Storage Media and Temperature Maintain Normal Anatomy of Cadaveric Human Skin for Transplantation to Full-thickness Skin Wounds

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Cadaveric human skin provides an optimal temporary cover after early excision of full-thickness burns; however, engraftment is reduced greatly by cryopreservation. Refrigerated skin is generally preferred because of its rapid revascularization, presumably caused by its greater viability. In this study, the effects of storage solutions, temperature, and the changing of the storage media on skin graft anatomy were evaluated as an indicator of graft viability. Split-thickness human skin grafts (0.012–0.015 mm) were retrieved from cadaveric donors and grafted to circumferential, full-thickness skin wounds on athymic mice. After clinical determination of engraftment 3 months after grafting, 6-mm punch biopsy samples of the human skin were harvested and separated into two groups. Biopsy samples were stored in either saline or Eagle's minimal essential medium. Media were not changed or were changed every 3 days. All groups were stored at either 4°C or room temperature (RT). After 5, 10, and 21 days of storage, biopsy samples were grafted onto athymic mice for 20 days. The biopsy grafts were then collected and prepared for histologic scoring on a scale of +4 (normal anatomy) to 0 (no epithelial cells). Significant differences in histologic scores were found by the nonparametric Kruskal-Wallis test followed by Wilcoxon pairwise comparison. Skin stored in media maintained better histologic anatomy than skin in saline, suggesting better maintenance of viability. There was also better preservation of anatomy after storage at RT for 21 days with media changes every 3 days when compared to unchanged media and all conditions at 4°C. These results support the hypothesis that increased availability of nutrients and increased storage temperature maintain higher viability of cadaveric human skin for transplantation to full-thickness cutaneous wounds. (J Burn Care Rehabil 2001;22: 393–396)

Viable human skin from cadaveric sources has proven to be a very effective biologic dressing to cover excised deep second-degree or third-degree burns if sufficient amounts of autograft skin are not available.[1,2] Refrigerated fresh allograft skin is superior to cryopreserved allograft with respect to the rate and strength of adherence to the wound, control of wound microbial growth on the wound, and ability to revascularize.[3] Fresh allograft also adheres better to wounds that contain moderate levels of microbial contamination.[3] Cadaveric human skin has been shown to be the optimal temporary wound cover following excision of full-thickness burns for over 4 decades; however, viability and engraftment appear to be reduced greatly by even the most current methods of cryopreservation.[3] In the present study, storage conditions for refrigerated human skin were evaluated to assess graft anatomy as an indicator of viability.

MATERIALS AND METHODS

Split-thickness human skin was procured from cadaveric donors from the Ohio Valley Tissue and Skin Center, Cincinnati, OH, with a Padgett dermatome set at a thickness of 0.012–0.015 mm in accordance with the standards of the American Association of

Tissue Banks[4] and placed in fresh Eagle's minimum essential medium (EMEM) at 4°C for transport. The skin was grafted on circumferential, full-thickness skin wounds in healthy male nude athymic mice[5] (BALB/C; nu/nu, 40–50 g; Harlan Industries, Indianapolis, IN) (n = 30). After clinical determination of engraftment at 3 months after grafting, 6-mm punch biopsy samples (n = 149) of the human skin were harvested and separated into four groups. Biopsy samples were stored in either saline (groups 1 and 2) or EMEM (groups 3 and 4); solutions were not changed (groups 1 and 3) or changed (groups 2 and 4) every 3 days. All groups were stored at either 4°C or room temperature (RT). The negative control biopsy sample was submerged in 100°C water, and the positive control was not stored. After 5, 10, or 21 days of storage, all biopsy samples were grafted onto athymic mice for 20 days. No samples were obtained for skin stored in unchanged and changed saline at RT. Biopsy samples were then collected, prepared for histology, stained with hematoxylin and eosin, and evaluated for histologic scoring using a scale of +4 (normal anatomy) to 0 (no epithelial cells) as outlined in Table 1. Statistical comparisons were performed by the nonparametric Kruskal-Wallis test and the pairwise Wilcoxon test.

RESULTS

Histologic scores of the various storage conditions are listed in Table 2. For the 4°C storage condition, skin stored in unchanged saline (group 1) yielded average histologic scores (n = 7) of 0.7, 0.6, and 0.0 at 5, 10, and 21 days storage, respectively (NS). The skin stored in changed saline (group 2) exhibited average histologic scores (n = 7) of 0.6, 0.5, and 0.0 at 5, 10, and 21 days storage, respectively (NS). In addition, the histology of skin stored at 4°C in either unchanged or changed saline was not significantly different across the storage time.

Skin stored at 4°C in unchanged EMEM (group 3) had average histological scores (n = 7) of 0.0, 1.6, and 0.0 at 5, 10, and 21 days storage, respectively (NS). The skin stored at 4°C in which the EMEM was changed (group 4) exhibited average histological scores (n = 7) of 3.3, 4.0, and 0.9 for 5, 10, and 21 days' storage, respectively. The values at 5 and 10 days' storage were both significantly greater than at 21 days (P = .0049). Statistical comparison of the unchanged EMEM to changed EMEM at 5, 10, and 21 days' storage time at 4°C storage condition showed that significant differences were found only at day 5 (P = .0089) and 10 (P = .0267).

For skin stored at RT, unchanged EMEM (group 3), the average histological scores (n = 7) were 1.5, 1.6, and 1.0 at 5, 10, and 21 days' storage, respectively (NS). The skin stored at RT with the EMEM changed every 3 days (Group 4) exhibited average histological scores (n = 7) of 3.3, 4.0, and 4.0 at 5, 10, and 21 days' storage, respectively (NS). Statistical comparison of the unchanged EMEM to changed EMEM at 5, 10, and 21 days' storage at RT revealed a significant difference only at day 21 (P = .0389).

DISCUSSION

These results suggest that human allograft skin should not be stored in saline at either 4°C or RT; however it may be stored for 5 days or longer in EMEM if the EMEM is changed every 3 days. The decreased average histologic score after storage in EMEM at 4°C for 21 days may be caused by the depletion of nutrients from the medium. In addition, the higher average histologic score for the changed EMEM at RT suggests that conditions for temporary skin storage are improved at RT and with the medium changes at least every 3 days.

Although fresh allograft is the prevailing standard for temporary burn wound coverage, determination of viability is subject to arbitrary and variable conditions of allograft procurement, processing, and storage among tissue banks and burn centers. Cadaveric skin is usually retrieved from tissue donors within 24 hours following the death of the donor. The time of skin acquisition postmortem is often outside of the control of the skin bank, as are the conditions of body storage prior to tissue recovery. Refrigerated allograft skin, if not used within 96 hours of procurement, must be cryopreserved or discarded, according to the Standards of the American Association of Tissue Banks.[4] It is often difficult for skin banks to supply fresh skin to burn centers because the availability of fresh donor tissues is inherently unpredictable. If fresh skin is available, the FDA requires that the tissue bank and user physician complete an exceptional release form and adhere to mutually agreed upon policies that consider patient safety and the immediacy of the allograft need.[6] For this reason, many skin banks

Table 1. Scoring of Histologic Sections

Score	Criteria
0	No epithelial skin cell (negative control)
1	Minimal epithelial cells
2	Thin epithelial cells
3	Moderate epithelial cells
4	Epithelial cells (positive control)

Table 2. Average Histologic Score of Human Cadaver Skin Stored in Unchanged and Changed Saline and EMEM

Storage Condition	4°C			Room Temperature		
	5 Days	10 Days	21 Days	5 Days	10 Days	21 Days
Group 1, Saline ⊿	0.7 ± 0.3	0.6 ± 0.2	0.0 ± 0.0	ND	ND	ND
Group 2, Saline Δ	0.6 ± 0.6	0.5 ± 0.2	0.0 ± 0.0	ND	ND	ND
Group 3, EMEM ⊿	0.0 ± 0.0	1.6 ± 0.5	0.0 ± 0.0	1.5 ± 1.5	1.6 ± 0.5	1.0 ± 0.4
Group 4, EMEM Δ	3.3 ± 0.5†	4.0 ± 0.0†	0.9 ± 0.3*	3.3 ± 0.5‡	4.0 ± 0.0‡	4.0 ± 0.0‡§

NOTE: Data are expressed as average histologic score.
ND, not done; Δ, changed; ⊿, not changed.
* $P < .005$ *v* 4°C at 5, 10 days storage.
† $P < .05$ *v* group 3 at 4°C.
‡ $P < .03$ *v* 4°C at room temperature.
§ $P < .05$ *v* 4°C at 21 days.

do not maintain a supply of refrigerated allograft, and many burn centers are unable to have the option of using fresh, refrigerated allograft skin in the treatment of severely burned patients

The efficacy of allograft skin as a temporary cover for burns has led to the establishment of standards and procedures for skin retrieval and storage to meet the demand for cryopreserved and refrigerated fresh skin.[7–11] Skin allografts are not intended to provide a permanent wound cover, yet the availability of viable allograft skin for use in burn patients remains a significant factor in patient care. The debate over the advantages of fresh versus preserved allograft has a long history.[12–15] Despite reports of the successful application of nonviable cryopreserved skin,[16–18] there is a consensus that the more viable the allograft, the better it will perform as a temporary wound cover in patients with large thermal injuries;[1,19,20] however, the impact of allograft usage and viability on clinical outcome remains undetermined.

Current American Association of Tissue Banks standards require that allograft skin to be cryopreserved is processed within 10 days of retrieval, provided the skin is placed in tissue storage media that is replaced at least every 72 hours. If the media is not changed, processing must be initiated within 96 hours of retrieval.[4] The introduction of controlled-rate freezing in conjunction with rapid rewarming has permitted the prolonged storage of viable allograft.[21] Extreme care must be maintained when warming skin to control not only the rate of rewarming but also the maximum temperature.[21] Storage at −80°C can be maintained for many months, while storage in liquid nitrogen (−160°C) maintains skin for up to 10 years.[22] Cryopreservation techniques are most damaging to epithelial cells, and thus the protective function of the dermal barrier can be impaired or lost. Cryopreserved allograft has been reported to un-

dergo early separation of the injured epidermal layer following its placement on the wound.[22] Cryopreserved allograft exhibits reduced wound adherence, reduced control of wound contamination, and reduced revascularization when compared to fresh allograft skin.[21] Although cryopreserved allograft may not vascularize as well as fresh allograft, many burn centers rely on cryopreserved skin because the supply of fresh allograft is limited or unavailable; therefore, the development of storage and cryopreservation techniques that maintain optimal skin viability could have a significant impact on engraftment and clinical outcome parameters.

The primary purpose of skin banking is to provide a continuous supply of the highest-quality allograft skin by preserving the tissue properties required for graft take. To achieve this goal, viability assays are used to identify the mechanisms and targets of damage in preserved skin so that protocols can be implemented to avoid the damage and ultimately to monitor those targets to acquire an estimate of post-preservation quality. The primary requirement that may be necessary for the engraftment of skin is the presence of living, functioning cells with a normal microscopic and macroscopic dermal structure.[15,21] An assay to be used routinely in skin banks should be able to measure these factors while being quick, easy, and inexpensive to obtain and perform. Ultimately, the assay should demonstrate specificity, precision, and a range of values between viable and nonviable controls to provide accurate, useful, and valid data.[23–25] Additionally, the utilization of two viability assays (oxygen consumption[26] and MTT reduction) meet these criteria and might ultimately be used to predict vascularization and engraftment of human allogeneic skin. Histologic, ultrastructural examination, and freeze substitution have been used to provide details of structural damage to the skin.[1,14,27]

Dye-exclusion tests with trypan blue[14,28,29] have also been used as indicators of membrane integrity and the extent of cell damage during preparation. We have previously shown that oxygen consumption for positive (fresh) and negative (boiled) samples remained constant. The oxygen consumption for the stored samples, which initially approximated fresh skin, gradually approached those of the negative control.[30] Samples stored at 4°C for the shortest interval (0–3 days) rapidly utilized oxygen from the medium. Samples stored for greater than 3 days showed progressively lower rates of oxygen consumption, intermediate between those stored for ≤ 3 days and negative controls. Interestingly, after the medium was changed on days 1, 3, and 10, there was an increase in oxygen consumption, suggesting stimulation of the viable cells in the tissue sample by an increase in nutrient availability. After 21 days of 4°C storage, oxygen consumption was comparable to the negative controls. These results suggest that stored samples progressively lose viability over 21 days despite replenishment of the storage media.[30] These in vitro results are consistent with our current in vivo findings that the viability of cadaveric human skin is improved by routine changes of a complete nutrient medium and by storage at RT rather than refrigerated. Future studies should focus upon the development of a media formulation[31] that is optimal for storage,[32] as well as new biotechnologies that will permit cryopreserved skin to engraft as consistently as autologous skin grafts.

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