Treatment of Chronic Wounds with Cultured Skin Substitutes: A Pilot Study

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Abstract: Chronic wounds in skin persist because the normal process of wound healing is obstructed. Failures occur in the inflammation and proliferation phases of wound healing that reduce formation of granulation tissue and prevent epithelial migration to close the wound, resulting mostly from vascular insufficiencies of multiple etiologies. Microbial contamination of chronic wounds also contributes importantly to the persistence of venous stasis, diabetic, and decubitus ulcers. Although slow healing can be stimulated by regular debridement and cleaning of chronic wounds, acceleration of epithelial closure has been demonstrated by application of sheets of cultured allogeneic keratinocytes. These studies have been extended by attachment of allogeneic keratinocytes to an implantable collagen–based sponge that is populated with cultured allogeneic fibroblasts. Because chronic wounds have low vascular competence and high probability for microbial contamination, skin substitutes are irrigated topically with a solution of nutrients and antimicrobial agents until epithelial engraftment. Initial case reports show that allogeneic epithelium can cover chronic wounds within seven to ten days, protective epithelium forms within one month, and long–term wound closure is accomplished, most probably by ingrowth of autologous epithelium. With this approach, cultured skin substitutes may be expected to promote healing of chronic wounds that have sufficient vascular competence to remain perfused. Together with surgical and non–surgical approaches to improvement of vascular function, cultured skin substitutes offer an alternative therapy for accelerated closure of chronic wounds.

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Introduction

Chronic ulcerations of the skin are responsible for a significant portion of health care expenditures. It was estimated in the U.S. in 1979 that 128,000 patients were hospitalized primarily for stasis ulceration. The average length of stay was 11.4 days with an estimated cost of 300 million dollars.[1] A survey by Meehan[2] of 148 hospitals reports a prevalence of pressure ulcers of 9.2%. A treatment that could safely and efficaciously reduce the healing time of chronic skin ulcers would be of great benefit to the patient and community.

Mechanisms of normal healing in skin wounds follow a timely and orderly sequence of hemostasis, inflammation, cell proliferation, development
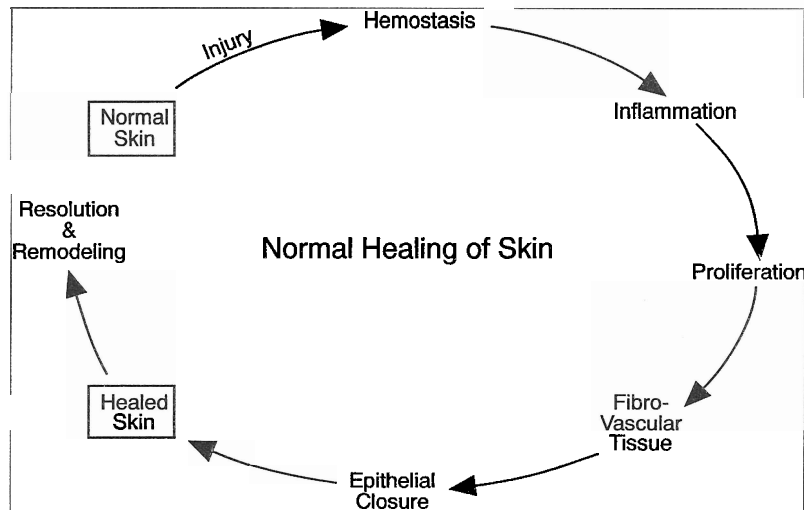
Figure 1. *Diagram of normal healing in skin. Wound repair proceeds by an orderly and timely process of hemostasis, inflammation, cell proliferation, development of fibrovascular tissue, epithelial closure, and resolution and remodeling of healed skin. Cosmetically–acceptable scar forms in healed wound.*
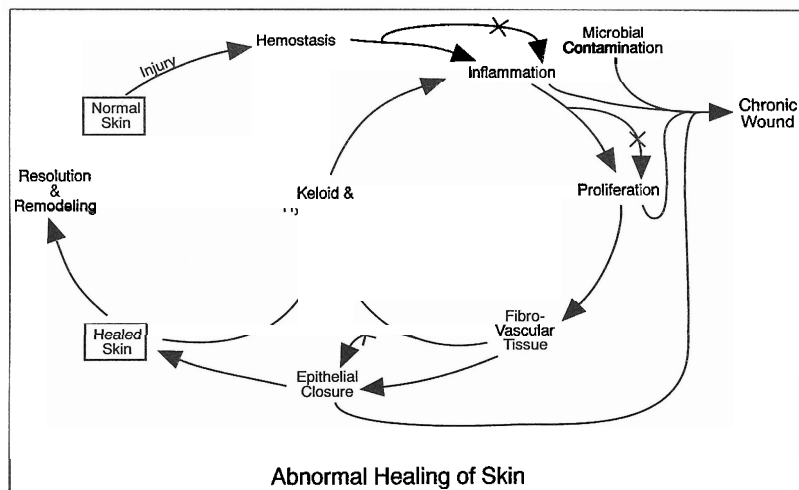
Figure 2. *Diagram of abnormal healing in skin. Chronic wounds are established by failures in inflammation, proliferation, or epithelial closure with superimposition of microbial contamination. Keloid or hypertrophic scarring result from failure of the healing process to resolve. Continuation of a cycle of inflammation and proliferation produces an excessive scar.*

of fibro–vascular tissue, epithelial closure, resolution and remodeling of repaired tissue[3] (Figure 1). In abnormal healing of skin, vascular insufficiencies or autoimmune conditions may cause failure of inflammation and cell proliferation which is compounded by microbial contamination to establish chronic wounds (Figure 2). Excessive healing that produces keloid and hypertrophic scarring is also abnormal, and results from failure of the inflammatory and pro-liferative phases of wound healing to resolve.

Successful transplantation of cultured epithelial autografts was first reported in 1981.[4] Similarly, cultured epithelial allografts have been demonstrated to reduce pain and to accelerate healing of chronic cutaneous wounds.[5] Cultured epidermal keratinocytes may be combined with collagen–based substrates as a vehicle for transplantation and to promote repair of connective tissue.[6] However, cultured keratinocytes used

alone are associated with greater fragility and bullae formation than the combination of cells with a collagen implant.[7,8] This report presents preliminary results of treatment of chronic skin wounds with cultured skin substitutes. The skin substitutes consist of collagen–glycosaminogly-can (GAG) sponges populated with cultured epi-dermal keratinocytes and dermal fibroblasts from allogeneic donors. Complete healing was obtained in two of four patients, partial healing in one, and no healing in one. Patients with com-plete healing were able to resume normal activi-ties, and healed wounds did not recur after more than one year of observation. These results sug-gest that cultured skin substitutes prepared from allogeneic human skin may provide an alterna-tive treatment for acceleration of healing in select-ed kinds of chronic wounds.

Materials and Methods

Cultured skin substitutes (CSS) were prepared from cultured skin cells and collagen–GAG sub-strates as previously described.[6] Dermal substi-tutes were prepared *in vitro* from bovine collagen and chondroitin–6–sulfate by freeze–drying[9] and sterilized gamma–irradiation. Separate cultures of human epidermal keratinocytes and dermal fibroblasts were prepared by isolation from human cadaveric skin obtained from the Ohio Valley Tissue and Skin Bank after confirmation of negative tests for transmissible pathogens.

Patients were enrolled by informed consent into a protocol approved by the Institutional Review Boards of the University of Cincinnati and the Jewish Hospitals of Cincinnati. All wounds treated were on the distal lower extremi-ty. Medical histories were obtained from subjects, and examinations were performed for evaluation of vascular competence. A summary of patient information is presented in Table 1. Wound areas ranged from 10–184 cm². Etiologies of wounds were peripheral vascular disease (ML), venous stasis (SM and JT), and dehiscence after saphe-nous veinectomy for coronary artery bypass surgery (JY). Patients were examined for perfu-sion of the affected leg by detection of dorsalis pedis pulse. Two patients (ML and SM) in whom no pulse was detected were further evaluated by Ankle/Brachial Pressure Ratios (A/BPR), and imaging of the affected leg by Impedance Plethysmography (IP) as described in Table 1. Duration of the wounds ranged from three to

Figure 3. *Histology of cultured skin substitute (CSS). CSS consists of a collagen–glycosaminoglycan sponge inoculated with human epidermal keratinocytes and dermal fibroblasts. CSS resembles split–thickness skin graft in total thickness and in separation of epithelial and connective tissues into distinct compartments. Scale bar = 0.1mm.*

twelve months. Patient JY received one applica-tion of CSS, patients ML and JT received two applications, and patient SM received three appli-cations.

Chronic wounds were debrided to viable sub-cutaneous tissue. CSS were overlaid with a porous, non–adherent dressing (N–Terface™; Winfield Laboratories). Cultured grafts were removed from dishes and transferred with N–Terface as a support and stapled to the wound. Grafts were dressed with cotton gauze and Op–Site™ (Smith & Nephew United), and CSS were irrigated with essential nutrients containing broad spectrum antimicrobials[10, 11] four times daily for seven days. On day seven, all dressings and staples were removed, and grafts were exposed to air for 30–60 minutes to dry the graft surface. Between days seven and fourteen, wounds were dressed with Adaptic™ (Johnson & Johnson Medical, Inc.) containing equal parts Neosporin™ (E. Fougera & Co.), Bactroban™ (Smith Kline–Beechum), and Nystatin™ (Schein Pharmaceuticals, Inc.) ointments. Grafted wounds were examined at two to three days and one week post surgery, and periodic intervals thereafter.

Results

Figure 3 shows the histologic appearance of cultured skin substitutes as used in this study. The porous side of the collagen–GAG sponge is populated with cultured human fibroblasts, and the non–porous side is covered with a stratified culture of epidermal keratinocytes. The total thickness of the cell–biopolymer implant is less

TABLE 1
Patient Information and Outcomes

Patient ID	ML	SM	JY	JT
Gender/Age	Female, 73	Male, 64	Male, 65	Female, 67
Wound Area (cm²)	36	184	10	20
Etiology	Peripheral vascular disease	Venous stasis	Saphenous veinectomy	Venous stasis
Associated Disease	Diabetes	Post phlebitic syndrome	Diabetes	Post phlebitic syndrome
Dorsalis Pedis Pulse	No*	No*	Yes	Yes
A/BPR	Rt, 0.84; **Lt, 0.60**	**Rt, 0.79**; Lt, 1.14	N/A	N/A
IP	Normal	Bilateral venous thrombosis	N/A	N/A
Wound Duration	4 months	12 months	3 months	12 months
Treatments	Debridements CSS X 2	Debridements CSS X 3, HBO$_2$	Debridement CSS X 1	Debridements CSS X 2, HBO$_2$
Outcomes	85% healed at 4 weeks after CSS #1 applied	10% healed at 1 week	60% healed at 1 week	50% healed at 4 weeks after CSS #2 applied
	Healed completely at 8 months after CSS #2 applied	3 applications of CSS failed. Amputation after 3 months	Healed completely at 4 weeks	
Recurrence	No	N/A	No	Yes

* Ankle/Brachial Pressure Ratios (A/BPR) and Impedance Plethysmography (IP) were performed only on patients in whom no Dorsalis Pedis Pulse was detected. A/BPR values in boldface type indicate treated side.

HBO$_2$ – Hyperbaric Oxygen

than 0.5mm, and epidermal and dermal components are organized similarly to a split–thickness skin graft.

Table 1 summarizes outcomes after treatment with CSS of three venous stasis and one diabetic leg ulcers. Patient ML healed approximately 85% of the wound area within five weeks of the first application of CSS. A second application of CSS to the remaining open area promoted complete closure of the wound by three months after grafting. Figure 4 shows clinical photographs of patient ML. At one week (Figure 4B), a translucent epithelium covers the wound. Epithelial coverage of the wound is accomplished over the majority of the wound surface within three weeks

after application (Figure 4C). In addition to epithelial closure of the wound, the patient reported reduced pain that allowed resumption of normal ambulation. Cosmetic appearance of the healed wound was very acceptable by eight weeks after grafting (Figure 4D).

SM received three applications of CSS, and all failed. Each set of CSS had initial adherence and epithelial survival during irrigations with nutrients and antimicrobials. After irrigations were discontinued on day seven, CSS degenerated rapidly with no healing of the wound by one month. After failure of all conventional and experimental alternatives for closure of this large wound, SM elected below–the–knee amputation.
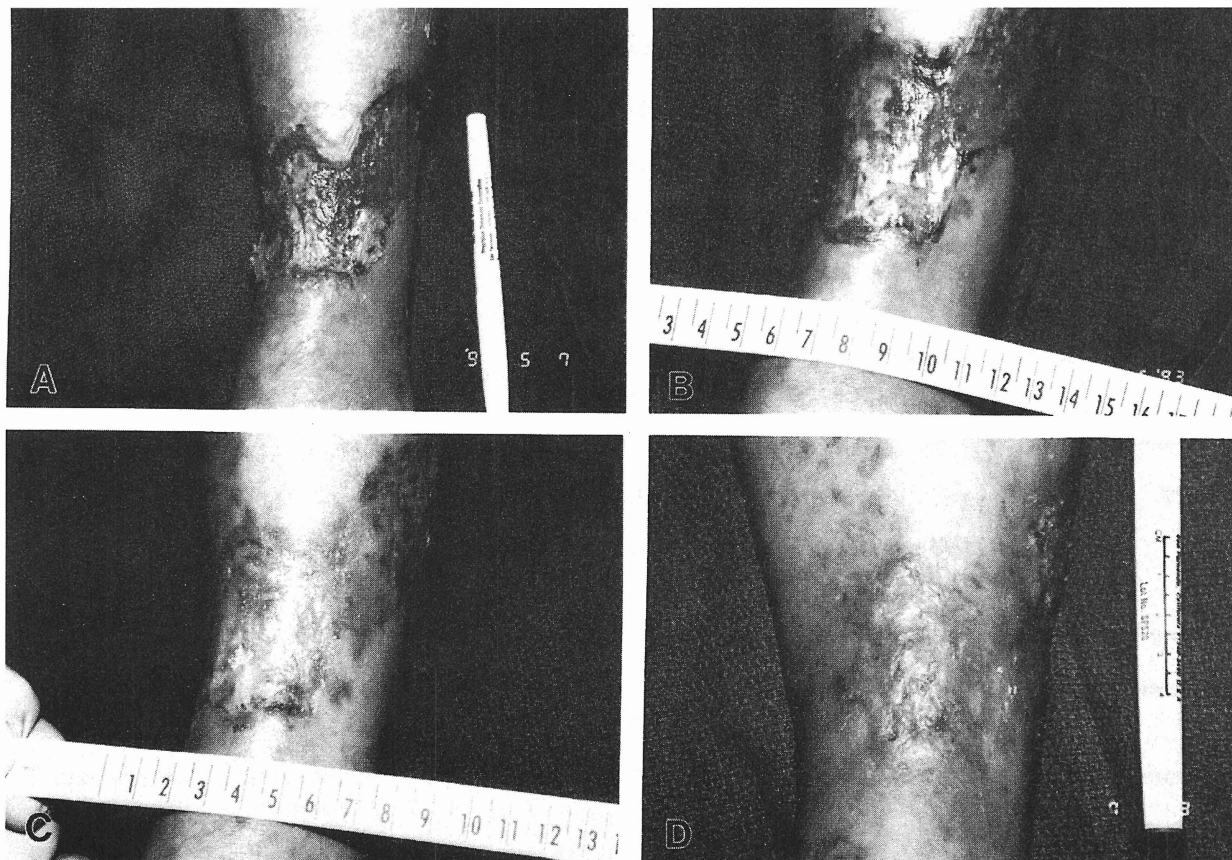
Figure 4. *Clinical photographs showing closure of a chronic diabetic ulcer with CSS. A) Chronic wound persisting for four months before treatment. B) One week after grafting with CSS shows development of a thin, translucent epithelium over the wound surface. C) By three weeks after grafting, stable epithelium has restored the protective barrier function of epidermis over >80% of the wound surface. D) After eight weeks, the wound is fully healed, and the patient can ambulate freely without pain.*

JY received one application of CSS to a non–healing, dehiscent wound subsequent removal of the saphenous vein for cardiac bypass. Complete healing was accomplished within four weeks, with no recurrence after one year. JT received two applications and had approximately 50% healing within four weeks after the second application. Gradual recurrence of the treated wound was associated with poor compliance with wound care protocols and continued use of tobacco and alcohol.

Discussion

Data presented here suggest that grafts of cultured allogeneic skin cells and biopolymers may accelerate healing of qualified chronic wounds. Qualifications of wounds include, but are not limited to, sufficient perfusion of the affected site and adequate debridement to viable tissue.

Additional qualifications of patient behavior are equally important and include compliance with wound care protocols, adequate nutrition, and management of associated disease. Two patients treated in this pilot study achieved wound closure in a shorter period of time than the duration of their wounds before treatment with CSS. This result indicates that CSS facilitated wound closure and may accelerate healing. However, determination of accelerated healing requires comparative investigation in a larger population of patients.

Closure of chronic wounds of large surface area within relatively short periods of time after grafting, e.g., one month, strongly suggests that transplanted allogeneic cells engraft directly. However, long term closure of chronic wounds is believed to result from replacement of allogeneic cells by autologous keratinocytes.[12] Within three to four months after grafting, no allogeneic ker-

atinocytes have been found, but wounds remain healed. This consistent finding in grafting of allogeneic keratinocytes suggests gradual replacement of allogeneic cells by circumferential ingrowth of autologous cells from the wound perimeter. This mechanism is consistent with the biological process termed "creeping substitution" in which parenchymal cells from the host repopulate the graft site, but allogeneic cells are not destroyed by T–cell cytotoxic action of the recipient. Reduced immunogenicity of cultured skin substitutes containing allogeneic keratinocytes is believed to result from depletion of donor leukocytes during culture of keratinocytes for transplantation.

Development of cell therapies will depend on culture and banking of allogeneic skin cells. Although use of cultured cells for transplantation is not regulated presently by FDA,[13] new guidelines for use of allogeneic skin tissue also apply to cells isolated from these tissues.[14] As with other experimental therapies, safety and efficacy of prospective therapies will be required. For cells cultured from allogeneic tissues, requirements for safety will include negative tests for viral and microbial pathogens, and no detectable disease after autopsy. Demonstration of efficacy requires comparative assessments of experimental therapies with prevailing standards of care to determine equivalence or superiority.[3] It may be expected that continued preclinical and clinical studies with allogeneic cells will lead to improvements in care of chronic wounds. Establishment of standards and guidelines for allogeneic cells may be expected to serve as a foundation for transplantation of genetically modified cells for targeted therapy of wounds or metabolic diseases. With these goals ahead, the findings of this study provide an important demonstration that advanced therapies with allogeneic cells will provide new alternatives to healing of chronic skin wounds.

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