The Use of Cryopreserved Human Skin Allografts in Wound Healing following Mohs Surgery

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BACKGROUND. *Immediate reconstruction has become the preferred approach to management of full-thickness cutaneous defects following microscopically controlled excision (MCE) of tumors. In a minority of patients, however, large reconstructive procedures are contraindicated, and a long-term biological dressing that stimulates healing while minimizing wound care is desirable.*

OBJECTIVE. *To assess the utility of cryopreserved human skin allografts (HSA) in wound care and wound healing following Mohs surgery.*

METHODS. *Sixteen patients were treated with HSA following MCE and followed postoperatively for evidence of infection, involution, or survival of HSA, and granulation tissue production. Follow-up was 2–26 months.*

RESULTS. *The use of HSA resulted in one of three general outcomes: rapid healing and rejection, subsequent full-thickness skin grafting, or persistence of HSA during prolonged healing.*

CONCLUSIONS. *HSA are a safe alternative to immediate reconstruction in a carefully selected population of skin cancer patients. They minimize wound care while providing continuous wound coverage during healing, and are an efficient bridge to full-thickness skin grafting.* Dermatol Surg 1995;21:615–620.

The incidence of nonmelanoma skin cancer has increased significantly over the past several decades.[1] Conventional therapy currently includes excision, with or without microscopically controlled margins, and the resultant defect is usually easily repaired with simple resurfacing techniques.[2,3] However, in some patients medical contraindications to large reconstructive procedures can pose a special challenge. In addition to these contraindications, patients may not be candidates for immediate repair because of wound size, depth, location, or lack of adequate donor tissue. In these cases, an intermediate to long-term biological dressing that can stimulate healing and minimize wound care is desirable. Cryopreserved human skin allografts (HSA) have long been successfully used in the management of thermally induced wounds,[4,5] however, their utility in the management of fresh surgical wounds has not been explored. We present our experience using HSA in 16 skin cancer patients. We conclude that HSA can provide a very efficient bridge to delayed full-thickness skin grafting while minimizing wound care, and may persist during protracted wound healing in some patients.

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Materials and Methods

Between 1991 and 1993, 16 patients 43–93 years old were treated with HSA following microscopically controlled excision (MCE) of basal cell or squamous cell carcinomas of the head and neck, back, or extremities (Table 1). Patients were considered for HSA because of the size or depth of their surgical defect, lack of adequate tissue for immediate repair, medical illness, or anticoagulation that precluded more extensive surgery. At the completion of tumor extirpation cryopreserved HSA (−196°C) were obtained within 1 hour from the Yale Skin Bank. All HSA were harvested, processed, and frozen by the Yale Skin Bank in accordance with the guidelines of the American Association of Tissue Banks. All potential cadaveric donors were screened for evidence of death complicated by or in the setting of infection, malignancy, multiple transfusions, poisoning, autoimmune disease, or chronic progressive neurological disorders. Donors were also screened for positive serology to hepatitis B surface antigen, hepatitis C, human immunodeficiency virus (HIV), human T lymphocyte virus type 1, and VDRL. Harvested skin samples were also sent for microbiological analysis, and only if there was no evidence of infection with pathogenic organisms was the tissue released for use.

Following extirpation of the malignancy all immediate reconstructive options were considered including placement of HSA. Only when both the surgeon and patient felt HSA was the best approach was allografting undertaken. Patients were advised of the risks and benefits of all available options and informed consent was obtained. HSA were quickly thawed within 2 minutes using cold tap water applied to the outside of the storage pouch, removed from the pouch, and submerged in sterile 0.9% saline at room temperature. The allograft was then trimmed to the surgical defect and sutured using running 5-0 or 6-0 monofilament suture. Basting sutures were used as needed. When necessary, full-thickness 5.0-mm incisions were made throughout the allograft to assist drain-

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Table 1. Characteristics and Past Medical Histories of Patients Treated with HSA

Patient	Age (Years)	Sex	Tumor	PMH	Site
1	78	M	BCC	Prostate cancer	L back
2	74	F	rec BCC	Breast cancer	L scalp
3	57	F	BCC	HTN, MI	Nasal tip
4	79	M	mor BCC	None	Mid back
5	92	F	BCC	IDDM	L temple
6	83	F	rec BCC	CAD	L scalp
7	63	F	BCC	CAD	Nasal bridge
8	51	M	SCC	None	L post auric
9	93	F	SCC	HTN	R shin
10	80	F	rec BCC	CAD	Nasal bridge
11	87	F	SCC	A fib, TIA	Nasal bridge
12	77	F	SCC	IDDM	L temple
13	74	M	rec SCC	HTN	R post auric
14	43	M	BCC	HTN	L med canth
15	76	F	rec BCC	HTN	L nasal ala
16	83	F	rec BCC	COPD, HTN	L temple

The mean age was 74 years; there were five males and 11 females. BCC, basal cell carcinoma; SCC, squamous cell carcinoma; rec, recurrent; mor, morpheaform; HTN, hypertension; MI, myocardial infarction; IDDM, insulin-dependent diabetes mellitus; CAD, coronary artery disease; A fib, atrial fibrillation; TIA, transient ischemic attack; COPD, chronic obstructive pulmonary disease.

age of wound exudate. Xeroform dressing was secured over the graft and removed at 24–48 hours. At this point wound care consisted of daily gentle washing with hydrogen peroxide or tap water and application of Polysporin ointment.

Patients were assessed at 24–48 hours postoperatively and then at 2–4-week intervals for evidence of infection. Persistent exudate, involution and survival of allograft, and granulation tissue were also monitored. If at any point in the postoperative period it was felt that a patient would benefit from, and/or tolerate a more standard form of repair, HSA tissue was removed and surgical repair was undertaken. In addition, if there was rapid HSA involution without complete healing, patients were reassessed for reconstruction or full-thickness skin grafting.

Results

The use of HSA resulted in one of three general outcomes: seven patients experienced rapid, progressive rejection of the allograft with concomitant healing by second intention (average survival of HSA in this group was 3 weeks); three patients underwent subsequent full-thickness skin grafting 2 and 4 weeks after initial allografting; and six patients experienced protracted wound healing with apparent persistence of allograft during healing (mean time to complete healing, 11 months). The follow-up time ranged from 2 to 26 months (Table 2). None of our patients experienced wound infection or other early complications such as hypersensitivity to, or immediate rejection of, HSA. Case histories and clinical photographs of representative patients are presented.

Table 2. Defect Size and Outcome of Patients Treated with HSA

Patient	Defect size (cm)	Depth	HSA persistence	Result	Follow-up
1	5 × 6	Fascia	10 mo	PH	10 mo
2	5 × 7	Periosteum	4 wk	RH	8 mo
3	2 × 2	Cartilage	Removed	FTAG	16 mo
4	10 × 10	Fascia	1 wk	RH	11 mo
5	4.5 × 5	Fascia	10 mo	PH	10 mo
6	8 × 9	Into bone	26 mo	PH	26 mo
7	2 × 3	Cartilage	Removed	FTAG	7 mo
8	3.5 × 3.5	Cartilage	3 wk	RH	10 mo
9	3 × 3	Fascia	3 wk	RH	7 mo
10	2 × 2	Cartilage	6 mo	PH	6 mo
11	2 × 2.5	Cartilage	3 wk	RH	6 mo
12	6 × 8	Into bone	7 mo	PH	7 mo
13	2.5 × 3	Cartilage	3 wk	RH	6 mo
14	3 × 3.5	Cartilage	6 mo	PH	6 mo
15	4 × 5	Cartilage	Removed	FTAG	2 mo
16	8 × 9	Periosteum	4 wk	RH	3 mo

The mean defect size was 4.4 × 5.1 cm. The mean HSA persistence was 3 weeks for rapid healing and 11 months for protracted healing. PH, protracted healing; RH, rapid healing; FTAG, full-thickness autologous grafting.

HSA Applied to Exposed Cartilage

DC is a 51-year-old white male without significant past medical history who was referred for excision of a squamous cell carcinoma of the posterior surface of the left ear. The cancer measured 1.2 × 1.0 cm, and there was no adenopathy. The tumor was completely removed in three Mohs stages with a resulting defect of 3.5 × 3.5 cm down to cartilage (Figure 1A). Reconstructive alternatives were discussed, which included full- and split-thickness skin grafting, adjacent tissue transfer, and healing by second intention. Due to the large area of exposed cartilage and the desire to minimize wound care the defect was covered with HSA as described above (Figure 1B). By 3 weeks postoperatively there was little residual allograft at the wound base, however, there was good granulation tissue formation. By 10 weeks there was complete healing by second intention (Figure 1C). The frequent side effects of erythema, swelling, and tenderness often seen over exposed cartilage of the ear were not noted in this case or in the other ear defects treated with HSA.

HSA with Subsequent Full-Thickness Skin Grafting

EE is a 63-year-old white female with a past medical history of coronary artery disease and hypertension who was referred for excision of a basal cell carcinoma of the left nasal tip. The clinical diameter of the tumor was 1.8 × 1.2 cm, and upon complete extirpation the defect was 2.3 × 2.8 cm down to cartilage (Figure 2A). Reconstructive options were discussed including full- and split-thickness grafting, a transposition flap, and healing by second intention. Due to the large area of exposed cartilage at the base of the defect the decision

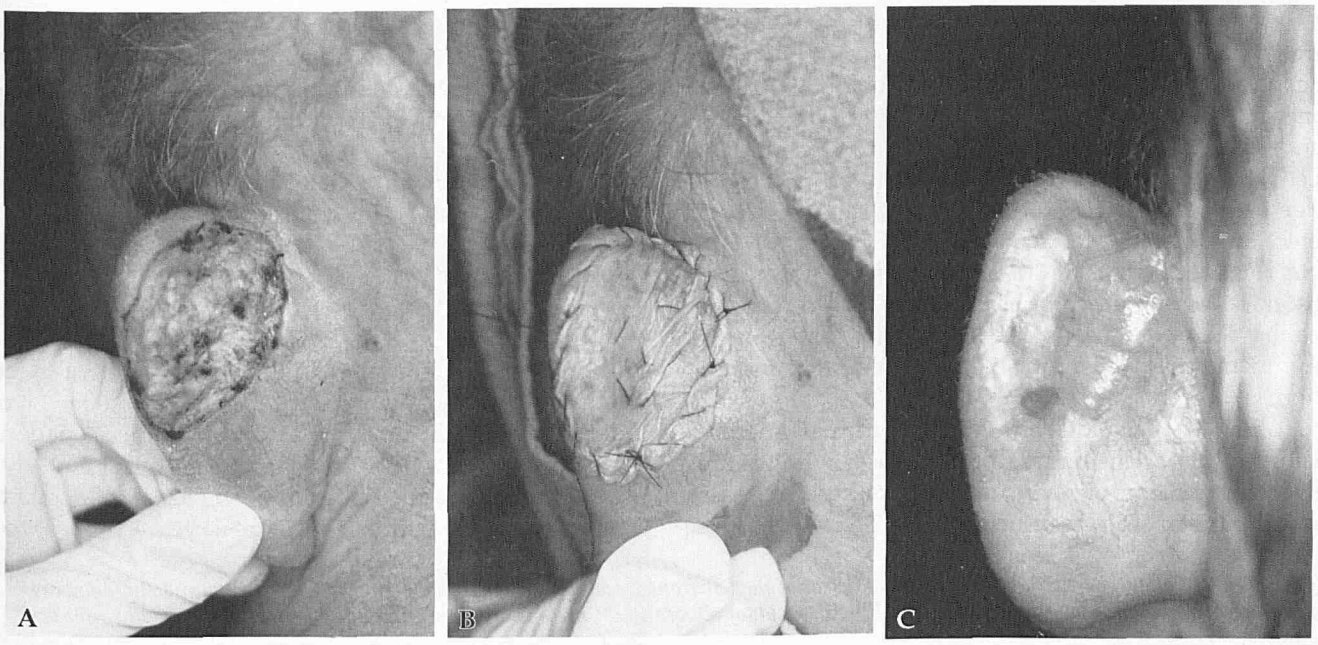


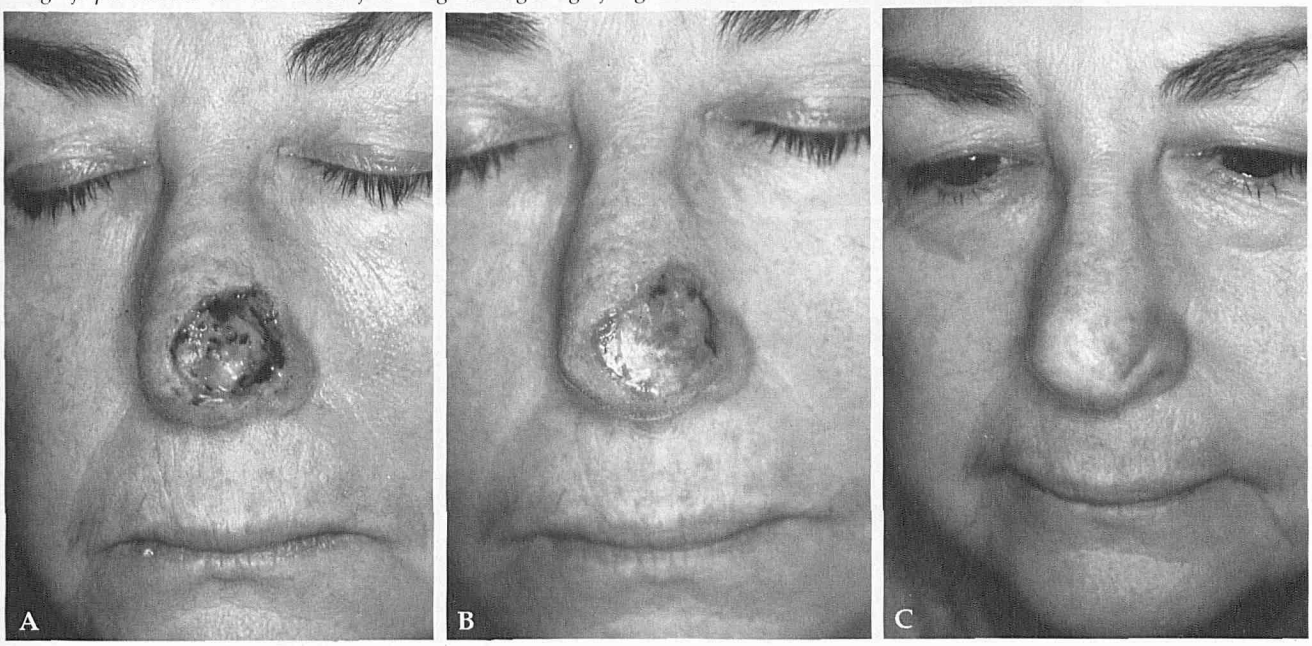
Figure 1. *A) Patient DC showing surgical defect with exposed cartilage immediately following MCE. B) Immediately following allograft placement. C) With complete healing following allograft placement.*

was made to cover the wound with HSA anticipating that granulation would permit final autografting with a more natural contour (Figure 2B). At 2 weeks postoperatively the patient's wound had granulated sufficiently for full-thickness skin grafting. The HSA was removed and full-thickness skin grafting was performed with an excellent final result (Figure 2C). In our experience, delayed full-thickness skin grafting over sites with exposed cartilage allowed to heal by second intention usually must be carried out after approximately 4 weeks in order to provide a natural contour. HSA, therefore appeared to hasten this process.

HSA Applied to Exposed Bone

CH is a 77-year-old white female with a past medical history of insulin-dependent diabetes mellitus who was

Figure 2. *A) Patient EE with nasal tip defect with exposed cartilage immediately following MCE. B) Two weeks following allograft placement. C) Final result following autologous grafting.*



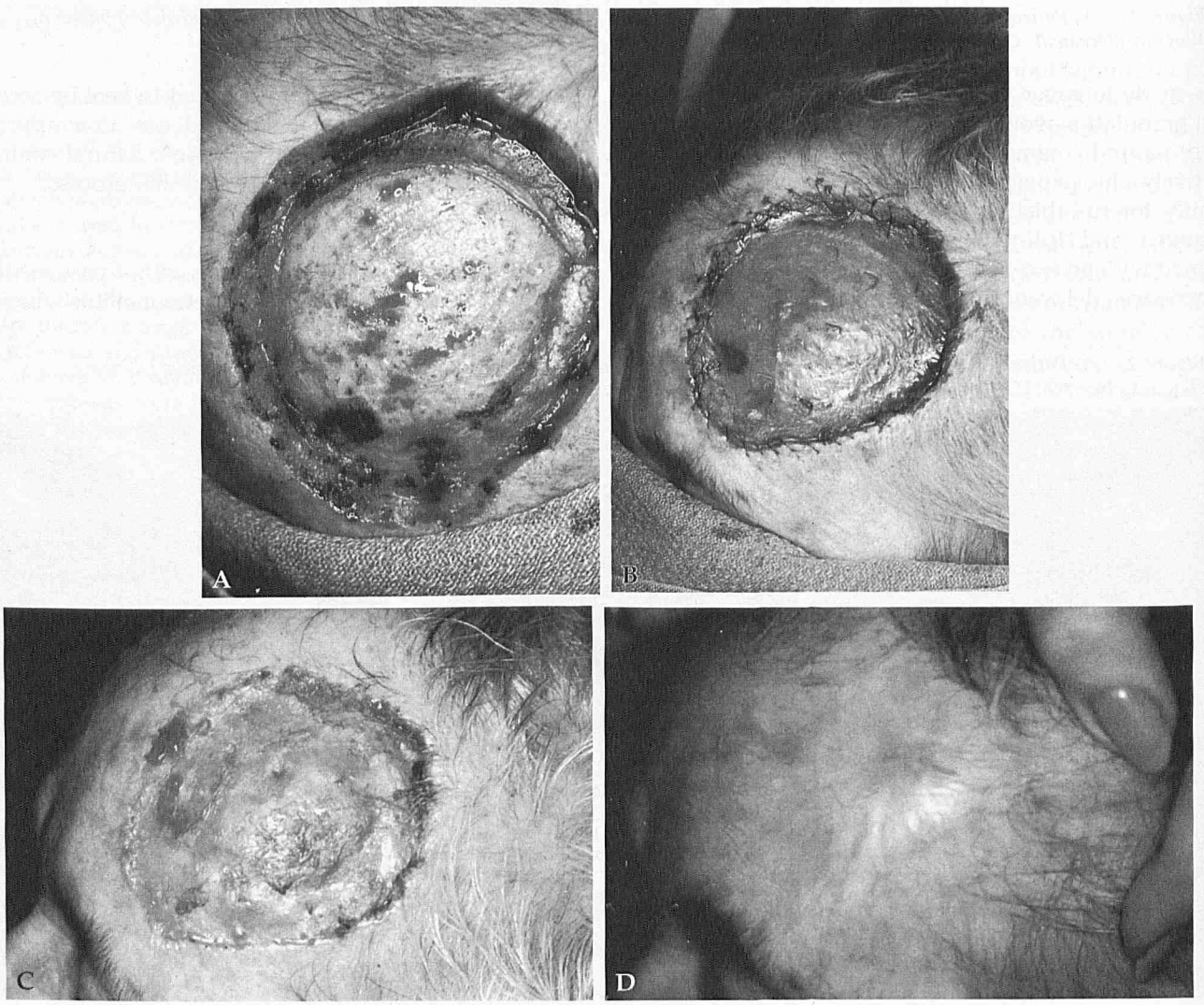
referred for excision of a biopsy-proven squamous cell carcinoma of the left frontal scalp measuring 5 × 6 cm in clinical diameter without regional adenopathy. Upon complete removal of the tumor the surgical defect measured 8 × 6 cm and extended to bone (Figure 3A). Reconstructive options were discussed and due to the large area of exposed bone present at the base of the defect the decision was made to cover the defect with HSA (Figure 3B). Use of HSA eventuated in persistence of some allograft at 2 months (Figure 3C), and complete healing with an excellent cosmetic result and contour despite the original depth of the defect at 7 months (Figure 3D).

AL is a 74-year-old white female with past medical history of hypertension and breast cancer who was referred for excision of recurrent basal cell carcinoma of the left parietal scalp measuring 2.5 × 2.5 cm in clinical diameter without regional adenopathy. After tumor extirpation the resulting defect measured 5 × 7 cm and extended to periosteum (Figure 4A). After consideration of all reconstructive options the patient opted for allograft placement (Figure 4B). This HSA rapidly involuted with accompanied rapid wound healing, and an excellent final result (Figure 4C) at 8 months.

Discussion

In our series of 16 patients, there was no obvious correlation between age, tumor type, size of defect, or underlying medical illness that could predict a patient's experience with HSA. The mean age of patients who experienced protracted healing was 82 years, while the mean age of the entire group was 74 years, (NS). Two of

Figure 3. *A) Patient CH with a large surgical defect of the left frontal scalp immediately following MCE. B) Immediately following HSA placement. C) Two months postoperatively with residual allograft still present at the healing site of surgery. D) Final result at 7 months.*



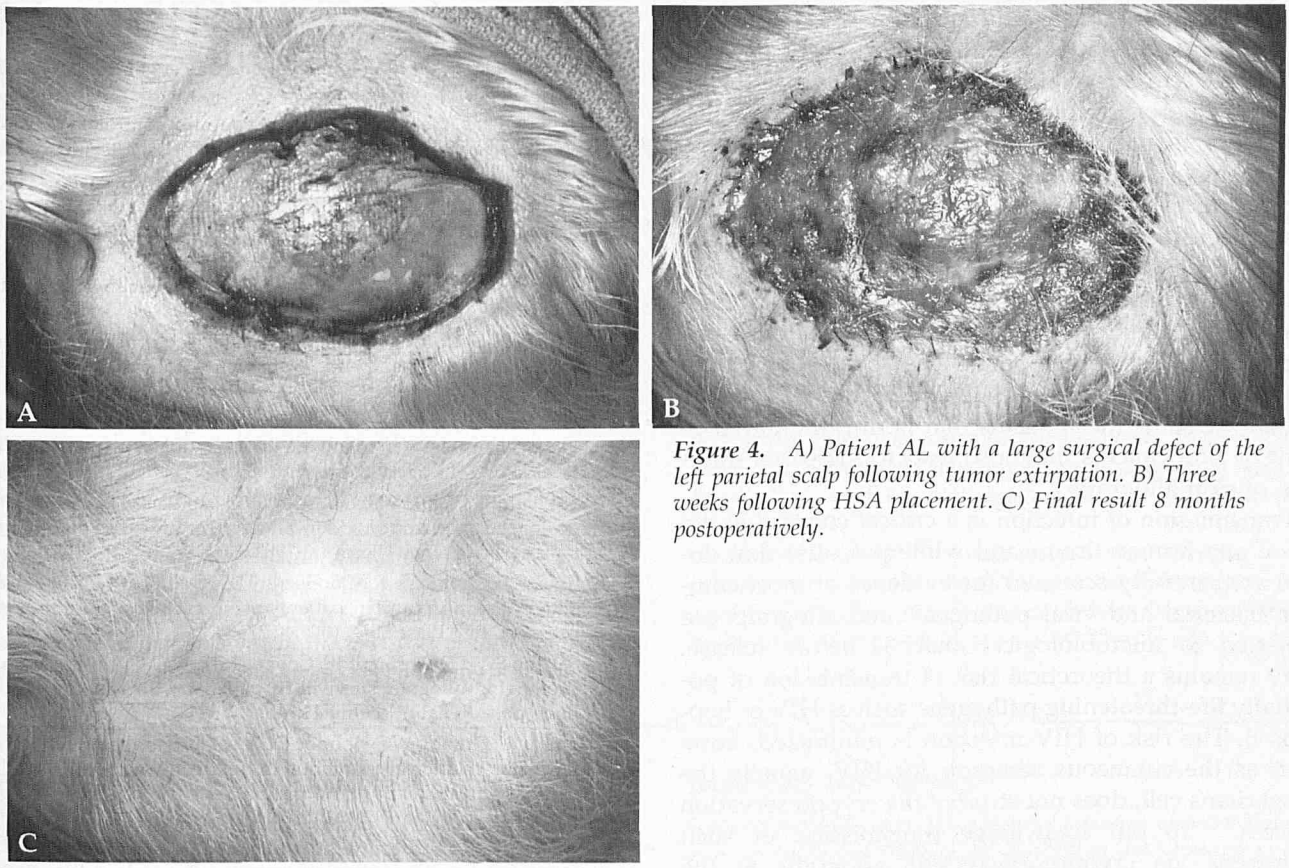


Figure 4. A) Patient AL with a large surgical defect of the left parietal scalp following tumor extirpation. B) Three weeks following HSA placement. C) Final result 8 months postoperatively.

the six patients who experienced protracted healing were insulin-dependent diabetics (patients 5 and 12), and one had a history of advanced prostate cancer treated with diethystilbestrol (patient 1). The only other patient in our series with a history of internal malignancy was patient 2, who had a left mastectomy for breast cancer more than 5 years before her current skin cancer was diagnosed and whose defect healed by second intention with rapid HSA involution. None of our patients was taking any form of immunomodulatory drug, however, one of our six patients who experienced protracted healing with persistence of HSA had a history of prior treatment of the primary skin cancer with local radiation therapy (patient 6). This therapy occurred several years before allografting, and any transient local immunosupression due to the radiation therapy should have resolved.

It is unclear why we have observed prolonged survival of HSA during healing in some patients, however, in patients with documented pathogenic or iatrogenic immunosupression long-term survival of HSA has been reported,[6–8] and this observation has led to attempts to enhance HSA survival in severely burned patients through the use of specific immunosupressive agents such as cyclosporine.[9,10] In addition, the process responsible for rejection and survival of these grafts is not completely known. Recent research points to an antigen-specific mechanism involving the host's cytotoxic T cells as the primary effector cell[11–13] and antigen presentation by host Langerhans cells.[14,15] It is unclear if any of these factors are affected in our patient population.

In this study long-term survival of HSA was inferred from persistence of tissue at the surgical site that had a distinct appearance readily discernible from native granulation tissue and surrounding skin. Patients were evaluated by two observers and if there was disagreement as to survival, the graft was considered to be rejected. We did not perform immunohistochemistry or chromosomal analysis of surgical site tissue to confirm the persistence of HSA during healing. We assume that by the time of complete healing, all HSA has been rejected or shed. Based on this clinical assessment of HSA survival, a minority of patients did experience persistence of HSA well beyond the mean survival time of 3.0 weeks. The high rate of long-term survival of HSA in our series may be due to the advanced age of our patient population, which coincides with natural senescence of the immune system. Our patients are presumably older than the average patient being treated with HSA for thermal injury, but the diverse medical problems in these two groups make analysis with historical

controls difficult. This age difference may explain why we have seen a relatively high proportion of patients with long-term survival of grafts compared with the sporadic reports of prolonged survival in burn patients. In addition, some authors have suggested that the development of skin cancer may be caused in part by aberrant local immune responses. For example, a decreased sensitivity to common contact allergens and a decreased peripheral CD4/CD8 ratio has been demonstrated in patients with multiple basal cell carcinomas,[16,17] and the development of second and third primary basal cell carcinomas has been shown to correspond with decreased CD4/CD8 ratios.[18] It may be that the same mechanisms that permit the development of skin cancers may also lead to increased tolerance of skin allografts.

Transmission of infection is a critical concern in the use of any human tissue, and while potential skin donors are carefully screened for evidence of most common bacterial and viral pathogens, and allografts are subjected to microbiological analysis before release, there remains a theoretical risk of transmission of potentially life-threatening pathogens such as HIV or hepatitis B. The risk of HIV infection is minimized, however, as the cutaneous reservoir for HIV, namely the Langerhans cell, does not survive the cryopreservation process.[19] To our knowledge transmission of such pathogens via cryopreserved skin allografts in the United States has not been reported. There remains only one case of HIV transmission via skin allograft in the world literature and that resulted from use of an allograft before the donor HIV antibody results were available.[20]

While conventional methods of repair are appropriate for the vast majority of patients undergoing MCE, we have demonstrated that HSA are an effective alternative in a carefully selected subgroup of patients. HSA are readily available on short notice in university settings or by overnight delivery and cost approximately $150.00 per application. As biological dressings they are ideal for large, deep defects in which exposed bone or cartilage is present, or in those patients unable to perform the daily wound care required for granulating surgical sites. In our experience there was less daily wound care involved for patients treated with HSA due to a decrease in the amount of exudate from the wound bed and a lack of exposed cartilage, bone, or new granulation tissue at the wound bed. In this setting they appear to provide continuous wound coverage until complete healing. They are also an effective bridge to delayed full-thickness skin grafting for deep defects providing rapid granulation and vascularization.

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