Human Skin Banking

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Skin autografting was first described by Reverdin in 1871 [1]. The clinical utility of allograft skin as a method for wound coverage followed shortly thereafter [2]. Girdner [3] was the first to report the use of allogeneic skin to cover a burn wound; however, it wasn't until 5 years later that Thiersch [4] described the histologic anatomy of skin engraftment and later popularized the clinical use of split-thickness skin grafts.

In 1874, Thiersch [4] published a report about a small series of patients on whom he later used partial-thickness grafts. Following the report, extensive trials led to cutting the grafts extremely thin to leave some of the surface epithelium behind to heal the donor site. This practice necessitated the use of little, thin chips of grafts, known in the English medical literature as "Thiersch grafts," "pinch grafts," "epidermis grafts," or "razor grafts"; these grafts were so generally unsatisfactory for resurfacing large areas that their use was soon restricted to application to small refractory ulcers.

Three developments in skin grafting brought about radical advances in the treatment of burn wounds. First, it became apparent that the dermal layer is the most important part of a skin graft in producing a new, tough, resilient surface. Second, it was demonstrated that the donor-site epithelium, after removal of a partial-thickness graft, regenerated from deep islands of hair follicle and sebaceous gland epithelium; thus, grafts could be cut thicker rather than as thin as possible, and the dermis could be transferred without interfering with the healing sites. Finally, because grafts could be cut much thicker, it became feasible to design instruments for cutting larger grafts. These thicker grafts were termed "split-thickness grafts," and for the first time large areas could be resurfaced satisfactorily in terms of square feet rather than square centimeters.

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These major advances in techniques permitted increased flexibility in the treatment of burn wounds, and consequently, the Thiersch, or pinch-graft method, and pedicle flaps in the treatment of contracted axillae and necks were no longer used. The use of split-thickness grafts permitted skin grafting to become a routine procedure [5].

Banking of human skin did not begin until the early 1900s. Wentscher reported the transplantation of human skin that had been refrigerated for 3 to 14 days [6]; however, it wasn't until the 1930s that blood and tissue banking took their place in the clinical practice of medicine. The clinical utility of allograft skin in burn wound coverage did not occur until 1938, when Bettman reported its success in the treatment of children with extensive full-thickness injuries [7]. Webster (1944) [8] and Matthews (1945) [9] described the successful take of skin autografts stored for 3 weeks at 4°C to 7°C, yet it wasn't until 1949 that the United States Navy Tissue Bank was established, signaling the beginning of modern day skin banking.

Baxter explored the histologic effects of freezing on human skin and identified the occurrence of ice crystal formation and tissue destruction in 1948 [10]. This was followed in 1952 by the pioneering research of Billingham and Medawar, demonstrating that skin could be effectively cryopreserved using glycerol [11]. Taylor subsequently demonstrated that the use of glycerol was associated with decreased ice crystal formation with freezing [12]. Shortly thereafter, Brown and Jackson popularized the use of allogeneic human skin grafts as biologic dressings for extensive burns and denuded tissue [13,14]. By 1966, Zaroff [15] reported the 10-year experience of the Brooke Army Medical Center with allograft skin in the treatment of thermally injured patients. In their report, the authors described the mechanical and physiologic benefits of allograft skin as a biologic dressing. Later that year, Morris [16] reported the beneficial effects of allogeneic skin in the treatment of infected ulcers and other contaminated wounds. In 1968, Cochrane [17] reported the first successful clinical use of frozen autologous skin grafts, having previously demonstrated the successful take of rat skin following controlled freezing in 15% glycerol and rapid rewarming before implantation. Shuck [18] further extended the potential use of allogeneic skin to include traumatic wounds based upon the Vietnam War experience. These increasing clinical uses of allograft skin led to further research into the mechanisms of its beneficial effects in wound healing, including the reduction of bacterial infection [19,20] and the stimulation of neovascularization [21].

Bondoc and Burke [22] are credited with establishing the first functional skin bank in 1971. Their experience with allograft skin led to a report of successful burn wound excision and allografting with temporary immunosuppression in children with massive injuries [23]. Today, allograft skin remains an ideal temporary cover for extensive or excised cutaneous or soft tissue wounds, particularly when the use of autologous tissue is not clinically indicated or when sufficient autograft skin is not available.

Clinical uses of allograft skin

Coverage of extensive full-thickness wounds

The clinical use of allograft skin in specialized burn care centers has been one of the driving forces behind the growth and development of skin banks in the United States. The general indications for its use in wound management are listed in Table 1. Allograft skin possesses many of the ideal properties of biologic dressings, and plays a major role in the surgical management of extensive wounds when autologous tissue may not be immediately available (Table 2). It reduces evaporative water loss and the drainage of protein-rich fluids, prevents wound desiccation, and suppresses microbial proliferation. Wound pain is lessened, and is associated with better patient compliance with occupational and physical therapy. By restoring a physiologic barrier at the wound surface, it reduces heat loss through the wound and mitigates the hypermetabolic stress response to burn injury. Fresh (viable) allograft skin represents the ''gold standard'' for biologic dressings employed for temporary wound closure (Table 3). Its use has become critically important for the surgeon faced with the immediate coverage of excised massive burn wounds. Allogeneic grafts are best applied unmeshed to maximize their effects as a temporary wound cover. Meshing of fresh allograft skin is rarely performed because reepithelialization of the interstices by allogeneic epidermis is uncommon. The allografts become well vascularized, stimulate neovascularization in the underlying wound bed, and provide temporary wound closure while preparing the recipient site for permanent coverage with autologous skin. In addition, fresh (refrigerated) allografts tolerate modest wound contamination and adhere better to the freshly excised subcutaneous fat than do cryopreserved grafts. The allogeneic skin is usually removed once the patient's donor sites are healed sufficiently for reharvesting or once autologous cultured skin is available for permanent wound closure.

When refrigerated (fresh) allograft is not available, cryopreserved skin is an excellent alternative for temporary wound coverage. Although frozen cryopreserved skin generally has less measurable viability than fresh skin, it is generally difficult to maintain continuous and ample stores of fresh skin beyond 14 days. It has therefore been a standard practice in skin banking to

Table 1
Indications for allograft skin use in wound management

Coverage of extensive wounds where autologous tissue is not available
Coverage of widely meshed skin autografts
Extensive partial thickness burns
Extensive epidermal slough
Testing for the ability to accept autograft
Template for the delayed application of keratinocytes

Table 2
Advantages of human allograft skin use

Reduce water, electrolyte, and protein loss
Prevent desiccation of tissue
Suppress bacterial proliferation
Reduce wound pain
Reduce energy requirements
Promote epithelialization
Prepare wounds for definitive closure
Provide dermal template for epidermal grafts

cryopreserve allograft skin that is not needed within 7 to 10 days rather than discard it. In fact, it is the potential and unpredictable demand for allograft skin in specialized burn care centers that has prompted the growth and development of local and regional skin banks throughout the world. Further research in allograft skin cryopreservation will be essential to maintain an ample supply of viable tissue for clinical use.

Coverage of widely meshed skin autografts

Another use of allograft skin has been its placement on top of widely expanded, meshed autologous skin grafts (Fig. 1). Originally, this technique was described using meshed allograft [24]. This method does not provide coverage for the entire wound; therefore, most burn surgeons currently performing overlay allografting use nonmeshed (sheet) allograft. This affords better protection of the open interstices of the autograft from desiccation and microbial contamination until epithelialization is complete. Although this technique may play a role in the management of massive excised full-thickness injuries, it should be used with discretion because many surgeons have expressed concern that the overlying allograft may induce an inflammatory rejection response that can retard the rate of reepithelialization of the underlying autografts. Some have therefore advocated the use of lyophilized allograft for this purpose as it is less viable and less antigenic.

Healing of partial-thickness wounds

Frozen allograft skin is an excellent wound cover when vascularization and adherence is not desired. Because it is usually less viable than fresh skin, it functions more as a biologic dressing than as a temporary skin replacement. Its adherence to the underlying wound bed results in the relief

Table 3
Advantages of fresh allograft skin

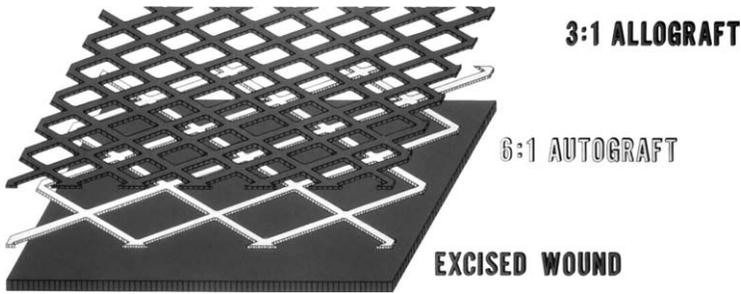| |
|---|
| Rapidity and strength of adherence to the wound |
| Control of microbial growth |
| Rapidity of revascularization |
| Reproducible clinical results |

Fig. 1. Diagrammatic illustration of meshed allograft overlay technique as described by Alexander [24]. The allograft is generally meshed 1.5:1 or 3:1 while the underlying autograft may be meshed 3:1 or greater.

of pain and the limitation of exudative and water losses, and reduces the need for frequent painful dressing changes. As the partial-thickness wound reepithelializes, the allograft slowly separates without disturbing the delicate autologous epithelium. Although this application is probably not cost-effective in the management of small second-degree burns or skin graft donor sites, it is often beneficial in cases of extensive partial-thickness wounds where its ability to prevent desiccation and promote epithelialization may reduce hospital stay or the need for autografting. Herndon and colleagues [25] have reported that scald burns of indeterminate depth are better treated with cadaver allograft than topical antimicrobial therapy. This study was followed by one in which 29 children were treated with debridement of the burn wound, and coverage with fresh or frozen cadaver skin meshed 2:1 [26]. The homografts were then covered with a mixture of Polymyxin and Nystatin ointments. Treatment with homograft significantly decreased length of stay compared with that achieved with topical antimicrobial therapy. Homografts acted as the best protection for damaged dermis, thus providing an environment for spontaneous epithelialization. Desai and colleagues [27], however, showed that scald burns of indeterminate depth were best treated with topical antimicrobials for 10 to 14 days followed by excision of areas that did not heal within 3 weeks.

Cryopreserved allograft is also an excellent biologic dressing for the management of patients with extensive cutaneous wounds resulting from drug reactions or superficial skin disorders (ie, toxic epidermal necrolysis, Stevens-Johnson syndrome, Staphylococcal scalded skin syndrome). When used for superficial wounds of this nature, allograft skin should be applied before exposing the wound to topical antibiotics because these agents tend to have an adverse effect on allograft adherence to the wound surface.

Testing for later acceptance of autograft

Both cryopreserved and fresh allografts have been used by physicians for the care of a variety of cutaneous and soft tissue wounds. In these instances,

allograft is used to provide a temporary biologic cover and to help predict the likelihood of an autologous graft later in the course of treatment. Allograft usage for this indication has been most common in the management of deep electrical burns in the process of demarcation of the escharotomy or fasciotomy sites, although it can also be used to temporarily cover extensive open abdominal and soft tissue wounds. Adherence or vascularization of the allograft is a reliable indication that the wound bed has sufficient blood supply to accept an autologous graft or flap.

Template for delayed application of keratinocytes

The clinical use of cultured epidermal autografts (CEA) in the care of burn patients was first described in 1981 [28]. Since that time, there have been numerous reports supporting its use as a permanent skin replacement for patients with extensive full-thickness burn injuries. This methodology has not been without problems, however, as many authors have described variable take rates and instability of these fragile grafts. Cuono [29] first advocated the use of allogeneic skin with CEA, allowing the allograft skin to vascularize before removing the antigenic epidermal layer by dermabrasion. Hickerson [30] reported his results on five burn patients, demonstrating over 90% CEA take on the allogeneic dermis, and supple, durable grafts up to 4 years postoperatively [30].

The past decade has also witnessed the development of an acellular dermal matrix (Alloderm) as a template for the simultaneous application of thin split-thickness autografts [31]. The potential advantage of the dermal template is reasoned to be the use of a thinner autologous skin graft donor site with its resultant quicker healing time and reduced donor site scarring. A recently completed multicenter clinical trial demonstrated equivalence of this technique with a standard split-thickness meshed autograft; however, autograft take rates were somewhat lower than that for controls, and varied from center to center [32]. In addition, the allogeneic dermal grafts measured only 36 to 116 cm^2, and were only evaluated up to 180 days postgrafting. Alloderm has also been used as a template for CEA; however, there have been only been anecdotal reports regarding the results of this potential application.

Micrografting techniques

Chinese surgeons have proposed the use of micrografts using both autologous and allogeneic skin [33]. This technique involves the mincing of autologous skin into pieces less than 1 mm in diameter. These micrografts are then used to seed the dermal surface of large sheets of allograft skin before transplantation onto the excised burn wound. As the autologous epidermal cells propagate on the wound surface, the allograft skin gradually separates in a manner similar to that observed with the overlay technique. This method, although resulting in an effective skin expansion ratio

approaching 1:18, has been shown to be associated with severe wound contraction that is often worse than that noted with meshed skin grafts.

Potential disadvantages of allograft use

Infection

Allograft skin has been reported to cause bacterial infection [34]. It is imperative that skin banks perform microbial cultures before release for transplantation (Table 4). Although White [35] has suggested that cadaver allograft containing $<10^3$ organisms/g of tissue can be safely used for wound coverage, current American Association of Tissue Banks (AATB) Standards [36,37] require that skin be discarded if pathogenic bacteria or fungi are present. This is particularly important given the immunocompromised status of the potential recipient and the likelihood of developing wound sepsis following such contamination.

There have also been reports of viral disease transmission by skin allografts. In 1987, Clarke [38] reported the transmission of HIV-1 to a burn patient from an HIV-positive donor; results of donor testing were not known before skin use. To date, there have been no other reported cases of HIV or hepatitis transmission from skin allografts.

Kealey [39] recently reported the transmission of cytomegalovirus (CMV) in cadaver skin allografts. Because 5 of 22 CMV-negative patients (22.7%) seroconverted, the authors recommended the use of CMV-negative allograft skin for seronegative burn patients. Although there is good evidence to support the transmission of CMV by allograft in burn patients, there is little evidence of CMV seroconversion [40,41]. Furthermore, Herndon and Rose [42] are of the opinion that the benefits of using cadaver allograft skin for the treatment of burn patients clearly outweigh the small risk described by Kealey and associates. In a subsequent study, Plessinger et al [40] demonstrated that 63% of the skin donors at their tissue bank were CMV-positive, and noted that the decision to limit the use of skin from the preponderance of donors could potentially result in a significant reduction

Table 4
Disadvantages of human allograft skin use

Potential infectious disease transmission
Bacteria
Viruses
HIV-1
CMV
Antigenicity
Rejection
Potential lack of availability

Abbreviation: CMV, cytomegalovirus.

in the supply of cadaveric allograft skin for burn patients. They concluded that the decision to use allograft skin from CMV-seropositive donors should be made by the surgeon caring for the patients.

Rejection

Athough demonstrating many characteristics of an ideal wound covering, allograft skin contains Langerhans cells that express class II antigens on their surface. These cells reside in the epidermis of the skin and will ultimately result in an immunologic rejection response. This typically results in an acute inflammatory reaction and may lead to the development of a deep wound infection. Vascularized allogeneic skin grafts typically remain intact on the wound of a burn patient for 2 to 3 weeks, although there have been reports of allograft skin survival for up to 67 days, due in part to the inherent immunosuppression of extensive burn injury [43]. Recent improvements in immunonutrition, critical care management, and a more aggressive surgical approach to definitive wound closure, however, have made the persistence of engraftment less predictable.

Efforts to prevent rejection have included methods that might reduce antigen expression by controlling Langerhans cell activity in the allograft skin. Treatment of the allografts with ultraviolet light irradiation and incubation of the skin in glucocorticoids has been reported to result in a modest prolongation of allograft survival compared with nontreated skin. However, these clinical trials were limited, and the utility of this methodology has not been substantiated. In addition, investigators have studied the effects of pharmacologic agents to induce immunosuppression in patients with major burn injuries [44]. Initial clinical trials reported improved allograft and patient survival in children treated with azathioprine and antithymocyte globulin. This regimen was associated with azathioprine-induced neutropenia, and patient outcome data has not been corroborated by others. More recently, the use of cyclosporin A has been demonstrated to prolong skin allograft survival in patients with extensive full-thickness thermal injuries [45,46]. Allograft rejection is generally observed within a few days of discontinuing treatment; however, there have been instances where engraftment has persisted after the completion of therapy. Further studies of these and newer immune suppressive agents appear warranted.

The growth of skin banking

The widespread use of allograft skin in the management of patients with extensive burn, traumatic, and soft tissue injuries has had a major impact on the number of skin banking facilities over the past 2 decades. Consequently, the majority of skin banks have been founded in close proximity to regional burn centers or within the burn center hospitals themselves. Skin banks must therefore maintain a close working relationship with regional burn

centers not only to meet the specific needs of the burn surgeon but also to help generate community support for skin donation through combined educational outreach programs. From 1969 to 1988, there was a steady growth in the number of active skin banks; however this appears to have reached a plateau during the past 10 years (Fig. 2). It is estimated that there are currently 54 operational skin banks in North America.

Role of the American Association of Tissue Banks

As skin banking facilities grew in number, it became apparent that policies and procedures required standardization. This was quite difficult initially as there was insufficient data to develop a consensus regarding standards of practice. As early as 1976 the AATB had begun to address this issue by the formation of the Skin Council. This provided a forum for the discussion of skin banking practices and was complimented by the activities of the American Burn Association's Skin Banking Special Interest Group. The Standards and Procedures Committees were created in 1977, and produced the first "Guidelines" for tissue banking in 1979. The first *Standards for Tissue Banking* was published in 1984, and tissue-specific technical manuals (including skin) were developed in 1987. Since that time, the Standards have been modified and refined based upon consensus and, where available, supportive scientific research.

Shortly after the development and promulgation of its *Standards for Tissue Banking*, the AATB created an inspection and accreditation committee in 1982, and began conducting voluntary, peer-reviewed inspections by 1986. To date, approximately 49 organizations involved in the banking of human skin have received accreditation, the majority of which are multitissue banking facilities.
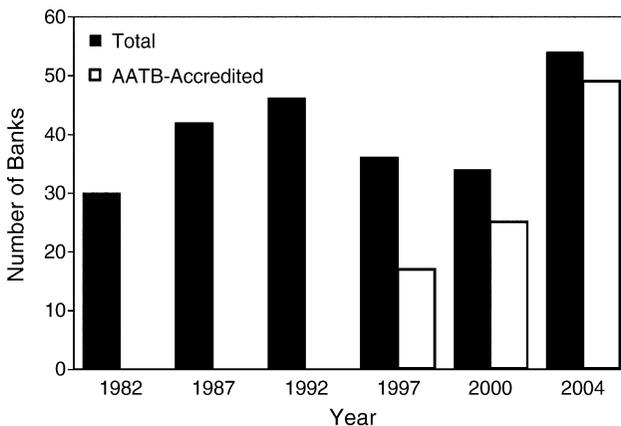

Fig. 2. Growth in the number of skin banks in the United States and Canada (from May 1990, and American Association of Tissue Banks).

Availability of cadaveric human skin allografts

Unlike organ transplantation, tissue donation rarely receives public attention. Although the supply of human skin should be infinite, there is a somewhat limited supply in the United States and Canada. With improved communications between burn and wound centers, skin and tissue banks, and the AATB, it should be possible to maintain an ample supply of allograft skin to meet the clinical need [47].

Skin banks must identify ways of increasing cadaveric skin donation while ensuring recipient safety from potential disease transmission. They must also reduce recovery and processing costs and maintain optimizing allograft quality despite the continuing need to use newer serologic and microbiologic tests to ensure tissue safety. To accomplish these goals, it may become necessary for skin banking operations to become regionalized. Such an undertaking could improve tissue availability and ultimately increase use by surgeons.

The tissue banking community has recently come under criticism by the media for its inability to maintain an adequate supply of human skin for the treatment of burn patients. Following these reports suggesting a national skin shortage, the AATB established a 24-hour hotline to address this issue. During the first 2 years of service, no calls were received from any burn or wound care center or physician, and there have been no further reports of skin shortages. In fact, according to the AATB's Skin Council, there has been a steady increase in the number of cadaveric donors from whom skin has been recovered. In 2002, there were nearly 7200 cadaver skin donors from whom approximately 10,500 ft^2 of skin was recovered and distributed to burn and wound care centers from AATB-accredited tissue banks (Fig. 3). Furthermore, in an effort to maintain adequate supplies of transplantable skin for patient use, only skin deemed unsuitable for transplantation has been made available for scientific research.

Technical aspects of skin banking

Donor screening

It is vitally important that complete and accurate medical information about the potential donor be obtained to ensure the safety of the tissue for transplantation. The AATB and the US Food & Drug Administration (FDA) require a comprehensive medical and social history of the donor, a physical examination of the potential donor, and a panel of serologic screening tests for viral diseases (HIV-1/2, Hepatitis B and C, and HTLV-1). Recently, the AATB has developed a "Donor Medical History and Behavioral Risk Assessment Questionnaire" with the cooperation and assistance of the FDA and other organ and tissue procurement organizations to help facilitate this process. It is also important that the time of
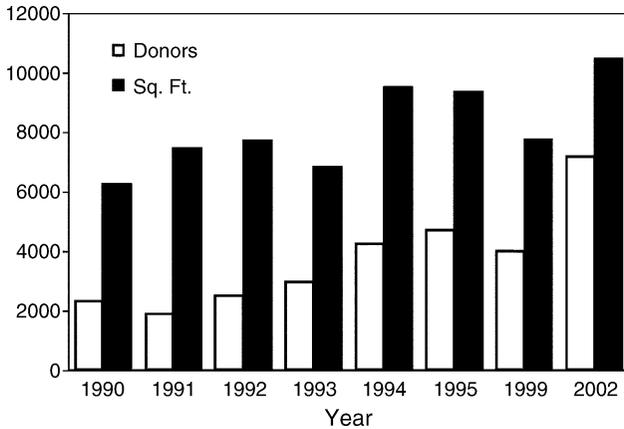
Fig. 3. Allograft skin donation and distribution from 1990–2002. (*Source*: American Association of Tissue Banks.)

death and body storage conditions be accurately documented, as these have a significant bearing upon skin viability and microbial contamination. In addition, a thorough physical examination is necessary to determine the quality of the skin and the technical feasibility of skin retrieval by evaluating the donor's size, overall health status, and skin condition. Table 5 lists those conditions that are commonly associated with deferral of a potential skin donor.

Skin recovery

Once donor screening is complete and proper consents have been obtained, the recovery team must arrange the time and location for skin removal in an appropriate facility (ie, hospital morgue or operating room, medical examiner's office, or the tissue bank). Skin retrieval should be performed expeditiously to minimize tissue deterioration and contamination. Current AATB Standards require that skin retrieval begin within 24 hours of death if the donor is refrigerated within 12 hours of asystole, or within 15 hours if refrigeration has not occurred.

In brief, the skin is removed under aseptic conditions. The areas from which the skin is taken are shaved of hair and cleansed with detergent solutions approved for use in operative procedures (ie, povidone–iodine, chlorhexidine). The retrieval technician puts on a cap and mask, performs a surgical scrub, and dons a sterile gown and gloves while the circulating technician prepares tissue and transport containers. This is usually followed by a chlorhexidine prep and rinsing with 70% isopropanol. The donor is then draped with sterile sheets and a blood sample is obtained by an intraventricular cardiac puncture. Following this, a thin layer of sterile mineral oil (or other sterile lubricant) is applied to the surfaces from which

Table 5
Disease states commonly associated with potential skin donor deferral

Extensive dermatitis
Acute burn injuries
Cutaneous malignancy
Poor skin quality
Extensive tattoos
Collagen vascular disease
Toxic chemical exposure
Skin infections
Extensive skin lesions
Extensive skin or soft tissue trauma

skin is to be removed. Split-thickness skin grafts are then removed using an electric dermatome at a thickness of 0.012 to 0.018 inches. The widths of the grafts generally range from 3 to 4 inches, but ideally should be determined by the preference of the transplanting surgeon. Skin retrieval sites are usually limited to the torso, hips, thighs, and upper calves. The amount of skin obtained per donor may vary depending on body habitus, skin defects or lesions, and body geometry; however, an average of 4 to 6 ft^2 per donor is not unusual. After tissue is obtained from the anterior surfaces, the donor is turned to the prone position, reprepped, and draped before completing the retrieval process. The skin is then placed in tissue medium and maintained at 1°C to10°C during transport to the skin bank for processing [48].

Skin processing

Processing environment

Skin is processed under aseptic conditions. Current AATB Standards indicate the need for processing in a class 10,000 laminar flow environment, yet recent data from our skin bank suggests that this offers no benefit over processing in a class 100,000 clean room [49].

Microbiologic testing

After returning to the skin bank, the procurement team obtains microbiologic cultures for aerobic and anaerobic bacteria, yeast, and fungi. Samples are best obtained before exposure of the skin to antibiotics [50]. It is generally recommended that a 1 cm^2 biopsy sample be taken for each 10% of the body surface area from which the skin has been removed [51,52]. Testing should be conducted in accordance with the National Committee on Clinical Laboratory Standards. Allograft skin should not be used for transplantation if it contains any of the following: (1) Coagulase-positive *Staphylococci*, (2) Group A, beta-hemolytic *Streptococci*, (3) *Enterococci*, (4) Gram-negative organisms, (5) *Clostridia* sp., or (6) yeast or fungi. Contamination can be minimized by meticulous donor preparation and adherence to aseptic techniques. Incubation in antibiotics is somewhat

controversial, because many antibiotics are unable to effectively kill microorganisms at 4°C, and there is the potential for exposing the recipient to resistant organisms. In addition, skin banking research has yet to determine which antibiotic combinations are effective against the commonly encountered organisms yet nontoxic to the cellular components of the skin.

Maintenance of viability

Maintenance of cell viability and structural integrity are key to the engraftment and neovascularization of allograft skin, yet there has been no quantification of the viability necessary for allograft "take." Postmortem time lapse appears to have the single greatest effect on skin viability. May has demonstrated that the functional metabolic activity of the skin rapidly declines if the donor is not refrigerated within 18 hours of death [52,53]. The ideal nutrient tissue culture medium has not yet been identified. Eagle's Minimal Essential Medium (MEM) and RPMI-1640 continue to be generally accepted; however, Cuono [54] has demonstrated the potential benefits of University of Wisconsin solution. Last, it remains unclear which cryoprotectants offer the greatest preservation of cell viability and structural integrity. Glycerol (10–20%) and dimethylsulfoxide (10–15%) have been reported to maintain skin viability following incubation times ranging from 30 minutes to 2 hours, yet the optimal concentrations of these cryoprotectants have not been identified nor have these agents been compared for efficacy. Donor factors such as age and gender do not appear to influence skin viability.

Koizumi et al [55] recently studied the effects of oxygen-enriched perfluorocarbon (PFC) on skin histology after prolonged storage. His studies demonstrated maintenance of normal skin histology at both 21 and 41 days of storage at 4°C with changing of the Eagle's MEM every 3 days, compared with skin stored without PFC, or at 25°C, or without media changes and concluded that the use of oxygen-enriched PFC may increase and prolong the viability of split-thickness skin compared with conventional methods.

Refrigeration

"Fresh" allograft skin is the preferred biologic dressing for the temporary coverage of excised extensive full-thickness burn wounds due to its more rapid adherence and rapid vascularization. The skin is stored at 4°C in tissue culture medium with or without antibiotics. Refrigeration slows the metabolic rate of the viable cells and nutrient tissue culture medium supports cellular metabolic activity. The major shortcoming of this storage method is the limited time that viability can be maintained. May [53] demonstrated that glucose metabolism declines at a rate of 10% to 15% each day during refrigerated storage; therefore, it has been common practice to cryopreserve skin within 5 to 7 days of refrigeration. Recent studies

suggest that skin viability can be maintained for up to 2 weeks at 4°C if the nutrient medium is changed every 3 days [56,57].

Cryopreservation

When skin is frozen for long-term storage, it is important that the methods used maintain cell viability and structural integrity. If the skin is not to be used "fresh," it should be cryopreserved within 10 days of procurement in media changed every 3 days. If not changed in the first 72 hours, it must be frozen in the first 96 hours. The skin is generally incubated in cryoprotectant solution for 30 minutes at 4°C. This is followed by slow-rate cooling at a rate of approximately −1°C per minute. Although computer-assisted, control-rate freezing is thought to be optimal [58], studies have demonstrated that cooling in a heat sink box at less than −2°C per minute is equally effective and does not compromise the metabolic activity of the skin [59]. The skin is frozen to −70°C to −100°C before placement in either a mechanical freezer or liquid nitrogen. Although this methodology has been reported to result in 85% retention of viability, there remains a need for research to determine the optimal technology for skin preservation [51,52].

Lyophilization

Skin can also be lyophilized by freeze drying or incubated in glycerol [60]. This process has been reported to decrease biologic degradation and antigenicity; however, this also results in epidermal cell destruction and the loss of barrier function. Moreover, lyophilized allograft skin has poor adherence to the excised wound bed and is far less effective than "fresh" skin or cryopreserved skin in controlling microbial growth [61]. Its clinical use has been further limited by its high cost of production compared with conventional allograft.

Skin storage

Refrigerated allograft skin should be stored in nutrient tissue culture medium at 4°C. The skin should be free floating in an aseptic container with 300 mL of medium per square foot of skin. The medium should be changed every 3 days to maintain optimal viability [62,63]. In 1995, it was reported [49] that the processing of allograft cadaver skin in a class-100 environment appeared to offer no additional benefit over processing the skin in a class-100,000 clean room. Skin that is to be frozen should be folded with fine mesh gauze or bridal veil covering the dermal surface before placement in a flat packet to ensure uniformity of the cooling process [64]. Skin stored in a mechanical freezer (−70 to −100°C) can be maintained for 3 to 6 months, whereas storage in liquid nitrogen (−150 to −196°C) has been shown to maintain viability for up to 10 years.

Transport

Refrigerated skin should be transported in tissue culture medium at wet ice temperatures (1–10°C) in an insulated container. Frozen allograft skin is transported on dry ice in an insulated container to prevent the skin temperature from rising to greater than −50°C. If the frozen skin is thawed at the tissue bank, it should be transported at wet ice temperatures.

Rewarming

Rewarming of frozen cryopreserved allograft skin must be performed in such a manner as to minimize cryodamage and preserve the structural integrity and viability of the skin. Early studies [65] demonstrated that warming rates of 50°C to 70°C/min resulted in 80% to 95% graft survival. Subsequent research [51,52] has revealed that warming should be performed over 2 to 4 minutes at a temperature of 10°C to 37°C (127–470°C/min). Rewarming in a microwave is not recommended due to uneven heating and excessive intracellular temperatures.

The future of skin banking

Skin banking must continue to evolve as engineered skin substitutes enter the clinical arena for the temporary and permanent coverage of partial and full-thickness wounds. Allograft skin has the potential to play a major role in permanent skin reconstruction after extensive thermal injury; however, this will require interactive research with the burn centers caring for these patients [66]. In addition, a number of skin substitutes have recently received FDA approval for use in the United States and have become part of the surgeon's armamentarium. Although they are generally more costly than allograft skin, such products possess a number of attributes including: (1) nonantigenicity, (2) ready availability, (3) sterility, and (4) the ability to provide a dermal equivalent as a template for the later application of thin (0.006 in) split-thickness autografts.

Allograft-based skin products

There is tremendous potential for human allograft-based skin products to be developed in the upcoming years; however, it will become increasingly important for skin banks to perform basic science and clinical research (in conjunction with burn and wound healing centers) to demonstrate the clinical indications and efficacy of allograft skin products in various clinical applications. Technologic advances may include modifications to reduce immunogenicity or the potential for disease transmission. Newer processing techniques could sterilize the skin without injuring the viable cellular elements or the structural integrity of the tissue. In addition, with continued research, deepidermized allograft dermis could become: (1) a source of

growth factors and antimicrobial agents, (2) a permanent full-thickness wound cover seeded with the patient's autologous keratinocytes and fibroblasts, or (3) a readily-available permanent wound cover preceded with nonantigenic allogeneic keratinocytes, fibroblasts, and melanocytes. Allogeneic skin provides a source of skin cells including keratinocytes, fibroblasts, melanocytes, and endothelial cells. These cells may be grown into large populations ($>10^{12}$ cells), cryopreserved in liquid nitrogen, recovered into culture, and combined with degradable biopolymers to form cultured skin substitutes [67]. Preclinical studies have shown organization of epidermal keratinocytes to form skin barrier [68,69], expression of pigment by melanocytes [70], and organization of endothelial cells into vascular analogs [71]. Clinical studies have demonstrated improvement of healing of chronic wounds with allogeneic skin substitutes [72], and permanent closure of excised burns with autologous cultured grafts [73]. However, cryopreservation of multilayered grafts with keratinized epithelium remains an unachieved goal in tissue transplantation. It may be expected that principles and practices of skin banking may contribute to eventual availability of unlimited supplies of skin grown in laboratories for treatment of a wide variety of skin-loss conditions. Collaborative research efforts may be necessary to achieve these goals in a timely and cost-effective manner as skin banks find themselves competing with the bioengineering industry.

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