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Principles and practices for treatment of cutaneous wounds with cultured skin substitutes

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Abstract

Background: Skin substitutes prepared from cultured skin cells and biopolymers may reduce requirements for donor skin autograft, and have been shown to be effective in treatment of excised burns, burn scars, and congenital skin lesions.

Data Sources: Cultured skin substitutes (CSS) generate skin phenotypes (epidermal barrier, basement membrane) in the laboratory, and restore tissue function and systemic homeostasis. Healed skin is smooth, soft and strong, but develops irregular degrees of pigmentation. Quantitative analysis demonstrates that CSS closes 67 times the area of the donor skin, compared to less than 4 times for split-thickness skin autograft.

Conclusions: CSS reduce requirements for donor skin autograft for closure of excised, full-thickness cutaneous wounds, and demonstrate qualitative outcome that is not different from meshed, split-thickness autograft. These results offer reductions in morbidity and mortality for the treatment of burns and chronic wounds, and for cutaneous reconstruction.

Keywords: Burns; Cultured skin; Wound closure; Skin grafts

Engineering of skin substitutes provides a prospective source of advanced therapies for treatment of acute and chronic skin wounds. Hypothetically, engineering of skin substitutes can allow deliberate fabrication of biologic materials with properties that address specific pathobiologic conditions (e.g. burns, scars, cutaneous ulcers, congenital anomalies). By design and incorporation of specific therapeutic properties in skin substitutes, reduction of morbidity and mortality from full-thickness skin wounds may be facilitated. Morbidity from grafting of autologous, split-thickness skin [1] occurs at both the treatment site and the donor site [2]. Acute wounds that require grafting include excised burns, burn scars, and congenital cutaneous anomalies (i.e. giant nevus). Patients with acute wounds, in general, do not have healing impairment, but may not have sufficient donor sites to cover their wounds if large total body surface areas (TBSA) are involved. Estimates for hospitalizations from burns range from 60,000 to 80,000 annually, and costs for recovery from acute injuries range from US$36,000 to 117,000 per patient [3–5]. Increased availability of skin grafts would prospectively provide advantages over conventional therapy including, but not limited to: reduction of donor site area required to close wounds permanently; reduction of surgical procedures and hospitalization time; grafting of patients who are poor candidates for donation of skin grafts; reduction of mortality and morbidity from scarring; and delivery of genetically modified cells [6–10].

The ultimate objective for skin substitutes is restoration of the anatomy and physiology of uninjured skin after treatment and healing of the wound. At present, only an autologous full-thickness skin grafts, free flaps or pedicle flaps [11,12] restore all of the structures and functions of uninjured skin but donor sites and treatment sites must be equal in size. Tissue expanders can stretch skin by an approximate factor of 2, but are associated with complications including rupture or infection of the expander, and necrosis of expanded skin before transplantation [13]. Meshed split-thickness skin grafts may be expanded by ratios of 1:4 with characteristic scarring of mesh interstices. Skin substitutes that contain cultured cells can provide large quantities of grafts for wound treatment, but restore only a subset of anatomic structures and physiologic functions of skin. Therefore, the full potential for engineering of skin substitutes has not yet been realized.

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Table 1
Comparisons of cell types in native, engineered, and grafted skin

Tissue type	Cell type	Uninjured skin	Split-thickness autograft	Engineered skin substitutes	Healed skin after grafting
Epidermis	Keratinocytes	+	+	+	+
	Hair follicle	+	−	−	−
	Sebaceous gland	+	−	−	−
	Sweat gland	+	−	−	−
	Melanocytes	+	±	±	±
	Immune cells	+	+	−	+
	Nerve	+	+	−	±
Dermis	Fibroblasts	+	+	±	+
	Endothelial cells	+	+	−	+
	Smooth muscle	+	+	−	±
	Immune cells	+	+	−	+
	Nerve	+	+	−	±

Requirements

Anatomic and physiologic

Uninjured skin performs a wide variety of protective (barrier, UV light absorption, immune surveillance, mechanical), perceptive (touch, temperature, pain), and regulatory (thermal, hydration, excretory) functions that maintain the homeostasis of the human body with the terrestrial environment [14,15]. Skin performs these functions by integration of epidermis and dermis that transduce energy though cellular and extracellular mechanisms to provide information to the brain for appropriate responses. Epidermal cells consist predominantly of keratinocytes (95%–97%) and adnexal cells (glands, hair, nails), but also include melanocytes, dendritic cells (i.e. Langerhans cells) of the immune system, and sensory structures of nerves (i.e. Merkel cells). Dermal cells include vascular components (endothelium, smooth muscle), fibroblasts, nerve cells (temperature, pain), immune-response cells (mast cells, histiocytes), and pilo-erector muscles. Most of the dermis consists of extracellular matrix (collagens, elastin, reticulin, polysaccharides) that provide the majority of the mechanical strength to the skin. Epidermis contains only very small volumes of extracellular matrix consisting predominantly of carbohydrate polymers [16] and stratum corneum lipids [17,18] that organize as a liquid crystal to form a barrier to permeability of aqueous fluids. Although the extracellular matrices of dermis and epidermis are essential to the function of skin, all extracellular matrices are synthesized by cells and organized into a correct anatomy. Because morbidity is defined and characterized by loss of tissue structure and function, full recovery of skin function may not be expected without full restoration of all cell types in engineered skin substitutes. Although cells from the wound bed are a source of fibro-vascular tissue, closure of full-thickness wounds requires transplantation of epithelial skin cells. However, epithelial closure alone does not restore full skin function. Therefore, complete restoration of skin function and medical recovery requires restoration of all types of skin cells from the graft and/or the wound, in an anatomically correct structure (Table 1). For example, no engineered model of skin substitute contains a functional vascular plexus, although experimental models have been described [19,20]. Consequently, both the mechanism and time for revascularization of engineered skin substitutes are protracted compared to grafts of native skin. This anatomic deficiency in skin substitutes causes them to become ischemic and nutrient-deprived after grafting that contribute to graft failure and secondary wound infection.

Surgical

The historic standard for rapid closure of full-thickness wounds with a skin substitute is split-thickness, autologous skin applied either as a sheet [21], or expanded by meshing [22]. Recovery of function and cosmesis is acceptable and allows return of patients to productive roles in society [23]. Successful outcome of skin grafts requires: adherence to wounds; histocompatibility; control of fluid loss and infection; absence of antigenicity and toxicity; mechanical stability and compliance; cost effectiveness; and availability [24,25]. Therefore, these requirements must also be satisfied by skin substitutes prepared by tissue engineering. However, anatomic deficiencies in engineered skin substitutes decrease the probability that these requirements can be met. Consequently, complications with use of skin substitutes prepared by laboratory fabrication compared to split-thickness skin graft have included, but have not been limited to: reduced rates of engraftment [26], increased microbial contamination [27,28], mechanical fragility [29], increased time to healing [30], increased regrafting, and very high cost [31] (Table 2). These complications may increase rather than decrease risks to patient recovery. Therefore, use of engineered skin substitutes may only be justified for use as an adjunctive therapy in cases without other alternatives until efficacy comparable to split-thickness skin is demonstrated. Nonetheless, complications with early models of

Table 2
Clinical limitations and considerations for use of engineered skin

Limitation	Consideration
Mechanical fragility	Special dressings and nursing care
Susceptibility to microbial contamination	Non-cytotoxic topical antimicrobial agents
Decreased rates of engraftment	Increased regrafting
Increased time to heal	Delay of recovery
Very high cost	Resource allocation

Table 3
Components of engineered skin

Dermal substitutes	Epidermal substitutes
Autologous cultured fibroblasts	Autologous cultured keratinocytes
Allogeneic cultured fibroblasts	Allogeneic cultured keratinocytes
Collagen-GAG, collagen gel	Thin epidermal graft
Acellular cadaveric skin matrix	Epidermal suction blisters
PLA/PGA, PEO/PBT	Epidermal cell suspensions

GAG = glycoaminoglycan; PLA = poly-lactic acid; PGA = poly-glycolic acid; PEO = polyethylene oxide; PBT = polybutylene terephthalate.

skin substitutes do not preclude their long-term potential for medical advantages in wound care.

Principles for tissue engineering of human skin

The capability to engineer skin substitutes removes constraints for the structure and function of skin for transplantation. Hypothetically, color, texture, pliability, tensile strength, barrier, matrix or cytokine expression may be altered by modification of the composition of engineered skin [32,33]. However, for the purpose of this discussion it is assumed that the ultimate standard of comparison for engineered skin is complete restoration of the anatomy and physiology of uninjured human skin. After this goal has been reached, modifications of the native structures and functions can be evaluated for whether any advantage is conferred to the recipient.

Components

Cells, biopolymers, and soluble mediators constitute the anatomy and physiology of human skin. Definitive to wound closure is restoration of epidermal barrier to provide protection from fluid loss and infection. Barrier is synthesized by the parenchymal cells of the epidermis, the keratinocytes [14]. Sheets of cultured keratinocytes were studied by many investigators for treatment of excised, full-thickness burns [34–38], and a consensus was reached that replacement of connective tissue was also required [29,39]. Fibrovascular tissue restores the mechanical strength and blood supply to attach and nourish epidermis. Therefore, repopulation of fibroblasts, endothelial cells and smooth muscle is required to form stable skin. Connective tissue cells may repopulate grafts from the wound bed, but multiple models of engineered skin also include cultured fibroblasts to facilitate predictable repair of connective tissue in treated wounds [28,40,41]. Pigment cells, the melanocytes, have also been cultured and transplanted for treatment of vitiligo [42] and burn scars [43], and added into cell-polymer constructs [44,45]. Nerve cells may extend dendrites into healing grafts of engineered skin, but full restoration of skin sensation has not been demonstrated either with split-thickness skin grafts or engineered skin [46,47]. Glands (sweat, sebaceous) and hair follicles have been transplanted experimentally [48,49], but neither engineered skin nor skin autografts restore these structures at present. Consequently, thermal regulation after healing of wounds treated with engineered skin is also deficient. However, these deficiencies do not reduce the importance of engineered skin for definitive closure of wounds, and therapeutic benefits to patients. Table 3 summarizes current materials used for engineered skin substitutes [50]. These materials range from cultured parenchymal cells (autologous or allogeneic) to tissue derivatives (i.e. acellular dermal matrix) to synthetic polymers (i.e., poly-lactic/poly-glycolic acid, polyethylene oxide/polybutylene terephthalate). Combinations of dermal and epidermal substitutes have also been reported to effectively close excised, full-thickness burns [51,52]. Commercial products and experimental models for dermal and/or epidermal repair have been configured from individual and combined materials as shown in Table 4.

Process

Engineering of skin substitutes implies deliberate design and fabrication according to specific functional objectives. Fabrication requires a process to result in a composition of matter that meets the design specifications. For engineering of tissues, including skin, recapitulation of ontogenesis would result in correct structures and functions. During ontogenesis, skin develops by sequential processes of: cytogenesis, morphogenesis, histogenesis, and organogenesis. However, recapitulation of ontogenesis in vitro is not currently possible. Conversely, the phenotype expressed by human skin cells in culture resembles most closely the wound healing physiology [53] which includes cytogenesis, morphogenesis, and histogenesis, but not organogenesis. Therefore, guidance of cultured cells to reiterate the wound healing process confers the greatest probability to restore those anatomic structures of skin that define wound closure. Organogenesis of skin, in which glands, follicles and nerve develop, does not occur during post-natal wound healing. Furthermore, scarring is characteristic of post-natal wound healing, but in minimal or absent in utero [54]. Table 5 summarizes the process steps for engineering of skin substitutes.

Table 4
Engineered skin substitutes*

Trademark name	Source	"Dermis"	"Epidermis"
EpiCel™	Genzyme Tissue Repair	Allodermis	Cultured auto HK
Integra™	Integra Life Sciences	Collagen-GAG & silicone	Thin autograft
AlloDerm™	LifeCell Corporation	Acellular dermal matrix	Thin autograft
DermaGraft™	Advanced Tissue Sciences	PGA/PLA+allo HF	Thin autograft
n/a	Univ Cincinnati/Shriners	Collagen-GAG+auto HF	Cultured auto HK
LaserSkin™	Fidia Biopolymers (Italy)	Hayluronic acid	Cultured auto HK
PolyActive™	HC Implants (The Netherlands)	PEO/PBT+auto HF	Cultured auto HK
ApliGraf™	Organogenesis, Inc.	Collagen gel+allo HF	Cultured allo HK
ORCEL™	Ortec International, Inc.	Collagen + allo HF	Cultured allo HK
TransCyte™	Advanced Tissue Sciences	Allo HF	BioBrane™

* The list of products is presented as neither all-inclusive, exclusive, or an endorsement.

Cytogenesis

Increased numbers of parenchymal cells are required to repopulate wounds and restore skin structure. Selective culture of keratinocytes, melanocytes, fibroblasts, and endothelial cells stimulates increases of cell populations as an exponential function, $(P_I)\ (2^n) = (P_F)$, where P_I is the initial population, n is the number of population doublings, and P_F is the final population [55]. By this function, a population of cells increases in number by an approximate factor of 1×10^6 in 20 generations. Growth rate, or doubling time, is the number of population doublings divided by the incubation time [55]. With an approximate doubling time of 1 day or less for keratinocytes and fibroblasts, very large populations of skin cells can be prepared in 2–3 weeks of culture. Therefore, rapid growth of cells in culture provides a fundamental basis for generation of tissue substitutes for skin repair. For keratinocytes, culture in serum-containing [56] or serum-free media [57] are practiced commonly.

Morphogenesis

After preparation of large populations of skin cells, organization into skin substitutes increases anatomic fidelity to native skin. Cultured human keratinocytes may be combined with dermal substitutes in vitro [58–60], and exposed to the air to stimulate epithelial stratification and cornification [61–63]. This culture condition provides a polarized environment with nutrient medium contacting the dermal substitute, and air contacting the epidermal substitute. Keratinocytes respond to this gradient by orienting proliferating cells toward the medium and cornified cells toward the air to reestablish the morphology of a stratified, squamous epithelium. Fibroblasts fill the biopolymer substrate, begin to degrade it and generate new extracellular matrix. Two biologic changes result from formation of skin substitutes that contain very high cell densities. First, the proliferation rates of the cells decreases by approximately an order of magnitude from the maximum rate of log-phase, subconfluent cells in selective culture. Correspondingly, the nutritional requirements per cell decrease. However, because skin substitutes may contain 10–100 fold more cells per unit area than selective cultures, the nutritional requirements of the entire population may increase. Second, increase of cell density causes an increase in concentration of secreted factors by cells in the tissue substitute. Higher concentrations of secreted factors often confers independence from exogenous growth factors in culture medium [64,65], and continued addition of mitogens under conditions of high cell densities may result in cytotoxicity. Keratinocytes and fibroblasts are known to secrete a wide variety of cytokines including inflammatory mediators, growth factors, matrix polymers and catabolic enzymes [32]. Combination of epithelial and mesenchymal cells may allow paracrine mechanisms between cell types to begin to operate. An example is the synthesis of competence factors (e.g. PDGF, TGF-α, bFGF) by keratinocytes, and progression factors (e.g. IGFs) by fibroblasts to support cell proliferation [66]. These and other factors are believed to stimulate the mechanisms of action for healing of skin wounds [67]. The same mechanisms have been demonstrated by topical applications of pure growth factors [68] produced by recombinant technologies [69,70]. However, cellular synthesis and delivery of these factors by engineered skin provides a continuous supply, and may regulate delivery of factors according to mechanisms endogenous to the wound.

Table 5
Process of engineering of skin substitutes

Process step	Definition	Current models
Cytogenesis	Exceed capacity of wounds to generate cells	+
Morphogenesis	Organize cells and matrix into an analog of skin	+
Histogenesis	Form stable and functional tissue in vivo	±
Organogenesis	Restoration of all functions of uninjured skin	−

Histogenesis

At present, no models of engineered skin substitutes reproduce the anatomy, physiology or biologic stability of uninjured skin. Stable recovery of skin function occurs only

after grafting, vascularization and healing of engineered grafts according to in vivo mechanisms. This step in the process of tissue engineering requires survival and engraftment of cells into the wound. Therefore, skin substitutes must respond to the regulatory mechanisms in the wound to restore function. In healing-competent wounds, skin substitutes must respond to the inflammatory process, and integrate with fibrovascular tissue to support grafted epithelium. If skin substitutes engraft, healed epithelium will develop by two weeks after application. Clinical characteristics of healed epithelium include repellence of water, suppression of granulation tissue, and capillary blanching and refill after punctate depression. Increases of epithelial area later than two weeks after grafting are attributable to secondary outgrowth of transplanted epithelium, and cannot be considered as engraftment.

Organogenesis

Recovery of all functions of uninjured skin is a current goal of tissue repair. However, neither split-thickness skin grafts nor engineered skin substitutes accomplish this goal at present. Only transplantation of full-thickness skin can restore organotypic functions including perspiration, hair growth and normal pigmentation [11,12]. Because development of epidermal appendages occurs in utero, but not in wound healing, these structures may be transplanted but cannot yet be prepared from postnatal cells after selective culture.

Clinical considerations and assessment

Multiple factors of clinical care can be decisive in whether or not skin repair results from treatment of wounds with engineered skin substitutes. Modification of care protocols for debrided, full-thickness wounds must compensate for the anatomic and physiologic deficiencies in alternative materials for skin repair. Currently available skin substitutes are avascular, slower to heal than skin autograft, and may be mechanically fragile. Among the factors that impact outcome with engineered skin are wound bed preparation, control of microbial contamination, dressings and nursing care, and survival of transplanted cells during vascularization of grafts.

Surgical considerations

Clinical complications with engineered skin result predominantly from anatomic and physiologic deficiencies that compromise responses to the wound healing process. Split-thickness skin graft contains a vascular plexus and adheres debrided wounds by coagulum, followed by inosculation of vessels in the graft to vessels in the wound within 2–5 days. Although healing is not complete within one week, native skin is engrafted and reperfused. In comparison, engineered skin substitutes with dermal and epidermal components are avascular, and reperfusion results from de novo angiogenesis. If the rate of vascularization is considered constant, then the time required for reperfusion is directly proportional to the thickness of the dermal component of the skin substitute, and is longer than reperfusion of split-thickness skin. The additional time required for vascularization may cause epithelial loss from microbial destruction and/or nutrient deprivation. Attachment of cultured epithelium to the dermal substitute in vitro confers an advantage to this kind of skin substitute because both epidermal and dermal components are applied in a single surgical procedure similarly to skin autograft. Biopolymers in skin substitutes are adsorbed, and cells reform functional skin tissue. Alternatively, dermal and epidermal components of skin substitutes may be applied in two stages with application of a dermal substitute followed by vascularization, and then an autologous epidermal substitute [39,71,72]. This approach increases the frequency of blood vessels and density of extracellular matrix in the graft bed, and has been reported to improve efficacy of cultured keratinocyte sheets. However, it requires two surgical procedures to accomplish permanent wound closure.

Topical antimicrobial agents have been shown to be more effective for control of wound contamination than parenteral antimicrobials [73]. Requirements for any topical antimicrobial include effective coverage of a broad spectrum of gram-negative and gram positive bacteria as well as common fungal contaminants. In burns these groups are represented most frequently by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. In addition, topical antimicrobials must have low histotoxicity to allow healing to proceed. It is also important that mechanisms of action of topical agents not overlap with parenteral drugs used for treatment of sepsis. If mechanisms overlap and resistant organisms develop against the topical agents, subsequent sepsis from a resistant organism may be untreatable. For example, if aminoglycosides are routinely used for parenteral treatment of sepsis, then they should not be candidates for topical use. Silver compounds (i.e. silver sulfadiazine, silver nitrate) are highly toxic because they act by precipitation of chloride from biological material, although very low concentrations have been reported noncytotoxic by McCauley et al. [74]. Parallel assays of cellular toxicity and antimicrobial activity were performed by Lineaweaver et al. [75], and determined concentrations of agents that were noncytotoxic, and retained antimicrobial activity. Kuroyanagi et al. [76] showed concentration-dependent inhibition of fibroblast growth by silver sulfadiazine in a two-point growth assay. This assay format has been adapted for determination of concentration-dependent response of proliferation of keratinocytes and fibroblasts to candidate agents for use as topical antimicrobials [77,78]. These studies have identified individual agents, and formulations of multiple agents that are not inhibitory to proliferation of keratinocytes and fibroblasts [79], and that remain effective against common wound organisms [80]. Investigative formulations

for management of microbial contamination include neomycin and polymyxin B for gram-negative organisms; mupirocin for gram-positive bacteria; and, nystatin or amphotericin B for fungi [79]. Quinolone drugs (i.e. norfloxacin, ciprofloxacin) may be added to broaden coverage of bacteria [78], if they are not part of the routine parenteral therapy for bacteremia, septicemia or sepsis. However, formulations of multiple antimicrobials for topical use are generally not available commercially as approved drug therapies.

In addition, the high costs of cultured skin substitutes remains a practical factor for clinical use. Estimates of cost of keratinocyte sheets range from $1,000 to $13,000/% body surface area covered [31,81]. Those costs can approximately double if a dermal substitute is also used [71,82]. Therefore, costs can become limiting for treatment of large (60–90%) total body surface area burns with cultured skin substitutes. At present, no studies have convincingly demonstrated a savings of total hospitalization costs by use of cultured skin substitutes of any kind. For contemporary treatment of burns, cultured skin substitutes remain an important adjunct to conventional skin grafting [29], but are not a primary modality of wound closure except in extreme cases.

Nursing considerations

Mechanical fragility of cultured skin grafts is an important source of failure from shear and maceration. For friable grafts, mechanical reinforcement may be added with a backing material that allows convenient handling and stapling to the wound. Cultured epithelial autografts are routinely attached to petrolatum-impregnated gauze for surgical application [83], but this material is not compatible with wet dressings. Alternatively, composite skin substitutes may be handled and stapled to wounds with a backing of N-Terface™ (Winfield Laboratories; Richardson, TX), a nonadherent, relatively strong and highly porous material [28,84]. A similar porous, non-adherent dressing called Surfasoft™ (Mediprof; Amsterdam, Holland) has also been used in Europe as a backing for cultured epithelial autografts by Teepe and colleagues [85]. Porous dressings allow both delivery of topical solutions, and drainage of wound exudate from grafts during the period of engraftment. To avoid mechanical disturbance, frequency of dressing changes is low (1–3) during the first week, and increases in frequency as mechanical strength of grafts increases after fibrovascular tissue and epidermal barrier develop. With attention to these surgical and nursing factors, closure of excised, full-thickness burns can be accomplished with reduction of requirements for donor skin autograft [86].

Assessment

After treatment of wounds with engineered skin substitutes, outcome must be measured to determine whether benefits of a prospective therapy justify any risks associated with the therapy, and whether risks associated are reduced for the disease being treated. Assessment may range from the level of the individual (e.g. survival) [4], to function (e.g. range of motion, return to work) [87], to tissue integrity (epithelial closure, scar formation) [1,88], to cellular and molecular markers (e.g. cell phenotypes, synthesis of proteins and nucleic acids) [35]. Although specific studies may collect quantitative data for assessment of outcome, routine practices of surgery and dermatology depend most heavily on the examination of a pathologic condition by the trained eye of the clinician. Clinical examination integrates multiple properties in the wounds according the perceptions of the physician. The Vancouver Scale for assessment of burn scar provides an ordinal score for properties of skin including pigmentation, vascularity, pliability, and scar height [89]. Similar comparative scales have been developed for engineered skin substitutes that show no statistical difference from skin autograft at one year after grafting [90]. These kinds of semi-quantitative scales provide a relative comparison for evaluation, but they are inherently subjective and dependent on the examiner. In addition, endpoints for quantitative assessments must consider sources of error. Wound area has been shown to correlate negatively with engraftment of cultured keratinocyte sheets [30]. Nonetheless, most studies with keratinocyte sheets quantify efficacy as “% take” [26]. However, Rue an coworkers [31] have illustrated astutely that superimposition of the negative correlation between wound area and % take introduces substantial error into the interpretation of a prospective clinical benefit. Conversely, endpoints without confounding variables such as “% TBSA covered” provide a more accurate measurement for assessment of efficacy of engineered skin substitutes.

Alternatively, objectivity may be increased by assessment of wounds with non-invasive instruments that measure biophysical properties in skin including size, vascular perfusion, epidermal barrier, pliability, color, and surface pH (Table 6). These instruments assess individual biophysical parameters of skin, and may be used to standardize the normal, healthy condition. Depending on the dermatologic disease, one or more of the parameters will be outside the normal range. In extreme conditions, such as full-thickness skin wounds, virtually all of the biophysical properties of skin are outside of the normal range, and can easily be distinguished statistically from uninjured skin. Measurement of surface electrical capacitance with a dermal phase meter confirms restoration of the definitive property of epidermal barrier, but it does not predict functional recovery. Nonetheless, this instrument is easily transferred to clinical assessment of wound closure for comparison of engineered skin and skin autograft [91]. Similarly, pigmentation of wounds treated with engineered skin substitutes can be measured quantitatively and kinetically with the chromameter, but it does not assess scar formation. Therefore, multiple parameters of skin function must be measured to quantify

Table 6
Noninvasive biophysical instruments for assessment of skin conditions

Cutaneous property	Endpoint	Biophysical instrument
Size/Shape	Area, volume	Planimetry, castings, laser scanner
Vascular perfusion	Blood flow, blood gases	Laser Doppler, transcutaneous PO_2, PCO_2
Epidermal barrier, surface hydration	Surface capacitance, trans-epidermal water loss	Dermal phase meter, evaporimeter
Visco-elasticity	Stress/strain, historesis	Cutometer, dermal torque meter
Color	Visible spectrum, grayscale	Chromameter, laser scanner
Heat	Temperature	Infra red camera
pH	Acid mantle of skin surface	Surface pH meter

whether epidermal barrier, blood flow, mechanical strength, and pigmentation are statistically distinguishable from uninjured skin. This kind of multiparameter, quantitative index is required for practice of dermatology in certain European countries [92], but is not yet part of skin assessment in the United States. Development of diagnostic and prognostic guidelines for noninvasive, biophysical instrumentation will require validation of individual endpoints for single assessments, followed by interrelation of individual endpoints according to multivariate mathematics. Several individual instruments have been validated including computer-assisted planimeters, laser Doppler flow meters, dermal phase meters, and instruments for in situ measurement of viscoelastic properties. However, integration of multiple instruments to substitute for the clinical examination has not yet been completed. Accomplishment of the goal may prospectively allow development of absolute standards for wound assessment that can be applied universally. Establishment of standards for assessment of wound healing with noninvasive biophysical instrumentation may proceed by the kind of disciplinary consensus used currently for chronic wounds [93].

Clinical experience

Cultured skin substitutes have been applied successfully for treatment of burns, chronic wounds, and reconstructive surgery of the skin. Each of these medical indications requires specific considerations to optimize the outcome after surgery. However, as described above, factors in the graft and factors in the wound affect greatly the quantitative and qualitative outcome of skin repair after grafting of CSS. Up-regulation of angiogenesis, basement membrane formation, and epidermal barrier, with simultaneous down-regulation of microbial growth, mechanical shear, and chemical cytotoxicity to the graft are essential for engraftment and survival of CSS. Attention to these factors provides an environment that promotes rapid and stable closure of cutaneous wounds.

Burns and burn scars

The most extensive experience with autologous CSS has been in treatment of burns of greater than 50% of the TBSA. Progressive improvement of the anatomy and physiology of CSS has correlated well with increased efficacy of wound closure. Favorable qualitative results were obtained by combination of cultured keratinocytes and fibroblasts with polymeric dermal substitutes [28]. Development of an irrigation solution consisting of nutrients and noncytotoxic antimicrobial agents increased rates of engraftment to about 50% [90]. Improvements to epidermal barrier and basement membrane by addition of ascorbic acid to the incubation medium further increased engraftment to greater than 80%. Another important advance was realized by the successful combination of auto-CSS with the dermal substitute, Integra Artificial Skin™ (Integra LifeSciences Corporation, Plainsboro, NJ) [51]. It was found that auto-CSS attach rapidly by connections between the “neo-dermis” of the Integra™ (Integra LifeSciences Corporation; Plainsboro, NJ), and the dermal substitute of the auto-CSS, and generate greater than 90% engraftment. These advances demonstrate the importance of formulation of experimental hypotheses on clinical responses to investigational therapies. By following the clinical needs of the patient for management of the wound, and following the natural composition of skin tissue as the model for CSS, the response of the wound to the graft is optimized and the clinical performance of the graft reaches therapeutic efficacy. Fig. 1 shows a pediatric patient with 86% burns who was covered over more than 50% of her TBSA with autologous CSS grafts. The healed skin is smooth, pliable, and strong. The anterior trunk [Fig. 1(A)] was treated with cadaveric allograft as a temporary cover, and the posterior trunk [Fig. 1(B)] was excised early and treated with Integra Artificial Skin™. The very smooth surface on the back is attributed to the use of Integra™ as a graft base. No epidermal blistering occurs after grafting due to the development of basement membrane in the laboratory. Protective skin barrier develops within one week after grafting due to the development of stratum corneum in CSS in vitro. Because the skin cells can be cryopreserved for several years in liquid nitrogen, additional CSS can be prepared at a later date with no additional donor site harvesting. Serial tracings of grafted areas over extended periods of time have demonstrated that skin healed with auto-CSS continue to grow as pediatric patients grow. This finding illustrates that connective tissue which develops

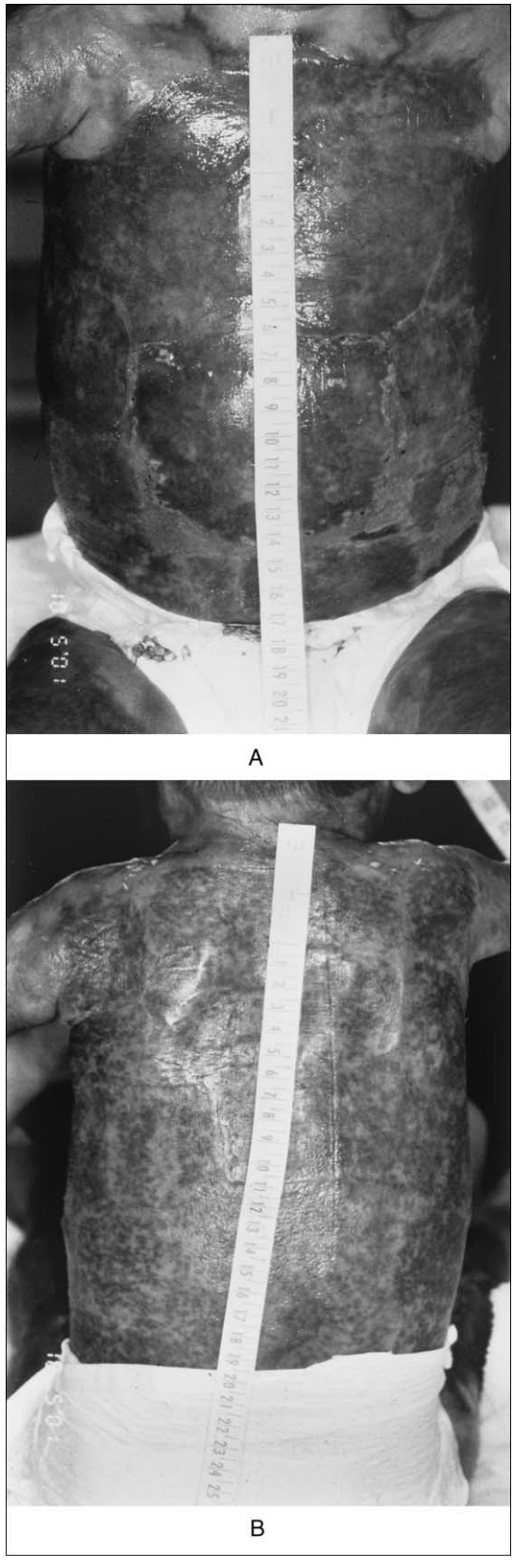

Fig. 1. Qualitative outcome at two months after grafting of excised, full-thickness burns with autologous cultured skin substitutes (CSS) in a patient with 86% total body surface injuries. (A) Anterior trunk grafted after treatment with cadaveric allograft prior to CSS. (B) Back grafted after treatment with Integra Artificial Skin™ show a very smooth surface which is attributed to uniform and rapid epithelial closure after development of a uniform layer of connective tissue by Integra™. Scale in centimeters.

after grafting of CSS may be distinguished from contracted bands of collagenous scar.

Chronic wounds

Chronic cutaneous ulcers have also been treated effectively with CSS in preliminary studies [94]. In this application, wounds are smaller than burns, and can be treated with CSS prepared from allogeneic cells which require no donor skin from the recipient. However, transplantation of allogeneic cells in any form requires compliance with regulations for pathogen testing before transplantation, and tracking of patients who receive the allografts. The allogeneic cells are known not to persist in the wound for more than a few weeks. Therefore, the mechanism of healing is indirect stimulation of connective and epithelial tissues from the wound base and perimeter. This is contrasted sharply with the direct healing of full-thickness wounds with auto-CSS. Two benefits are derived from closure of chronic wounds with allo-CSS. First, the patient receives a direct benefit from closure of the wound with relief of pain and disability. Second, the restoration of function reduces or eliminates the needs for in-home care and social services related to the patient's medical needs.

Giant nevus

Auto-CSS have also been studied in treatment of elective cases of reconstructive surgery, such as congenital giant nevus. These lesions require excision of the full-thickness skin to subcutaneous fat or fascia. Because these subcutaneous tissues are less well vascularized than dermis, the application of cadaveric allograft for up to one week has been found to stimulate vascular development in the wound bed. Also, because these wounds are surgical wounds generated by elective procedures, the issue of microbial contamination is negligible. Consequently, with proper quality assurance of the CSS grafts, efficacy of closure of the wounds is greater than 90% at postoperative day 14 at which time the patient may be discharged home. Fig. 2 shows a pediatric patient with a giant nevus covering her entire back, and extending anterior onto flanks, thorax, and abdomen. The left back had been treated previously with unmeshed autograft skin from the scalp [Fig. 2(A)]. The remainder of the lesion on the back was excised, and treated with auto-CSS. Healing before discharge at postoperative day 15 was greater than 95%, and no regrafting was required. The skin was stable, soft, and strong at one year after surgery [Fig. 2(B)], but had irregular pigmentation. The lesion on the flanks and anterior surfaces was removed

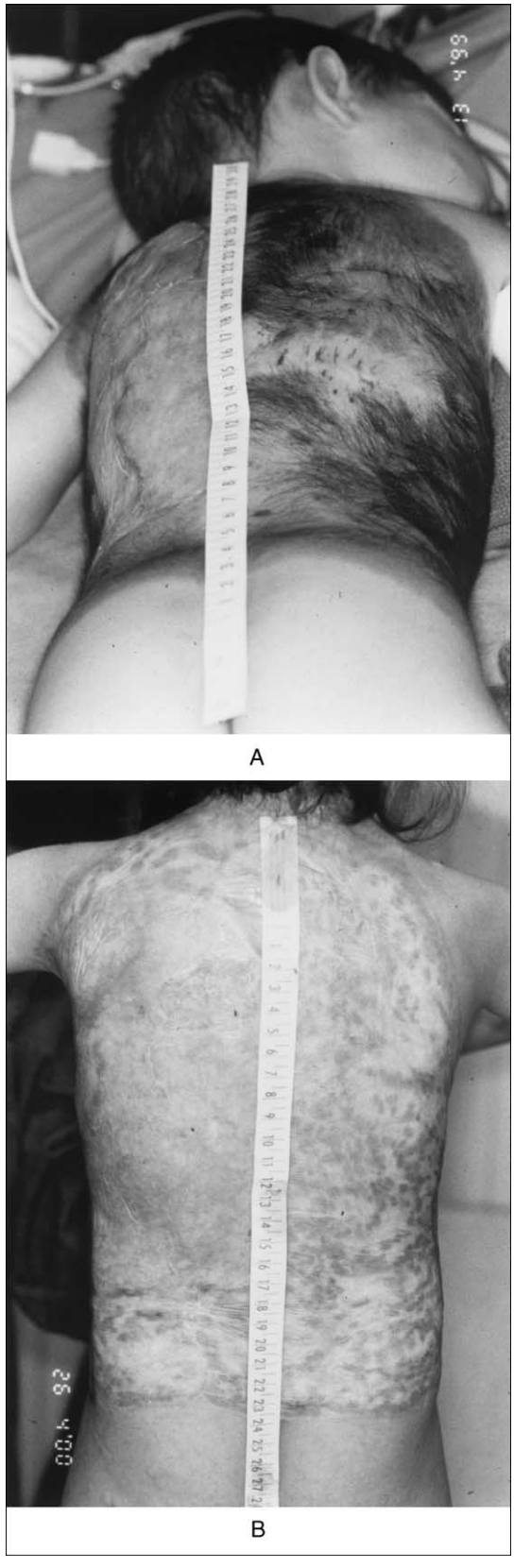

Fig. 2. Effective treatment of a giant congenital nevus with autologous CSS. (A) Partial treatment of the left back with unmeshed autograft from the scalp. The lesion on the back was excised, grafted for one week with cadaver allograft, followed by autologous cultured skin substitutes (CSS). (B) One year after treatment, skin healed with CSS is soft, strong and has irregular pigmentation. The remaining lesion on the flanks was treated with CSS prepared from cryopreserved cells which eliminated the requirement for donor skin in the second procedure. Scale in centimeters.

several months later, and treated with CSS made from cells that were cryopreserved at the time of the first procedure. This case demonstrates the comparable qualitative result of auto-CSS to sheet autograft skin, and the reduction in donor skin required to complete the removal of this extensive cutaneous lesion by the use of auto-CSS. These medical benefits constitute a reduction in morbidity from cutaneous wounds that require skin grafting.

Conclusions and future directions

As anatomy and physiology of engineered skin substitutes improve, they will become more homologous to native skin autograft. Improvement of skin substitutes will result from inclusion of additional cell types and from modifications of culture media, biopolymer substrates and physical environment (i.e. humidity, mechanical tension, electrical properties) that promote greater fidelity to native skin. Better homology may be expected to reduce stringency for clinical use of cultured skin substitutes, and accomplish the efficacy of skin autograft. After predictable efficacy is shown with autologous cells, successful models will become platforms for testing of chimeric grafts, and genetically modified cells [6–8,10,95,96]. Gene therapy for treatment of local or systemic conditions is feasible with cultured skin substitutes [8,9,95,97–99]. For wound healing applications, transient expression of selected gene products may be best, whereas, constitutive gene expression may be required for systemic deficiencies. Delivery of gene products systemically probably will require physiologic regulation to be efficacious [100].

As engineering of skin and other tissues makes a transition from research to clinical practice, then members of its community will become compelled to think and act like engineers. Therefore, like other engineering disciplines, uniform standards for quantitative analysis of cultured skin substitutes must be established for evaluation of materials composition and performance. This goal will require interdisciplinary review and development of consensus. For this purpose, instrument-based, noninvasive assessment of outcome will be as important as materials analysis. Instrumental assessments may include measurement of epidermal barrier [91,101], blood flow [102], pigmentation and erythema [103,104], visco-elastic properties [105,106], and surface texture [107]. Establishment of these engineering standards will provide a platform from which validation and introduction of skin and other tissue substitutes will be expedited.

Although the term "tissue engineering" describes a relatively new interdisciplinary field, it has its roots many years past in the fields of cell culture and polymer chemistry [108–110]. If past progress in engineering of cultured skin substitutes is an indication of progress ahead, it is easy to predict the reduction of medical morbidity and mortality by use of engineered skin substitutes.

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