the processing methods used had not been validated. Human error can also lead to release of contaminated tissues. In one case, a technician failed to follow standard procedures, resulting in release of tissue labeled as having been subjected to irradiation when, in fact, no irradiation had occurred.\textsuperscript{25} However, a wound infection in a tissue recipient, or even disseminated bacteremia, does not necessarily imply the donor tissue. Site infections are a well-known risk of many surgical procedures and can result from the use of contaminated solutions or equipment insufficiently sterilized between procedures.\textsuperscript{26}

**Risk reduction procedures**

After death has been declared and authorization for donation has been given, the process of determining the suitability of the potential deceased donor begins. The risk of disease transmission is minimized by collection and careful review of (1) information obtained during interviews with family member(s) or other knowledgeable historian(s) and healthcare providers; (2) available medical records; (3) findings of a physical assessment; (4) results of an autopsy, if performed; and (5) results of blood tests for infectious disease markers. Procedures for processing and, in some cases, sterilizing tissues also contribute to tissue safety.

**Donor history screening**

Evaluation of the health and behavioral risk history of the prospective donor is an important step in establishing the suitability of tissues for transplantation and preventing the transmission of infectious diseases. Family members may be able to provide reliable medical and behavior information, but friends and associates may provide relevant information not known to family members, especially the legal next of kin. Medical personnel may also be aware of significant medical and risk information. General donor eligibility criteria include the absence of systemic infection or any infectious or malignant disease transmissible by tissues and of behavioral risks for HIV infection or viral hepatitis. Malignancy generally disqualifies the donor, unless the malignancy is nonmetastatic or not known to metastasize to the tissue to be recovered (e.g., virtually no cancer is known to metastasize to the eye or skin), and there is no suspicion of direct regional spread. The donor history screening is specifically designed to reject those at high risk for viral hepatitis or HIV (e.g., nonmedical injected drug users, men who have had sex with another man in the past five years, persons who exchanged sex for money or drugs, persons with hemophilia or related clotting disorders, and persons with recent significant incarceration or with symptoms suggestive of a current viral infection).\textsuperscript{11}

Beyond the general selection criteria for donors, there are specific eligibility criteria for each tissue type, to facilitate selection of tissues that will function adequately, as well as those that will not transmit disease. For bone and soft tissue, these include such factors as: donor age, for bone that is intended to be used for weight-bearing functions; no evidence of significant metabolic bone disease or a connective tissue disorder; and no exposure to toxic substances that could accumulate in the tissue to be recovered. Donors of cardiac tissues are screened for a history of significant valvular disease or cardiac infection, and vascular donors may be determined ineligible if there is a history of diabetes, vasculitis, varicose veins, or significant atherosclerosis. Cardiac and vascular tissue donations are also limited by age restrictions per AATB standards and individual tissue bank policy. When skin is recovered, areas of skin exhibiting signs of a skin infection, or where a rash, nevus, or tattoo is present, are avoided. Cornea donors cannot have a history of refractive corneal procedures, such as radial keratotomy. Also per EBAA and AATB standards, tissue recovery sites in deceased donors are evaluated for trauma and infection and tissue should not be recovered from sites found to be damaged or contaminated. Donors of reproductive cells or tissues are screened for evidence or risk of inheritable diseases, and there are age restrictions. Extraembryonic tissue such as amnion and umbilical vein require the delivery to be full term; meconium staining of amniotic fluid is not acceptable, and there can be no current pelvic or vaginal infection in the mother. Consent is obtained from both parents.

In an effort to improve the efficacy and uniformity of the donor history acquisition process, a multiorganizational project team was
established to develop a standardized donor history questionnaire and materials, modeled after the acceptable full-length donor history questionnaire and accompanying materials for blood donors. The project team included members from the AATB, Association of Organ Procurement Organizations, EBA, NATCO (formerly the North American Transplant Coordinators Organization), Centers for Disease Control and Prevention (CDC), FDA, Health Resources Services Administration (HRSA), United Network for Organ Sharing (UNOS), and Health Canada. The goal was to eliminate questions that do not contribute significant information, and to develop simple and concise questions that employ terminology easily understood by the lay public. The effort produced a standardized form to serve as a guide for donor history information collection by tissue banks. Use of the form is not intended to be mandatory, but is designed to facilitate effective history collection, in compliance with regulatory and professional standards requirements and is highly recommended. The “Donor Risk Assessment Interview” form was made available by the AATB in 2012.

**Donor physical assessment**

The physical assessment seeks evidence that is consistent with risk for infectious diseases that would disqualify the donor. Such findings include unexplained jaundice or icterus; enlarged lymph nodes; unexplained white lesions in the oral cavity; blue or purple spots on the skin (possible Kaposi sarcoma); signs of nonmedical injected drug use; unexplained hepatomegaly; genital lesions consistent with a sexually transmissible disease; signs of systemic infection (generalized rash or petechiae); tattoos, piercing, or other body art that appears recent; trauma to intended recovery sites; corneal scarring; and a rash, a scab, or a lesion that is suspicious for vaccinia. The physical assessment is fully documented so that it can be reviewed, along with medical/social/behavioral history, medical records, and records of an autopsy (if performed). AATB offers a sample tissue donor physical assessment form and instructions for its use (see http://www.aatb.org/Guidance-Documents), to facilitate the performance and documentation of this assessment in a thorough manner by tissue banks.

**Tissue recovery**

Tissues are collected aseptically in an operating room, an autopsy room, or other suitable location where aseptic procedures can be performed. AATB standards require control of recovery site parameters including size/space, location, traffic, lighting, plumbing/drainage, ventilation, cleanliness of the room and furniture surfaces, absence of pests, absence of other activities occurring simultaneously, freedom from sources of contamination, and capacity to permit proper handling of contaminated equipment and disposal of biohazardous waste. In addition, all working surfaces must be disinfected. Following a surgical scrub, the person performing the recovery dons proper attire (gown and gloves). Body preparation, including shaving body hair, cleansing the skin with antimicrobial agent(s), and surgical draping of the body, is performed consistently with aseptic technique. Using aseptic technique, recovery of tissue is sequenced, and well-defined zone recovery methods are employed. Recommended recovery methods are described in AATB’s Guidance Document, “Prevention of Contamination and Cross-contamination at Recovery: Practices and Culture Results” (http://www.aatb.org/Guidance-Documents). Generally, recovered tissues are not processed at the time of recovery, Tissues are often cultured at recovery, then individually packaged in sterile wraps, labeled with a unique donor identifier, placed on wet ice, and sent without delay to tissue banks that will process them. Following donation, the donor body is usually reconstructed to permit normal funeral arrangements and viewing. The organ/tissue donor coordinator’s responsibilities continue after the transplant procedure, in that a letter of appreciation may be sent to the next of kin, giving general information about the use of the donated organs and tissues. This communication serves as a liaison between the donor’s family and the organ/tissue procurement agency. Later, contact may be reestablished to assist in amelioration of grief and bereavement. However, some donor families specifically request that there be no postdonation communication. AATB standards require formal establishment of donor family services. AATB has published a guidance document, “Providing Service to Tissue Donor Families,” which describes support that should be offered (http://www.aatb.org/Guidance-Documents).

**Infectious disease testing**

Infectious disease marker testing includes HIV-1 and HIV-2 antibodies, HCV antibody, hepatitis B surface antigen (HBsAg), and syphilis. In addition, nucleic acid testing (NAT) is performed for HIV-1 and HCV. Tissue banks also screen donors for antibodies to hepatitis B core antigen (anti-HBc), which, although a confirmatory test is not available, may indicate HBV infection even when HBsAg is not detectable. Tissue banks may perform HBV by NAT. Whenever possible, infectious disease marker testing is performed on pretransfusion/preinfusion blood samples to avoid false-negative results caused by hemodilution in the event that transfusions and/or infusions had been given shortly before the time of death. In the case of posttransfusion/infusion specimens, algorithms for determining the extent of plasma dilution are available. Testing of postmortem (postasystole) blood specimens may be complicated by hemolysis or the presence of sediment, which can cause false-positive results in some HBsAg enzyme immunoassays (EIAs) and false-negative results in some HIV, HBV, and HCV NAT assays. The tests for HIV and hepatitis must have been validated for use with postmortem specimens and approved by the FDA for use in tissue donor screening (www.fda.gov/cber/products/testkits.htm). When practical, tissue from living donors, such as semen donors, is preserved and quarantined until the donor is restested for HIV and hepatitis viruses in order to rule out seroconversion during the period of storage. Additionally, both semen and oocyte donors must be found negative for Neisseria gonorrhoea and C. trachomatis and are usually tested for carriage of one or more genetic disorder, as indicated by donor racial and ethnic background. Donors may also undergo specific testing for rare genetic disorders if the recipient couple seeks a donor known to be negative for a particular gene mutation.

**Tissue sterilization**

Tissue sterilization is defined as the killing or elimination of all microorganisms from allograft tissue, whereas disinfection refers to the removal of microbial contamination. The Association for the Advancement of Medical Instrumentation (AAMI), a standard-setting organization for the medical instrumentation and technology industry, defines Sterility Assurance Level (SAL) as the probability that an individual device, dose, or unit is nonsterile (i.e., one or more viable microorganisms being present) after it has been exposed to a validated sterilization process. While absolute sterility theoretically would represent an absence of any pathogen, SAL is generally applied only to the level of possible contamination.
with bacteria or parasites. In contrast to log reduction of viruses determined in assessments of virus reduction methods, SAL is an absolute determined by the ability of the method to eradicate or reduce microorganisms, the susceptibility of organisms that may be present to the sterilization method applied, and the maximal bioburden that could occur in the initial material. For example, a SAL of $10^{-6}$ means that there is less than a 1 in 1,000,000 chance of a viable microorganism remaining after the sterilization procedure. The FDA requires that medical devices be sterilized using a method validated to achieve a SAL of $10^{-6}$. A medical device derived from or that includes a biological product component must also meet a SAL of $10^{-6}$ if it is to be labeled sterile. A SAL of $10^{-3}$, or a 1 in 1000 chance of a viable microorganism being present, is a more achievable goal selected by some processors for aseptically processed tissues if the processor has been unable to validate their process to the more stringent SAL of $10^{-6}$ level or if the tissues are unable to withstand the harsh treatment needed to achieve a more restrictive SAL without an impairment of tissue function. Such tissues may not then be labeled as sterile.

The complex physical structures and density of musculoskeletal tissues pose challenges for adequate penetration of antimicrobial agents to eradicate microorganisms. Allografts will not tolerate methods usually applied to metal and plastic medical devices because such treatment would impair the mechanical and biologic properties necessary for clinical utility. As an alternative, sterilization of tissues has been accomplished by several methods, including heat, chemicals, ethylene oxide gas, supercritical CO₂, and gamma or electron beam irradiation. However, not all sterilants have adequate tissue penetration. This is particularly the case for gases and liquids. The initial bioburden, which may be high in some tissues, must be considered. Some tissues are treated with antibiotics in vitro before storage, but this treatment decontaminates only the surface and may be effective against bacteria only.

A variety of methods, including chemical treatments and irradiation, have been used to reduce or eliminate pathogens in tissue intended for transplantation. The introduction of bone sterilization by ethylene oxide gas simplified bone processing and facilitated the widespread use of sterilized air-dried and lyophilized bone products. The effects of ethylene oxide treatment on the biomechanical and osteoinductive capacity of bone allografts have been questioned, although animal studies have yielded inconsistent results. These concerns, combined with those regarding the carcinogenic potential of ethylene oxide and its breakdown products, have largely led to abandonment of this method in the United States and the United Kingdom.

First introduced over 40 years ago, γ-irradiation of bone is still used widely, usually employing a cobalt-60 source. The γ-rays penetrate bone effectively and work by generating free radicals, which may have adverse effects on collagen and limit utility in soft tissues unless performed in a controlled dose fashion at ultra-low temperature. The minimal bactericidal level of γ-irradiation is 10 to 20 kGy (1 kGy = 100,000 rad). Uncontrolled human studies have shown irradiated, calcified, and demineralized bone grafts to be clinically effective. Numerous studies have shown that mineralized bone allografts irradiated at 25 to 30 kGy are also clinically effective, with success rates of 85% to 91% reported. In controlled studies, the clinical effectiveness of bone allografts subjected to 25 kGy irradiation was comparable to that of nonirradiated bone grafts, although doses exceeding 25 kGy for cortical bone and 60 kGy for cancellous bone have been found to induce cross-linking of collagen and to impair mechanical function in a dose-dependent fashion. There is in vitro evidence that high irradiation reduces osteoclast activity and increases osteoblast apoptosis, and that residual bacterial products induce inflammatory bone resorption following macrophage inactivation. However, the clinical significance of these findings has not been established. Newer processes employing radioprotectants have preserved bone allograft integrity when doses ≥25 kGy are applied and controlled-dose methods permit successful irradiation at lower doses (see proprietary chemical sterilization methods to follow). Irradiated demineralized bone has active osteoinductive activity and has been effective in non-structural clinical applications.

Concerns about pathogen transmission and the limitations of irradiation, especially for soft tissues, have prompted improvements in sterilization methods and in the validation of these methods. A number of proprietary chemical–based processing methods have been developed with aims of effectively penetrating tissues and reducing, killing, or inactivating microorganisms and viruses without unacceptable adverse effects on the tissue’s biomechanical properties. Additionally, for use in transplantation, the agents must either be able to be effectively removed or be nontoxic. All methods in current use are applied only to tissue from donors who have met stringent criteria for medical history and behavioral risk assessment as well as negative results on infectious disease marker testing. Some popular methods used are described in this section.

The Tutoplast process (Tutogen Medical, Gainesville, FL) was the first process to sterilize and preserve tissue without affecting biological or mechanical properties. The process has been in use for over 30 years, for a variety of hard and soft tissues, including bone, fascia lata, pericardium, skin, amniotic membrane, and sclera. Initially, lipids are removed in an ultrasonic acetone bath that also inactivates enveloped viruses and reduces prion activity. Bacteria are destroyed using alternating hyperosmotic saline and purified waterbaths that also wash out cellular debris. Soluble proteins, nonenveloped viruses, and bacterial spores are destroyed in multiple hydrogen peroxide baths, and a 1 N sodium hydroxide treatment further reduces prion infectivity by 6 logs. A final acetone wash removes any residual prions and inactivates any remaining enveloped viruses. Vacuum extraction dehydrates the tissue before the grafts are shaped and then double-barrier packaged. Terminal sterilization using low-dose γ-irradiation yields a SAL of $10^{-6}$.

The BioCleanse process (Regeneration Technologies, Alachua, FL) employs six steps: (1) bioburden control, (2) bioburden assessment, (3) minimization of contamination during processing, (4) rigorous cleaning, (5) disinfection steps, and (6) a final step of low-temperature, controlled-dose γ-irradiation. The process has been validated to achieve a SAL of $10^{-6}$.

The Allowash XG process (LifeNet Health, Virginia Beach, VA) employs six steps: (1) bioburden control, (2) bioburden assessment, (3) minimization of contamination during processing, (4) rigorous cleaning, (5) disinfection steps, and (6) a final step of low-temperature, controlled-dose γ-irradiation. The process has been validated to achieve a SAL of $10^{-6}$.

The BioCleanse process (Regeneration Technologies, Alachua, FL) employs low-temperature addition of chemical sterilants, such as hydrogen peroxide and isopropyl alcohol, which permeate the tissue’s inner matrix, followed by pressure variations intended to drive the sterilants into and out of the tissue. Regeneration Technologies reports a SAL of $10^{-6}$ for soft tissues without adverse effects on the initial allograft mechanical properties.

The Clearant process (Clearant, Los Angeles, CA) is designed to avoid the negative effects of γ-irradiation through addition of free radical scavengers, employing dimethylsulfoxide (DMSO) and propylene glycol as pretreatment radioprotectants. Although the process subjects tissue to 50 kGy of radiation and achieves a SAL of $10^{-6}$ for bacteria, fungi, yeast, and spores, the tissue’s biomechanical properties are retained.
The Musculoskeletal Transplant Foundation (Edison, NJ) uses a series of chemicals, including nonionic detergents, hydrogen peroxide, and alcohol, to treat cortical and cancellous bone grafts. For soft tissues, such as bone-patellar tendon allografts, an antibiotic mixture containing gentamicin, amphotericin B, and primaxin is added, and then washed out to a nondetectable concentration. The Musculoskeletal Transplant Foundation claims a SAL of $10^{-3}$ for its products. Incoming tissues whose bioburden exceeds prescribed parameters are pretreated with low-dose γ-irradiation.

NovaSterilis (Lansing, NY) has developed a sterilization technique that uses supercritical carbon dioxide at low temperatures and relatively low pressures, resulting in transient acidification, which is lethal to bacteria and viruses, with good penetration reported. However, this technique only recently became available for clinically available allografts, and data on clinical efficacy and retention of allograft mechanical properties are limited.

**General principles of tissue preservation and clinical use**

Except for bone, which is usually lyophilized (freeze dried), the most common method of storing processed tissues is at cold temperatures, either by refrigeration or by freezing (Table 40.3). For short-term storage, refrigeration at about 4°C often suffices, whereas long-term storage usually requires a frozen state. Several types of tissues can be preserved by multiple methods. Bone, dura mater, and amnion can be effectively cryopreserved or lyophilized. Much of the lyophilized and cryopreserved human tissue used in transplantation is intended to serve a structural purpose and maintenance of cell viability is not necessary. Tissues of this type, which include dermis, are composed of an extracellular matrix (such as collagen) with few or no viable cells present to support the matrix after transplantation, although they can contribute growth factors to facilitate remodeling. Even when the processing method used is intended to preserve cell viability, the donor cells will die following transplantation. The extracellular matrix, whether transplanted containing viable cells or devoid of them, is repopulated following transplantation. The extracellular matrix, whether transplanted containing viable cells or devoid of them, is repopulated.

**Bone**

Bone allografts have many uses, including provision of acatabular and proximal femoral support for replacement of failed prosthetic hip joints, packing of benign bone cysts, fusion of the cervical or lumbar spine to correct disk disease or scoliosis, restoration of alveolar bone in periodontal pockets, reconstruction of maxillofacial deficits, and replacement of bone that has been resected because of a bone malignancy, such as osteosarcoma (see Table 40.1). The last procedure is accomplished with large osteochondral allografts that permit tumor resection and achievement of a cure without limb amputation.

Historically, bone allografts have been prepared by cutting a larger graft using simple techniques. Today’s technology allows the cutting, machining, and piecing together of allografts via precision instrumentation, and has resulted in stronger and more versatile grafts that can withstand the challenges of new surgical techniques. Linear grooves, notches, or crosshatchings may be incised into bone surfaces to make the bone graft less likely to slip or become dislodged after placement. Many bone allografts, especially those used in neurosurgical applications, are now placed using precision instrumentation that not only ensures exact placement, but also enhances stability. Allografts can be cut or shaped to precise angles that accommodate, for instance, lordosis of the cervical and lumbar spine. Advanced processing methods are being developed to improve availability while retaining or improving function.

Fresh autograft can be taken from the patient’s own iliac crest during surgery, but this practice is becoming less common. Fresh bone autograft is preferred by some surgeons, but preserved allografts are practical and accepted alternatives that approximate the

| Table 40.3 Human tissue storage conditions and duration |
|-----------------------------------------------|-----------------|-----------------|
| **Human Tissue** | **Storage Condition** | **Temperature (°C)** |
| Musculoskeletal | Frozen, (cryopreserved and non-cryopreserved) long term | −40°C or colder |
| | Frozen, (cryopreserved and non-cryopreserved) temporary storage for 6 months or less | 40 to −20°C |
| | Refrigerated, short term | 1–10°C |
| | Lyophilized, long term | Ambient temperature** |
| Skin | Frozen, short term | −40°C or colder |
| | Refrigerated, long term | 1–10°C |
| | Lyophilized, long term | Ambient temperature** |
| Cornea | Refrigerated, short term (defined by method) | 2–8°C |
| | Lyophilized, long term | Liquid nitrogen (liquid or vapor phase |
| | | −100°C or colder |
| | Lyophilized, long term | Ambient temperature** |
| **Semen** | Frozen, long term | −100°C or colder |
| **Cardiac, vascular** | Frozen, cryopreserved | 4°C or colder |
| **Dura mater** | Lyophilized, long term | Ambient temperature** |

*Or as defined and validated by the processor.
**Ambient temperature monitoring not required for lyophilized tissue.
Source: Adapted with permission from Dock et al. 11 and Trainor.38
results obtained with fresh bone autograft. In some patients, an autograft is not an option because sufficient high-quality bone is not available. In addition, the use of bone allografts reduces operating room time and the number of operative sites, leading to reduced morbidity and lower cost. The use of bone allograft does carry the risk of donor-to-recipient transmission of infectious disease, although this risk is minimized through careful donor selection and testing, and disinfection and sterilization of tissue during processing as described earlier.

Frozen bone
Frozen bone, collected under aseptic conditions and then frozen or cryopreserved, is available in a wide variety of shapes and sizes from deceased donors, or as a femoral head or tibial plateau obtained from a living donor undergoing total joint replacement in an operating room (now an uncommon practice in the United States, but continuing in Canada, Australia, and Europe). This frozen bone is largely free of bacteria, but it does carry the risk of viral transmission. Diseases known to have been transmitted by unprocessed bone include HIV infection, conditions associated with HTLV, tuberculosis, and hepatitis.

Frozen bone can cause alloimmunization from exposure to antigens on the attached connective tissues, marrow, and blood, although such alloimmunization apparently does not affect the graft’s efficacy. Detailed reviews addressing the role of histocompatibility and the immune response in bone allograft transplantation have been published. Antibodies to histocompatibility antigens, blood group antigens, and bone matrix proteins have been induced by transplanted frozen bone. In order to avoid Rh alloimmunization, bone from an Rh-negative donor is usually selected when using bone that has not been processed to remove red cells and the recipient is an Rh-negative female of childbearing potential.

Bone that otherwise would be discarded can be collected from living donors during surgical procedures (total hip or knee replacements with prostheses). The eligibility of a volunteer living donor is determined by a careful health history review, a directed physical examination if indicated, and donor testing for anti-HIV-1 and -2, HIV-1 NAT, HBsAg, anti-HBc (total), anti-HTLV-I/II, anti-HCV, HCV NAT, and syphilis. In some countries, if NAT assays are not performed, the donated tissue is quarantined, and the living donor is retested six months later for infectious disease markers. This quarantine and retesting process is intended to eliminate donors who were in the early stage of viral infection but were antibody negative at the time of donation (in the window period). A follow-up anti-HBc test may detect donors who had been infected with HBV shortly before donating but who no longer have detectable HBsAg. Generally, culturing for bacteria is performed on each bone to be fresh frozen or on each batch/lot of cryopreserved bone.

Allograft bone is further prepared by removal of extraneous tissue; it is then packaged, and immediately either shipped to the processing tissue bank on wet ice or sent to freezer storage at −40°C or colder. Frozen bone allografts are available as whole bones or cut into usable shapes and sizes. Frozen bone can generally be stored up to 5 years at −40°C or colder, but the maximal storage duration and expiration date may vary based on processing and storage methods, as validated by the tissue bank. There is no evidence that the biomechanical or osteoinductive properties decline during frozen storage. However, in the absence of cryopreservation, frozen bone does not maintain cellular viability. Thus, frozen bone is used for structural support that depends on an intact calcified extracellular matrix or is used as filler to promote new bone formation.

Lyophilized bone
Following aseptic recovery, deceased donor bone can be placed on ice for transport to storage in a freezer and maintained frozen at −40°C or colder, and then can later be sent to a tissue processor with dry ice as a refrigerant. Alternatively, immediately after recovery, the tissue can be placed on wet ice and expedited directly to the processing tissue bank where, within 72 hours of recovery, it is frozen at −40°C or colder until processing. Such processing includes removal of surface tissues and internal fat, blood, and marrow by means of mechanical agitation, high-pressure water jets, or alcohol soaks. It can also include detergents or other solutions as part of a proprietary process. Then the bone is milled into clinically useful shapes and sizes. This may include computer-guided milling and use of assemblies that result in complex mechanical structures. Conventional allografts include corticocancellous strips, wedges, and dowels; cortical struts and rings; and cancellous and cortico-cancellous cubes and chips. Bone can also be ground into a powder and be available as DBM and products that include it. DBM, which is also known as demineralized freeze-dried bone allograft, is derived from cortical bone and is available in combination products in the form of gels, pastes, putties, and flexible strips or sheets. DBM itself may be obtained in specific granule or particle sizes, as a powder, or in entangled, twisted fiber configurations. DBM primarily provides growth factors, but accompanying collagen can help play a structural role as a scaffold for future bone growth. The combining of DBM with approved polymer carriers results in moldable grafts that are user friendly for the surgeon, do not migrate after placement, and whose bone content does not dissolve following transplantation. Such grafts can readily be applied to completely fill bony defects and to act as a scaffold for ingrowth of the recipient’s own cells, or they can be used to enhance other structural repair devices, such as dental implants, vertebral body spacers and cages, or support devices such as rods, screws, and plates.

The bone allografts are lyophilized to a residual moisture content of <6% or 8% (depending on measurement method) and packaged into jars, peel packs or “boat” packaging. Routine quality control testing of bone is designed to monitor safety, rather than potency or efficacy. Sterility is assessed by the testing of samples of each batch, or by another acceptable method. If a robust validated sterilization process is used, sterility may need to be assessed only at established intervals, such as quarterly. Potency can be evaluated by using assays for osteoinductive capacity and biomechanical properties, but these analyses usually are conducted only when there is a change in the production process. Lyophilized bone is brittle unless fully rehydrated before use. Lyophilized bone can usually be stored at ambient temperature for up to five years if the graft’s package integrity and its vacuum are maintained, depending on validation performed by the tissue processor.

While the purpose of lyophilization is to allow for convenience, other preservation methods exist that also allow for ambient temperature storage of grafts. Some tissues can be dehydrated via chemicals (such as acetone), some kept in saline (e.g., costal cartilage), and some are packaged with a humectant (such as glycerol). The last two examples are considered prehydrated and are “ready to use” off the shelf. Expiration dates for all of these alternate methods are established by validation of the packaging by the tissue processor.

Bone collected aseptically in an operating room and processed aseptically can be lyophilized without use of a sterilant. Because the bone, theoretically, is bacteria-free, final sterilization with
γ-irradiation may not be necessary. Although the bone should be free of bacteria, it still has the potential to transmit disease. Despite this risk, some physicians have preferred aseptically processed, nonsterilized lyophilized bone because it was thought to have better osteoinductive capacity than sterilized bone. However, controlled-dose low-temperature radiation has been found to have no significant effect on osteoinductive capacity.47

Ear ossicles
Ear ossicles are used as a special kind of bone graft to correct selected cases of deafness in which the patient’s own ossicles have suffered congenital, traumatic, or postinfectious damage.48 Ear ossicles are procured by removal of the temporal bone en bloc or as a core with a bone-plug cutter. The temporal bone can be stored temporarily, for months if frozen, or up to two weeks if preserved in formalin; the tympanic membrane and ossicular chain are then dissected. Ossicles have been stored for up to two months in cialit (an organomercuric compound), and for up to one year at room temperature in buffered formaldehyde. Alternatively, ossicles are dissected at the time of collection, lyophilized, and then sterilized by γ-irradiation. Lyophilized ossicles can be stored at ambient temperature for up to five years.

Connective tissue
Cartilage and meniscus
Human cartilage can be transplanted at weight-bearing or non-weight-bearing sites. For non-weight-bearing uses such as nasal reconstruction and mandibular or orbital rim augmentation, the graft provides structural support and need not be viable. Costal cartilage can be recovered for this use. The cartilage can be sterilized by γ-irradiation and stored in saline at refrigerated temperatures, or it can be lyophilized and stored at ambient temperature.

Articular cartilage can be transplanted to weight-bearing articular surfaces to replace focal cartilage defects caused by trauma or degenerative disease, particularly in the knee. Cartilage in an osteochondral or osteoarticular allograft can be obtained as a femoral hemicondyle, a tibial plateau or fragment, or a measured segment removed with a template cutter that can be press fitted into a similarly cut area in the recipient. Osteochondral allografts avoid autograft site morbidity and are advantageous when the focal articular cartilage defects being repaired are large (>2.5 cm).49 It has been assumed that, in weight-bearing applications, chondrocytes must survive the collection and preservation process and remain viable, producing normal cartilage matrix to maintain mechanical properties. It appears that chondrocytes deep within the cartilage matrix resist cell-mediated immune responses by the recipient and, if kept viable during storage, are able to survive after transplantation. Cartilage grafts from histo-incompatible donors, stored <24 hours at 4 °C, have survived for as long as seven years after transplantation, if the grafts developed a sound union and if conditions for correct biomechanical functioning were present.50 Articular cartilage collected in a sterile manner can be stored at 4 °C in saline or electrolyte solutions with or without 10% fetal (bovine) calf serum and antibiotics.51 Screening procedures are now in place to reduce the risk of source animals that may have bovine spongiform encephalopathy (BSE). Osteoarticular and osteochondral allografts can be stored refrigerated for up to 28 days with successful clinical outcomes. If such grafts have been cryopreserved, expiry for these allografts can be extended to one year.

The use of a large osteochondral allograft, such as the femur with the articular cartilage attached, is thought to require preservation of cartilage viability in order to maintain biomechanical properties. To accomplish this, grafts have been stored at refrigerated temperatures in electrolyte solutions for up to one month, or have been frozen in 10% glycerol or 15% DMSO and stored at −70 °C or colder.52 Following transplantation in humans and animals, the surface of the articular cartilage allograft undergoes degenerative changes within a few years. These grafts carry the same risk of disease transmission as other fresh tissue allografts.

Menisci are C-shaped disks of fibrocartilage interposed between the femoral condyle and tibia. The presence and integrity of the meniscus are essential for knee mechanics and biochemical functions. Loss or disruption of the meniscus is associated with pain, joint laxity, and degenerative arthritis. Meniscal transplantation has been proposed as a method of providing a biologically and biomechanically acceptable structure to replace a damaged or removed meniscus, with a goal of relieving pain, decreasing stress on the anterior cruciate ligament, and preventing late arthritis, although evidence of allograft tissue being chondroprotective is lacking. Although there have been unpublished reports of successful transplantation of menisci stored <24 hours at 4 °C, fresh menisci are not usually available. Cryopreserved menisci are used successfully, with good outcomes (including reduced pain and increased knee function) reported.53,54

Tendon and ligament
The knee is the joint most frequently involved in sports-related injury. Arthroscopic methods for replacing the anterior or posterior cruciate ligaments with autografts, allografts, or artificial tendons and ligaments are frequently used. Despite the attendant need for sacrifice or weakening of normal structures, the use of autografts appears to have a high success rate and low incidence of complications. However, allografts may be indicated for multiple ligament knee injuries, anterior cruciate ligament revisions, or posterior cruciate ligament reconstruction, and when extensor mechanisms are impaired (as with previous tendon tears). It is also sometimes preferable to avoid the morbidity associated with autograft. In addition, there are occasions when sources of adequate autograft tissue are not available.55 Allografts used to replace the injured anterior cruciate ligament are usually derived from deceased donor patellar ligaments, tendons of the leg (e.g., tibialis, semitendinosus, gracilis, and peroneus longus), or Achilles tendons. Ligament and tendon allografts are usually stored frozen, but some are stored lyophilized. In vitro biomechanical properties of tendons do not seem to be greatly affected by freezing, lyophilizing, or ethylene oxide sterilization.56 However, many surgeons eschew lyophilized tendon allografts because of experiences with clinical failure. Frozen tendon allografts are commonly sterilized by γ-irradiation, although this can reduce their mechanical strength, particularly if performed at room temperature or if the dose exceeds 20 kGy.57 There is no evidence that maintenance of cellular viability during processing and storage is important to clinical effectiveness. The effect of irradiation on the biomechanical properties of human tissue has been explored extensively, with inconsistent results. This is probably because the studies failed to use uniform irradiation methods and comparable study designs. A key study found a difference in average stress at failure between nonirradiated and γ-irradiated tendons; that difference is likely a consequence of the free radicals generated, which can cause minor crosslinking of collagen fibers and alteration of the tendon’s material properties.58 In order to eliminate the potential for elongation of irradiated grafts after implantation, the authors encouraged pretensioning of grafts before insertion.
Fascia lata
The fascia lata is a broad fibrous membrane surrounding the thigh muscles. The thick lateral portion acts as a flattened tendon, and its muscular insertions helping to maintain the trunk in an erect posture. Fascia lata can be removed and transplanted as an autograft or allograft. As an allograft, fascia lata has been used to suspend the upper eyelid to correct ptosis, as a covering for bone grafts in dental surgery, to replace injured anterior cruciate ligaments, to provide support for bladder suspension, and to repair ankle, hip, and shoulder suspensions (e.g., repair of a ruptured shoulder rotator cuff). Fascia lata usually is preserved by lyophilization, resulting in a residual moisture of <6% or 8% (depending on measurement method), the graft is then sterilized by γ-irradiation and stored for up to five years at ambient temperature. After rehydration, the graft’s biomechanical properties equal those of fresh frozen fascia lata. The use of fascia lata has become less popular because of the availability of alternative products, such as decellularized skin.

Dura mater
Dura mater is the outermost, toughest, and most fibrous of the three meningeal membranes covering the brain and spinal cord. The intracranial portion is collected, processed, stored, and distributed for several clinical applications; the most common use is the closure of dural defects caused by resection of tumor or the repair of traumatic injury. Human dura allograft is most commonly preserved by lyophilization. Ethylene oxide and γ-irradiation are effective in preventing transmission of viruses and bacteria; however, Creutzfeldt–Jakob disease (CJD) has been transmitted by dura mater treated by these methods. Following findings by Brown and coworkers,59,60 in 1986 The Committee on Health Care Issues of the American Neurological Association recommended using 1N NaOH for one hour or steam autoclaving for one hour at 132 °C as standard sterilization procedures for CJD-infected tissue or contaminated materials. Donors with a history of clinical dementia or other central nervous system disorders are not accepted as donors. Lyophilization and sterilization treatments do not lessen the effectiveness of dura mater allografts. Reconstituted freeze-dried dura mater is thick and strong, holds suture well, and is incorporated into normal surrounding tissue without rejection. Because of the risks and resulting decreased demand, dura mater is currently processed in the United States by only one tissue bank.

Skin
Human skin allograft is the dressing of choice for temporary grafting onto deep burn wounds whenever sufficient amounts of autograft skin are unavailable. Early excision of burned tissue and covering of the wound with deceased donor skin allograft has shortened hospitalization and decreased mortality more than has any other treatment.61 A skin allograft provides temporary coverage and acts as a barrier against loss of water, electrolytes, protein, and heat. It reduces opportunities for the invasion of bacteria and speeds re-epithelialization. Skin allografts are replaced periodically until the patient’s vascular bed is reestablished. Skin allografts also are used for unhealed skin defects (decubitus ulcers, autograft skin sites, pedicle flap sites, and traumatically denuded areas). Although skin has historically been used only as a covering, decellularized (mechanically and chemically treated) skin offers the opportunities for use of a collagen matrix that can be implanted and be remodeled within the site with the recipient’s own cells. It is used for such applications as bladder suspension surgery, tendon repair, post-mastectomy breast reconstruction, oral reconstruction, and repair of large defects, such as postoperative hernias and dehisced wounds. In an analogous fashion, Taylor and colleagues40 have used perfusion-decellularized cardiac tissue matrix in an attempt to develop a bioartificial heart.

After collection, fresh skin can be stored in medium at 1 to 10 °C for up to 14 days,11 but fresh skin is seldom used today. Skin also can be frozen using a method that retains cell viability, in order to improve availability. Because cell viability declines during refrigerated storage, results are best when cryopreservation is performed within 2 to 3 days after recovery. Cryopreserved skin can be prepared as strips (often 3-inch by 8-inch sections), either unmeshed or meshed (most commonly with a 1:1.5 expansion ratio, which triples the area that can be covered). The skin is then covered in fine-mesh gauze and laid flat, packaged, and then cryopreserved with glycerol or DMSO at a concentration of 10% or 15% as a cryoprotectant. Cryogenic damage is minimized by controlling the rate of freezing to between −1 and −5 °C/minute. Many tissue banks use a “heat sink” freezing method, rather than one that employs computer-controlled freezing chambers. Heat sinks involve aluminum plates combined with styrofoam-insulated boxes; these are placed directly into a −70 °C mechanical freezer. This simple process provides a slow, controlled freezing rate that is acceptable for skin and that also maintains cellular viability.62 AATB standards permit frozen storage in a mechanical freezer at −40 °C or colder, in the vapor phase of liquid nitrogen, or submerged in liquid nitrogen.11 The maximal allowable storage period in the frozen state during which viability and structural integrity are maintained has not been determined. Cryopreserved skin allograft usually is transported from the tissue bank to the hospital with dry ice in order to maintain a frozen environment until use.

Skin for use in burn applications generally is not preserved by lyophilization because this method decreases clinical efficacy. However, lyophilized skin is sometimes used by oral surgeons to cover oral mucous membrane defects and to speed re-epithelialization. Lyophilized (and prehydrated) acellular dermal matrix is also available, in several thicknesses for different applications, and can serve as a natural biological matrix for soft tissue augmentation in soft tissue defects and in periodontal peri-implant soft tissue management. Following hydration, lyophilized skin has multidirectional strength and can adapt to surface contours, and it then is resorbed over 4–6 months, depending on the site, defect size, patient age and health status, and the biomechanical load on the graft. Depending on processing method and packaging configuration, lyophilized skin can be stored as long as five years at ambient temperature or it may require refrigeration.

Ocular tissue
Cornea is one of the most frequently transplanted tissues; >46,000 corneas were transplanted in the United States in 2013. Corneal transplantation has become highly effective because of improvements in suture materials, surgical instruments and techniques, and medications to prevent and reverse rejection. It is considered a standard therapy for a variety of conditions. However, early in this century, demand decreased because of improvements in cataract surgery techniques compared with those in use in the 1990s, during which time use for complications of cataract surgery had supplanted keratoconus as the leading indication. In the last 10 years, there has been a paradigm shift from full thickness keratoplasty to selective keratoplasty, where only the diseased layers of the cornea are replaced. One such selective keratoplasty techniques is Descemet’s stripping endothelial keratoplasty (DSEK), which transplants only
the innermost portion of the cornea; the tissue adheres to the host cornea with the use of an air bubble. The benefits of this technique are a smaller, stronger wound with minimal disruption of the interior curvature of the cornea, resulting in faster recovery improved visual acuity, as well as a reduction in the occurrence of adverse effects such as graft rejection and vision threatening intraoperative and postoperative complications. As a consequence, DSEK for Fuchs endothelial dystrophy has become the preferred surgical therapy at much earlier stages of the disease, resulting in a resurgence of demand for cornea allografts. Currently the most common indications for corneal transplantation are keratoconus, Fuchs dystrophy, post–cataract surgery corneal edema, and corneal regrafting. Donor cells in the avascular full-thickness cornea graft enjoy long-term survival without the aid of histocompatibility matching because the recipient site is also almost completely avascular. Because of the avascularity of the cornea, routine immunosuppression is accomplished with topical corticosteroids. However, systemic immunosuppression may be used in conjunction with topical agents for high-risk cases. Some experts believe that the failure rate of 5% to 10% might be improved by HLA matching; recipients known to be sensitized to HLA antigens have rejection rates higher than nonsensitized recipients. The possibility of allogeneization is of particular concern in patients who are undergoing repeat grafting procedures because of graft failure or who have ocular infections, as the corneal rim may become quite vascularized. Sclera may be used in the repair of ocular defects, in orbital reconstruction following enucleation, and in some dental applications.

Ocular tissue can be recovered by enucleation or by in situ excision of the cornea, with a rim of sclera. It is preferable that recovery be performed within 10 hours after death. The oldest method of storage for whole globes was at 4°C in a moist chamber; this method appeared to maintain viable endothelial cells sufficient for graft efficacy for as long as 48 hours after recovery. Because it yields improved viability, a more common method today is storage of the cornea, with attached rim of sclera, at 4°C in a modified tissue culture medium, based on that developed in 1974 by McCarey and Kaufman. One example commonly used is Optisol-GS (Bausch & Lomb, Irvine, CA), which contains dextran (as an osmotic agent), chondroitin sulfate, gentamicin, and streptomycin. Storage of corneas in the medium, at 2 to 8°C, can maintain endothelial viability for as long as 14 days, and can maintain functional integrity for eutopic graft applications not requiring visual acuity for even longer storage periods. Grafts are usually used within seven days. Although they have been treated with antibiotics, allograft corneas are not considered sterile. Organ culture stored corneas may be used internationally (particularly in the European Union), but US banks do not use this method. Rarely, corneas are frozen with cryoprotectants. Sclera is usually preserved in ≥70% ethyl alcohol; such a method yields a shelf-life as long as two years.

Cardiac and vascular tissue
Cardiac and vascular tissue includes heart valves, patches, nonvalved outflow tract arteries and vessels that can be used as conduits. Donor medical history requirements differ, so AATB has established separate standards for cardiac tissues and for vascular tissues. Since their introduction nearly five decades ago, human heart valve allografts have been shown to be an alternative for patients needing heart valve replacement for whom mechanical and xenograft valves are contraindicated. Human heart valve allografts do not require recipient anticoagulation, have a lower incidence of thromboembolism, and appear relatively resistant to infection. After valve allograft transplantation, donor endothelium is not maintained, but donor fibroblasts may remain for an undetermined period. Because anticoagulation is unnecessary, human valve allografts are the graft of choice for children, females of childbearing potential, and patients with cardiac infection in the aortic root. The use of allograft valves has been slowed, however, because implantation is more technically difficult compared to modern versions of stented prosthetic valves. In addition, their availability is limited, especially for pediatric use. Additionally, clinical results with transplantation of xenograft tissue valves have improved, although these are not available in the small sizes required by many pediatric patients.

Technical impediments have made it impossible to successfully produce man-made (either completely artificial or modified xenograft) replacement heart valves for use in neonates and other pediatric patients who require very small grafts. Only donated human heart valves from newborns or small children offer unobstructed blood flow through such a small annulus. Also, the tissue's pliability renders human allografts adaptable to the ingenuity of cardiothoracic surgeons who repair congenital defects by using allografts to replace underdeveloped or otherwise defective valves or outflow tracts, or to construct valves and tracts that may be absent. Complex repairs may need to be staged over many years or may be only palliative. Demand for clinical use of nonvalved conduit sections of cardiac allografts (mostly from the main pulmonary artery and/or its branches) has shown superior results in use as treatment of defects of the right ventricular outflow tract.

On a global scale, availability of cryopreserved pediatric allograft heart valves has historically been low and unable to meet demand. To obtain cardiac allografts, hearts are recovered aseptically, immersed in a sterile isotonic solution within a sterile container, placed on wet ice, and transported expeditiously to a tissue processing facility. The pulmonic and aortic valves, along with their intact outflow tracts and/or small pieces of these conduits, are dissected free of the heart within 48 hours of donor asystole, and then placed in tissue culture medium amended with a low-dose antibiotic cocktail. Studies demonstrate that cryopreservation of heart valves allow successful banking of valves of various sizes and types while retaining the intact matrix and having a low clinical incidence of valve degeneration, rupture, leaflet perforation, and valve-related death. For these reasons, human heart valves generally are cryopreserved, with a method that includes an initial exposure to antibiotic solutions for 12 to 24 hours. Cryopreservation then follows, using a 10% DMSO solution tissue culture medium that is often amended with 10% fetal calf serum. Freezing is accomplished using a computer-assisted controlled rate of −1°C/minute to −40°C. Valves generally are stored in the vapor phase of liquid nitrogen. Theoretically, heart valves can be stored indefinitely in liquid nitrogen, although the nature of any deterioration during storage is not well characterized. The viability of cryopreserved connective tissue matrix cells is maintained, but at a lower level than that of fresh valves, and endothelial viability is lost. In addition, noncellular matrix elements are maintained.

The aorta and iliac arteries can be preserved using the same methods applied to heart valves. Frequently, the aortic arch is preserved with the aortic valve intact; such grafts are intended for transplantation as a valved conduit. Preservation and storage methods are similar to those for valves. Synthetic grafts are often the graft of choice, but such grafts may be less effective in an infected field. Aortoiliac arteries are used successfully as conduits in mycotic aneurysm repairs, when synthetic grafts have become infected, and for aortoenteric fistulas in an infected field.
Arterial or venous segments of vascular organs may be recovered in order to provide a source of vascular “conduits” for use in organ transplants when the organ’s attached vessels are damaged or inadequate. Vascular conduits that have been recovered and transplanted under these conditions are not considered tissues under the FDA’s human cells, tissues, or cellular or tissue-based products (HCT/P) rules, but they are regulated as organs under 42 CFR Part 121. Donor screening and testing, as well as labeling and storage requirements, are identical to those for donor organs specified in a federal contract with the Organ Procurement and Transplantation Network.

Autograft veins are used in cardiac and peripheral vascular bypass graft procedures whenever it is possible, but veins from deceased donors that have been recovered under aseptic conditions may be used for revascularization when autologous vein grafts are not available. Cryopreservation of allograft vessels is similar to that of cardiac allografts. Well-established tissue bank procedures are designed to retain venous endothelial cells during recovery, processing, and preservation, but these cells are rapidly sloughed off the lumen after the vein is transplanted into the high-pressure arterial system. Retention of endothelial cells during recovery and processing does aid, however, in reduction of the risk of thrombosis or failure after implantation through protection of the integrity of the vessel’s basement membrane and acellular matrix. Although not proven to be necessary for successful clinical outcome or to prevent alloimmunization, ABO- and Rh-compatible allograft valves and vessel conduits are usually requested. One recent case series, involving limb salvage utilizing allograft saphenous veins, showed significantly better results in cases with ABO blood type compatibility. Some studies have shown that the use of these tissue allografts carry a risk of HLA antigen sensitization.

Peripheral nerve

Fresh autografts of peripheral sensory nerves are used in nerve repair, but this practice is hampered by collection morbidity and resulting limitations on the amount of autologous nerve tissue that can be made available. Although allografts ideally might repair peripheral nerve defects without requiring the sacrifice of the patient’s own nerve, frozen, irradiated, and lyophilized allografts have not functioned well. New animal studies using nerve allografts cold-preserved for seven weeks have shown promising results, as have cultured Schwann cells added into synthetic conduits. Axogen, Inc. (Alachua, FL), has developed a thermally acellularized nerve allograft scaffold called Avance that is treated with chondroitinase in order to degrade chondroitin sulfate proteoglycan. Such grafts have been shown to inhibit both aberrant growth and retrograde regeneration in the absence of any immunosuppressive therapy. Animal studies employing such an approach demonstrated enhancement of nerve regeneration. The first human Avance nerve allograft was implanted in 2007 into a 38-year-old man who had suffered a traumatic facial nerve injury; a single nerve allograft was used to connect the severed facial nerve root to three nerve branches. The surgeons informally reported that the graft’s handling characteristics were superior to those of autograft tissue.

Parathyroid

Hypercalcemia, kidney stones, and other complications associated with hyperparathyroidism can be treated by surgical removal of the parathyroids. Hyperparathyroidism often is caused by a single parathyroid adenoma, but in 10% of cases, generalized parathyroid hyperplasia is found, rendering the removal of all four parathyroids necessary. Postoperatively, the lack of parathyroid hormone can result in permanent hypocalcemia in 5% of patients. To prevent this outcome, autotransplantation of a small amount of parathyroid tissue is performed during total parathyroidectomy in order to provide a controlled source of parathyroid hormone. Cases of parathyroid cell allotransplantation have been reported, with durable results in selected cases where the recipient was being immunosuppressed to prevent rejection.

The parathyroid tissue is placed in the sternocleidomastoid muscle, flexor muscle groups, or subcutaneous tissue of the forearm. The remaining parathyroid tissue can be divided, placed in vials containing chilled tissue culture medium, and then cryopreserved using autologous serum, RPMI, and DMSO. The excess tissue then can be frozen under controlled conditions and stored in liquid nitrogen at $-196^\circ$C. Frozen parathyroid autograft can be retrieved for subsequent use if the tissue implanted at the time that the parathyroid was resected proves to be insufficient, fails to function, or becomes infected. Cryopreservation of parathyroid tissue maintains cell viability and graft function. This is illustrated by postimplant amelioration of hypocalcemia and sustained elevation of parathyroid hormone in the venous effluent of the grafted forearm compared with that of the nongrafted forearm.

Postthaw viability can also be demonstrated in vitro by the suppression of parathyroid hormone secretion by the addition of increasing calcium concentrations. The maximal duration of cryopreserved parathyroid tissue storage has not been determined.

Reproductive tissue

Semen

Assisted reproductive technology procedures and artificial insemination of a female with her partner’s previously stored semen or with donor semen are established therapies for the clinical management of infertility or when a woman does not have a male partner. Cryopreserved semen can be stored by a man, termed a client depositor, who may become sterile as a consequence of therapy for testicular malignancy or for another reason, for later use with his wife or other “intimate partner,” or even with a gestational carrier. Sperm can even be collected postmortem, but such a practice poses ethical issues regarding the lack of consent. According to the FDA, there are approximately 706 registered establishments that provided cryopreserved semen storage in the United States in 2015. The vast majority of these sites are fertility clinics that process and store semen for their patients’ own use with their partner. Each ejaculate can be separated into several vials or straws for separate storage that can be retrieved and thawed for use when needed.

Semen banks, or sperm banks, usually offer a library of donors from whom donated frozen semen specimens are available. According to a survey conducted by the American Association of Tissue Banks in 2014, there were 26 sperm banks in the United States that offered, as their main business, donor sperm for use by patients in need of donor sperm. The offering of a selection of donors facilitates the matching of donor’s hair and eye color, race, and other genetically determined characteristics with those of the intended father or co-parent or with those of both parents. These donors are usually “anonymous”; such a donor’s identity is known only to a few of the semen bank staff. However, some semen banks offer a program through which some donors have agreed to disclose their identities when offspring reach the age of 18 or, sometimes, even before use of the semen. Sexually transmitted diseases, including HIV infection, can be transmitted by donor semen to women undergoing artificial insemination. Cryopreservation permits extended storage and the retesting of donors at least 6 months after
the donation of specimens to be released. This process is intended to prevent use of semen donated by a recently infected man, before development of detectable antibody or viral nucleic acid (during the "window period"). Other diseases and organisms transmissible by donor semen include hepatitis B, gonorrhea, *U. urealyticum, M. hominis, T. vaginalis,* and *C. trachomatis.* Transmission of HTLV-I, syphilis, HCV, and human papillomavirus may also be possible.23

The basic practices and techniques of semen cryopreservation have changed little since the cryopreservative glycerol was discovered accidentally by Polge and coworkers82 in 1949. Glycerol remains the standard cryoprotectant, with storage in liquid nitrogen. Freezing methods in use have been designed to control the rate of temperature decline, and to prevent thermal shock by cooling the semen, slowly, in air or in a waterbath, to 5 °C before initiation of the actual freezing process. This takes place in the vapor phase of liquid nitrogen, or in a programmable controlled-rate freezing device. After freezing, semen can be stored in the liquid phase of liquid nitrogen indefinitely. The longest period of semen cryo-storage, followed by documented birth of healthy offspring, is 40 years.83

Although a defective pregnancy as a result of sperm injury during the freezing–thawing process is a theoretic concern, such an effect has not been demonstrated. Cryopreservation of semen does not influence the frequency of abortions or multiple births, or the infant's gender, body size, or intelligence.84 In fact, there is some evidence that indicates that favorable outcomes of cryopreserved semen actually might exceed that of fresh semen. One study reported finding birth defects in 0.7% of offspring and spontaneous abortion of 7.7% of pregnancies achieved using cryopreserved semen, whereas in the general population the frequency of birth defects is 6% and the frequency of spontaneous abortion is 10% to 18% of pregnancies.85

Oocytes and embryos
Since the birth of Louise Brown, the world’s first "test tube baby," on July 25, 1978, there has been an explosion in the use of assisted reproductive technologies, such that several techniques have been accepted as standard medical therapy. According to the Centers for Disease Control and Prevention's 2012 National ART Success Rates report, there are at least 456 assisted reproductive technology programs in the United States.5 Although the technology to freeze unfertilized oocytes reliably only recently moved from the research setting, embryos are routinely cryopreserved. In 2012, these programs performed nearly 135,000 embryo transfers. Among embryos created using nondonor oocytes, 69% were employed fresh, while 31% had been frozen. Many of those embryos were created using donor semen and/or donor oocytes. Donor oocytes were used in approximately 13% of embryo transfers carried out in the United States in 2012.5 Many of these were embryos created for recipients >35 years of age, using an oocyte donor much younger than the recipient. Embryo transfer success rates have been shown to be influenced far more by the age of the oocyte source than by the age of the uterus into which embryos are transferred. Medical history and infectious disease testing requirements similar to those for semen donors apply, although a quarantine period and retesting are not required.

Extraembryonic tissue preservation and transplantation
Extraembryonic tissues that have been used occasionally for transplantation include the amnion and the umbilical vein. Fetal amnion, which is the smooth, slippery, glistening membrane lining the fluid-filled space surrounding the fetus, has been used as a covering for nonhealing chronic leg ulcers, burns, and raw surfaces following mastectomy, and in major oral cavity reconstruction and vaginoplasty. Amnion also has been used as a pelvic peritoneum substitute following pelvic exenteration and as a source of replacement enzymes for infants with inborn errors of metabolism.86 Most of the fetal amnion is covered on the maternal side by the chorion, a slightly roughened membrane. Amnion is sterilely collected during cesarean section. The amnion’s epithelium and basement membrane can be separated by blunt dissection from the underlying chorion immediately after collection or after temporary storage. The amnion is then cryopreserved or lyophilized. Human umbilical vein allografts previously were used occasionally as vascular substitutes to provide venous access for hemodialysis or as an arterial bypass graft, but such allografts proved to be inferior to saphenous vein autografts. Such umbilical vein grafts are no longer available, following application of FDA device manufacturing requirements to their recovery and processing.

Donor–recipient matching
For most tissues, donor–recipient HLA matching is not necessary and is rarely done. Tissues such as bone, fascia, tendon, cartilage, and dura mater are not preserved or transplanted in a viable state; rather, they serve as a support or matrix that the recipient’s own cells can enter and gradually replace. Immunologic rejection, therefore, is not a significant concern, and matching of blood group or HLA antigens is considered unnecessary. There are exceptions, however. Immunologic rejection can occur in patients who have received a repeat cornea graft; therefore, efforts are made to use HLA-matched corneas in these patients.87 HLA sensitization has also been reported in recipients of vascular allografts or allograft heart valves.88

The ABO antigens are a significant consideration in transplantation because they constitute very strong histocompatibility antigens. Because they are expressed on vascular endothelium, major ABO mismatching can cause rapid graft rejection resulting from endothelial damage by ABO antibodies and subsequent widespread thrombosis within the graft. Therefore, ABO matching is important to the success of vascularized organ grafts (i.e., kidney, heart, liver, and pancreas). ABO matching is not important for a successful outcome when using most tissue grafts (i.e., fascia, bone, heart valves, skin, and cornea). However, hypersensitivity to antigens expressed by fresh or cryopreserved donor tissue is a rare occurrence and appears to be dependent on an undetermined unusual immune response by the recipient.

Alloimmunization to RhD, Fy5, and Jk5 red cell antigens following transplantation of frozen unprocessed bone has been reported.45,46 Consequently, frozen unprocessed bone allografts usually are matched with the donor for the D antigen if the recipient is a female of childbearing potential, in addition to being matched for ABO group.

Transfusion service support of tissue transplantation
Hospital transfusion services have been greatly affected by transplantation, and have encountered new and increased demands for services. They are involved in transplantation in several ways, including (1) providing traditional blood components; (2) providing new or special blood components; (3) taking responsibility for
tissue acquisition, storage, distribution, and tracking; and (4) providing specialized services. For organ transplants, the major demand is for traditional blood components, although special preparation may be required.

FDA regulations pertaining to tissue (see below) cover donor selection and testing, tissue recovery, processing, storage, labeling, and distribution to the "consignee" (21 CFR Part 1271). The consignee can be a distributor, a surgeon in a hospital operating room, a dentist in his or her office, or a designated individual or department in a hospital or other healthcare institution. Tissue "banks" that are located in hospitals are not regulated by the FDA if they serve only to store and dispense tissues provided by comprehensive tissue banks or distributors. A hospital tissue service can be centralized in a support area for the operating suite, hospital central supply service, or hospital transfusion service.

Alternatively, tissues can be handled using a decentralized system and be ordered, received, and stored by each functional area of the hospital in which they are used. However, in the absence of centralization, records of storage and recipient identification may be inadequate. In one case involving an HIV-infected donor, the recipients of five of the tissues could not be identified from hospital records. Other examples of inadequate traceability exist.90 The Joint Commission standard90 on recordkeeping and traceability of tissues, College of American Pathologists Transfusion Medicine Checklist,91 and AABB Standards for Blood Banks and Transfusion Services92 require that the institutions’ records permit tracing of any tissue from the donor or source facility to all recipients or other final tissue disposition. However, New York State is the only government regulatory agency that requires tracking of tissues to the recipients with records kept separate from patient charts.

The hospital transfusion service has the capacity, experience, and skills to act as a central depot and distribution point for all human tissues, such as bone, tendon, fascia, and cartilage, as well as cellular tissues of both reproductive and nonreproductive organs. Such tissues may ultimately be reimplanted in the original location (e.g., calvaria) or in a heterotopic location (e.g., limbs for parathyroid gland). A transfusion service operating as a central tissue repository and dispensing service may also be called upon to manage autologous tissue, such as calvaria (skull bone flaps), bone, skin, and parathyroid gland. Such tissues may require preparation and packaging before storage. Testing is not required, but careful labeling and recordkeeping are essential. Such tissues may ultimately be reimplanted in the original location (e.g., calvaria) or in a heterotopic location (e.g., limbs for parathyroid gland). It is prudent to establish time limits for storage either on an individual basis as specified by the surgeon or on a generalized basis, because stored tissues may not be claimed if the tissue was not needed because the patient died or for other reasons. The tissue dispensing service may also be called upon to package tissues in an appropriate, qualified, properly labeled transport container for transport to another institution.93

Reimbursement
Reimbursement for tissue transplantation is similar to that for blood transfusion. The tissue bank recovers expenses through a service fee (per tissue) billed to the hospital. This service fee includes such costs as services rendered by the organ/tissue recovery agency; recovery supplies and logistical support of the recovery agent that may be provided by the tissue processor; the tissue processor’s operating costs associated with processing, storage, and distribution, as well as research and development; and overhead costs incurred with support of all operations. Healthcare insurance carriers reimburse hospitals for most tissue service fees. Current procedural terminology codes specific to allograft transplantation procedures are available and used routinely.

Oversight
With the rapid growth of all areas of tissue banking, there has been an increasing need for accountability and for measures that ensure that safe, quality tissues are available for clinical use. Quality improvement can be effected through voluntary standards, and most tissue banks have incorporated the achievement of high standards into their goals. The AATB has established comprehensive standards for donor screening, recovery and processing of musculoskeletal, cardiac, vascular, and skin tissues, and reproductive cells.91 Additionally, the standards contain institutional requirements; descriptions of required functional components of a tissue bank; requirements for construction and management of records and development of procedures; requirements for informed consent, tissue labeling, storage, and release; expectations for handling adverse outcomes, investigations, and tissue recalls; requirements for establishment of a quality program; specifications for equipment and facilities; and guidelines for tissue dispensing services and tissue distribution intermediaries. AATB’s Standards for Tissue Banking are consulted not only by tissue bankers, but also by end-user healthcare facilities, other standard-setting organizations, and regulators worldwide. In 2015, 124 tissue banks in North America held AATB accreditation. Best practice for checking a tissue bank’s accreditation status is to perform an accredited bank search on the AATB website (www.aatb.org).

AAB Standards for Blood Banks and Transfusion Services92 address tissue inspection, handling, storage, preparation and dispensing, handling adverse events, and recordkeeping, which must provide traceability to each recipient or other disposition. The Joint Commission has standards for storage and issuance of tissue for hospitals and ambulatory surgery centers. These standards apply to bone, tendon, fascia, and cartilage, as well as cellular tissues of both human and animal (xenograft) origin. The standards address key functions, including the need to develop procedures for tissue acquisition and storage, recordkeeping and tracking, and follow-up of adverse events and suspected allograft-caused infections, which must be reported to the tissue bank from which the tissue was obtained. Similar to federal regulations and AATB Standards, the minimal record retention period is specified to be 10 years from the date of transplantation, distribution, other disposition, or expiration, whichever is latest. The College of American Pathologists’ Laboratory Accreditation Program’s Transfusion Medicine Checklist includes several questions on storage and issuance of tissues, including accountability; procedures for proper storage, handling, in accordance with the source facility’s directions; procedures for investigating recipient infections and adverse events, and handling lookback notifications from a supplier; and recordkeeping, which allow for tracking from donor to recipient and vice versa.
FDA authority to create and “enforce regulations necessary to prevent the introduction, transmission, or spread of communicable diseases between the States or from foreign countries into the States” under section 361(a) of the US Public Health Service Act (42 USC 264) applies to human tissue intended for transplantation. Formal enforcement policy and regulations did not exist until December 14, 1993 (codified in 21 CFR Parts 16 and 1270), when the “Interim Rule: Human Tissue Intended for Transplantation,” which required donor screening, infectious disease testing and recordkeeping “to prevent transmission of infectious diseases through human tissue used in transplantation,” was adopted in response to reports of HIV transmission by human tissue and of potentially unsafe bone imported into the United States.

These regulations were supplanted by a series of federal regulations, published in stages, first announced in the Proposed Approach to the Regulation of Cellular and Tissue-Based Products in March 1997. A final rule, “Human Cells, Tissues, and Cellular and Tissue-Based Products: Establishment Registration and Listing,” published in January 2001, required organizations that are engaged in tissue recovery, donor qualification, tissue processing, and/or tissue-related laboratory testing to register as a tissue establishment with the FDA. The rule (21 CFR Part 1271) became effective for all tissue banks on March 29, 2004.


In addition to requirements for establishment registration, donor eligibility screening and testing, and good tissue practice, the regulations set forth requirements for adverse reaction reporting and also define inspection and recall authority. To improve tissue safety and surveillance, the FDA Current Good Tissue Practice Rule, effective May 25, 2005, requires that tissue establishments report infectious adverse events after allograft transplantation to the FDA through its MedWatch adverse event reporting system. More than half of the reports filed by tissue banks have been flagged to indicate possible recall of tissue(s). Of these flagged reports, the majority pertained to acceptance of ineligible donors for whom one or more components of the donor qualification process was not performed or was insufficiently documented. The FDA also encourages health-care professionals, patients, and consumers to voluntarily report tissue adverse reactions through the MedWatch system (see http://www.fda.gov/Safety/MedWatch). There is an effort to develop the Transplantation Transmission Sentinel Network, which is intended to facilitate recognition of adverse events associated with transplanted allografts. The system is being developed by UNOS in collaboration with several stakeholders, including the AATB, EBAA, Association of Organ Procurement Organizations, American Academy of Orthopaedic Surgeons, American Orthopaedic Society for Sports Medicine, Society of Thoracic Surgeons, Health Resources Services Administration, and FDA.

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Key References
A full reference list for this chapter is available at: http://www.wiley.com/go/simon/