Skin substitutes from cultured cells and collagen-GAG polymers

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Abstract—*Engineering skin substitutes provides a potential source of advanced therapies for the treatment of acute and chronic wounds. Cultured skin substitutes (CSS) consisting of human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates have been designed and tested in preclinical and clinical studies. Cell culture techniques follow general principles of primary culture and cryopreservation in liquid nitrogen for long-term storage. Biopolymer substrates are fabricated from xenogeneic (bovine) collagen and glycosaminoglycan that are lyophilised for storage until use. At maturity in air-exposed culture, CSS develop an epidermal barrier that is not statistically different from native human skin, as measured by surface electrical capacitance. Preclinical studies in athymic mice show rapid healing, expression of cytokines and regulation of pigmentation. Clinical studies in burn patients demonstrate a qualitative outcome with autologous skin that is not different from 1:4 meshed, split-thickness autograft skin, and with a quantitative advantage over autograft skin in the ratio of healed skin to biopsy areas. Chronic wounds resulting from diabetes or venous stasis have been closed successfully with allogeneic CSS prepared from cryopreserved skin cells. These results define the therapeutic benefits of cultured skin substitutes prepared with skin cells from the patient or from cadaver donors. Future directions include genetic modification of transplanted cells to improve wound healing transiently or to deliver gene products systemically.*

Keywords—*Cultured skin substitutes, Wound healing, Keratinocytes, Fibroblasts, Biopolymers*

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1 Objectives

ENGINEERING SKIN substitutes provides a potential source of advanced therapies for the treatment of acute and chronic skin wounds. Hypothetically, the engineering of skin substitutes can allow deliberate fabrication of biological materials with properties that address specific patho-biological conditions (e.g. burns, scars, cutaneous ulcers, congenital anomalies). Through the design and incorporation of specific therapeutic properties in skin substitutes, reduction in morbidity and mortality from full-thickness skin wounds may be facilitated. Morbidity from the grafting of autologous, split-thickness skin (ROBSON *et al.*, 1992) occurs at both the treatment site and the donor site (MCHUGH *et al.*, 1997).

Acute wounds that require grafting include excised burns, burn scars and congenital cutaneous anomalies (giant naevus). 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 (TBSAs) are involved.

Estimates for hospitalisations from burns range from 60000 to 80000 annually, and costs for recovery from acute injuries range from US$36000 to 117000 per patient (PRUITT and MASON, 1996; BRIGHAM and MCLOUGHLIN, 1996; SAFFLE *et al.*, 1995). Pressure ulcers in the United States are estimated to have a prevalence of 9.2% in hospitalised patients (BERGSTROM and BRADEN, 1992; MEEHAN, 1990) and to incur an annual cost of treatment exceeding US$1.3 billion (MILLER and DELOZIER, 1994).

Increased availability of skin grafts would prospectively offer advantages over conventional therapy including, but not limited to: reduction in the donor site area required to close wounds permanently; reduction in surgical procedures and hospitalisation time; grafting of patients who are poor candidates for donation of skin grafts; reduction in mortality and morbidity from scarring and chronic wounds; and delivery of genetically modified cells (FENJVES *et al.*, 1994; KRUEGER *et al.*, 1994; EMING *et al.*, 1996; MORGAN *et al.*, 1987). Skin substitutes that contain cultured cells can provide large quantities of grafts for wound treatment, but restore only a subset of the anatomical structures and physiological functions of skin. Therefore the full potential of the engineering of skin substitutes has not yet been realised.

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2 Principles of tissue engineering of skin

The capability to engineer skin substitutes removes constraints on the structure and function of skin for transplantation.

Hypothetically, colour, texture, pliability, tensile strength, barrier, matrix or cytokine expression can be altered by modification of the composition of engineered skin (BOYCE, 1996*a*; 1997). 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 on the recipient.

2.1 *Components*

Cells, cytokines and the extracellular matrix constitute the anatomy and physiology of human skin. Definitive to wound closure is restoration of the epidermal barrier to provide protection from fluid loss and infection. The barrier is synthesised by the parenchymal cells of the epidermis, the keratinocytes (ELIAS, 1983).

Sheets of cultured keratinocytes have been studied by many investigators for treatment of excised, full-thickness burns (COLEMAN and SIWY, 1992; HERNDON and RUTAN, 1992; CLUGSON *et al.*, 1991; COMPTON *et al.*, 1989; GALLICO III *et al.*, 1984), and a consensus was reached that replacement of connective tissue was also required (DESAI *et al.*, 1991; CUONO *et al.*, 1987). Fibrovascular tissue restores the mechanical strength and blood supply to attach and nourish the epidermis. Therefore repopulation of fibroblasts, endothelial cells and smooth muscle is required to form stable skin. Connective tissue cells can 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 (PARENTEAU *et al.*, 1992; HANSBROUGH *et al.*, 1992*a*; *b*; BOYCE *et al.*, 1993*a*; *b*; *c*). Pigment cells, the melanocytes, have also been cultured and transplanted for treatment of vitiligo (LERNER *et al.*, 1987) and added to cell-polymer constructs (SWOPE *et al.*, 1997; BOYCE *et al.*, 1993*c*).

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 (WARD *et al.*, 1989; WARD and TUCKETT, 1991). Glands (sweat, sebaceous) and hair follicles have been transplanted experimentally (JAHODA *et al.*, 1996), 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 1 summarises the current materials used for engineered skin substitutes (BOYCE, 1997). These materials range from culture parenchymal cells (autologous or allogeneic) to tissue derivatives (i.e. xenogeneic collagens, acellular dermal matrix) to synthetic polymers (i.e. polylactic/polyglycolic acid, polyethylene oxide/polybutylene terephthalate). The results described here combined cultured human keratinocytes, fibroblasts and melanocytes with substrates made from lyophilised bovine collagen and glycosaminoglycan (GAG) (BOYCE *et al.*, 1988; 1990; 1993*c*).

2.2 *Process*

Engineering 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 the 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, complete 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 (CLARK, 1991), which includes cytogenesis, morphogenesis, and histogenesis, but not organogenesis. Therefore stimulation of cultured cells *in vitro* to express the wound-healing physiology offers the greatest probability of restoring those anatomical structures of skin that define wound closure. Organogenesis of skin, in which glands, follicles and nerves develop, does not occur during post-natal wound healing. Furthermore, scarring is characteristic of post-natal wound healing, but is minimal or absent *in utero* (ADZICK and LORENZ, 1994). Table 2 summarises the process steps for engineering skin substitutes.

2.2.1 *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 in 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 (BOYCE and HAM, 1983). By this function, a population of cells increases in number by an approximate factor of 1×10^6 in 20 generations. With an

Table 1 Materials for engineered skin substitutes

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
Polylactic acid/polyglycolic acid; polyethylene oxide/polybutylene terephthalate	epidermal cell suspensions

Table 2 Process of engineering skin substitutes

Process step	Definition	Current models
Cytogenesis	exceed capacity of wounds to generate cells	+
Morphogenesis	organise cells and matrix into an analogue of skin	+
Histogenesis	form stable and functional tissue *in vivo*	±
Organogenesis	restoration of all functions of uninjured skin	−

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 (RHEINWALD and GREEN, 1975) or serum-free media (BOYCE and HAM, 1985) is practised commonly.

2.2.2 *Morphogenesis:* After preparation of large populations of skin cells, organisation into skin substitutes increases anatomical fidelity to native skin (Fig. 1). Cultured human keratinocytes can be combined with collagen-GAG *in vitro* (SAINTIGNY *et al.*, 1993; PARENTEAU *et al.*, 1991; SLIVKA *et al.*, 1993; BOYCE and HANSBROUGH, 1988) and exposed to the air to stimulate epithelial stratification and cornification (PONEC *et al.*, 1995; PRUNIERAS *et al.*, 1983; BOYCE and WILLIAMS, 1993; BERTHOD and DAMOUR, 1997; BERTHOD *et al.*, 1996, 1997). This culture condition provides a polarised environment with nutrient medium contacting the dermal substitute, and air contacting the epidermal substitute. Keratinocytes respond to this gradient by orienting proliferating cells towards the medium and cornified cells towards the air to re-establish the morphology of a stratified, squamous epithelium. Fibroblasts fill the biopolymer substrate, begin to degrade it and generate a new extracellular matrix.

Two biological changes result from the formation of skin substitutes that contain very high cell densities. First, the proliferation rate 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. Secondly, an increase in cell density causes an increase in the concentration of factors secreted by cells in the tissue substitute. Higher concentrations of secreted factors often confer independence from exogenous growth factors in the culture medium (CHEN *et al.*, 1995; BOYCE *et al.*, 1993*b*), and the continued addition of mitogens under conditions of high cell densities can 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 (BOYCE, 1996*b*).

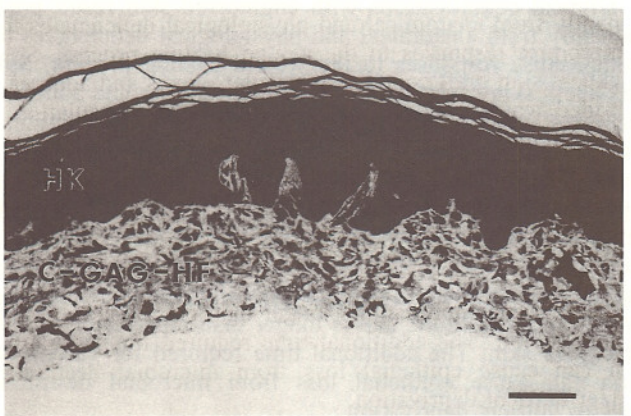

Fig. 1 *Photomicrograph of cultured skin substitute. Cultured human keratinocytes (HK) in vitro organise to form stratified squamous epithelium attached to dermal substitute composed of cultured human fibroblasts and collagen-GAG substrate (C-GAG-HF). Scale bar = 100 μm*

A 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 (STILES *et al.*, 1979). Cellular synthesis and delivery of these factors by engineered skin provide a continuous supply, and may regulate the delivery, of factors according to mechanisms endogenous to the wound.

2.2.3 *Histogenesis:* At present, no models of engineered skin substitutes reproduce the anatomy, physiology or biological stability of uninjured human skin. Stable recovery of skin function occurs only after grafting, vascularisation and healing of engineered grafts according to *in vivo* mechanisms. This step in the process of tissue engineering requires the 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 in the epithelial area later than two weeks after grafting are attributable to secondary outgrowth of transplanted epithelium and cannot be considered as engraftment.

2.3.4 *Organogenesis:* The 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 (ISENBERG and PRICE, 1996; MAST and NEWTON, 1996). Because development of epidermal appendages occurs *in utero*, but not in wound healing, these structures can be transplanted but cannot yet be prepared from post-natal cells after selective culture.

3 Preclinical studies

The histologic similarity of cultured skin substitutes *in vitro* to native skin (AUGUSTIN *et al.*, 1997) may satisfy an observer that certain cutaneous structures are reformed, but it does not confirm that wound healing will be accomplished. To demonstrate wound healing, athymic mice have provided an important grafting model of human skin substitutes for several laboratories (HIGOUNENC *et al.*, 1994; HANSBROUGH *et al.*, 1992*a*; EMING *et al.*, 1996; FENJVES *et al.*, 1989; BANKS-SCHLAGEL and GREEN, 1980). Cultured skin substitutes as described here have been grafted orthotopically (BOYCE *et al.*, 1991), inoculated with melanocytes to generate pigment (BOYCE *et al.*, 1993*c*) and tested for degradation of collagen-GAG (HARRIGER *et al.*, 1996) and the efficacy of topical antimicrobials (BOYCE *et al.*, 1997*a*). Expression of pigment in cultured epithelium after grafting has been shown by multiple groups. This laboratory has regulated pigmentation in cultured skin substitutes by cytometric sorting of keratinocyte populations to remove pigmented melanocytes. Subsequently, pigment was restored by inoculation of melanocytes into skin substitutes.

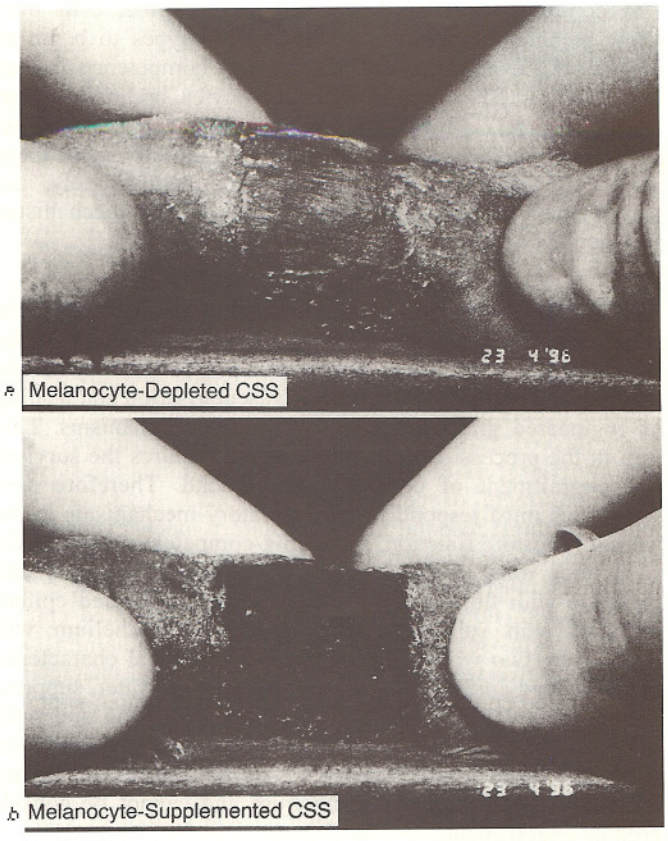

Fig. 2 *Photographs of athymic mice with regulated pigmentation in cultured skin substitutes (CSS). (a) Melanocyte-depleted CSS; (b) melanocyte-supplemented CSS*

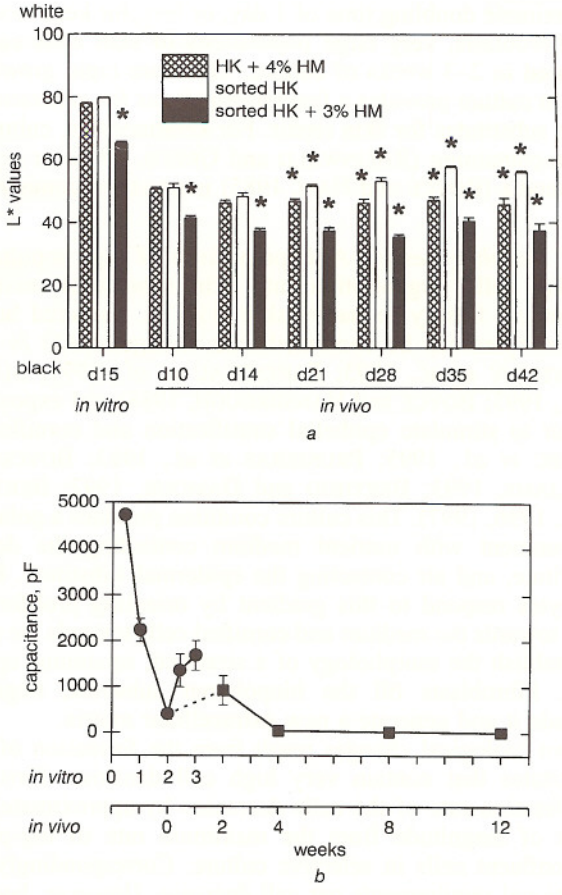

Fig. 3 *Non-invasive biophysical assessment of wound healing with CSS in athymic mice. (a) Plot of L* values (grey-scale) from serial measurements with Minolta Chromameter 300. Intensity of pigmentation is inversely proportional to L* values. (*) $P < 0.05$. (b) Plot of surface electrical capacitance from serial measurements with NOVA Dermal Phase Meter 9003. Epidermal barrier is inversely proportional to barrier formation and becomes stable after grafting. (–●–) CSS in vitro; (– – –) extrapolated values; (–■–) CSS in vivo*

An example of hypo- and hyper-pigmented CSS on athymic mice is shown in Fig. 2. Although this degree of pigmentation is easily distinguished, pathological aberrations in skin pigmentation may be more subtle. To increase objectivity in assessment, skin pigmentation can be measured with a biophysical instrument* (SWOPE *et al.*, 1997) (Fig. 3*a*). By this kind of analysis, statistical differences can be determined between pigment *in vitro* and *in vivo*, as well as kinetic changes in an individual or a population of subjects. Biophysical instruments can also be used to evaluate the surface hydration of grafted skin that represents formation of a dry, keratinised epithelium. These measurements can be performed with a meter† (BOYCE *et al.*, 1996) (Fig. 3*b*) to quantify kinetic rates of epithelial healing.

4 Clinical studies

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 anatomical and physiological 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 the outcome with engineered skin are wound bed preparation, control of microbial contamination, dressings and nursing care, and survival of transplanted cells during vascularisation of grafts.

4.1 *Surgical considerations*

Clinical complications with engineered skin result predominantly from anatomical and physiological deficiencies that compromise responses to the wound healing process. Split-thickness skin graft contains a vascular plexus and adheres to debrided wounds by coagulum, followed by inosculation of vessels in the graft to vessels in the wound within 2–5 days. In comparison, engineered skin substitutes with dermal and epidermal components are avascular, and reperfusion results from *de novo* angiogenesis. If the rate of vascularisation 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 vascularisation can cause epithelial loss from microbial destruction and/or nutrient deprivation.

Irrigation of wounds with topical nutrients (BOYCE *et al.*, 1995*c*) promotes engraftment of cultured cell-biopolymer grafts by transient nutritional support during vascularisation. Attachment of cultured epithelium to the dermal substitute *in vitro* confers an advantage on composite skin substitutes,

* i.e. Minolta Chromameter

† Nova Dermal Phase Meter

because both epidermal and dermal components are applied in a single surgical procedure, similar to skin autograft. Biopolymers in skin substitutes are adsorbed, and cells reform functional skin tissue.

Topical antimicrobial agents have been shown to be more effective for control of wound contamination than parenteral antimicrobials (MONAFO and WEST, 1990). Requirements for any topical antimicrobial agent 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 do not overlap with parenteral drugs used for the 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 sulphadiazine, silver nitrate) are highly toxic, because they act by precipitation of chloride from biological material, although very low concentrations have been reported non-cytotoxic (MCCAULEY *et al.*, 1989). Parallel assays of cellular toxicity and antimicrobial activity have been performed (LINEAWEAVER *et al.*, 1985, DAMOUR *et al.*, 1992) and have determined concentrations of agents that were non-cytotoxic and retained antimicrobial activity. KUROYANAGI *et al.* (1991) showed concentration-dependent inhibition of fibroblast growth by silver sulphadiazine in a two-point growth assay. This assay format has been adapted for determination of the concentration-dependent response of proliferation of keratinocytes and fibroblasts to candidate agents for use as topical antimicrobials (BOYCE *et al.*, 1995*d*; BOYCE and HOLDER, 1993). These studies have identified individual agents and formulations of multiple agents that are not inhibitory to proliferation of keratinocytes and fibroblasts (BOYCE *et al.*, 1995*e*) and that remain effective against common wound organisms (HOLDER, 1989).

Investigative formulations for the 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 (BOYCE *et al.*, 1995*e*). Quinolone drugs (i.e. norfloxacin, ciprofloxacin) can be added to broaden the coverage of bacteria (BOYCE *et al.*, 1995*d*), if they are not part of the routine parenteral therapy for bacteraemia, septicaemia or sepsis. However, formulations of multiple antimicrobials for topical use are generally not available commercially as approved drug therapies.

4.2 *Nursing considerations*

The mechanical fragility of cultured skin grafts is an important source of failure from shear and maceration. For friable grafts, mechanical reinforcement can 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 (COMPTON, 1993), but this material is not compatible with wet dressings. Alternatively, composite skin substitutes can be handled and stapled to wounds with a backing of N-Terface, a non-adherent, relatively strong and highly porous material (BOYCE *et al.*, 1993*a*; HANSBROUGH *et al.*, 1989). A similar porous, non-adherent dressing called Surfasoft has also been used in Europe as a backing for cultured epithelial autografts by TEEPE *et al.* (1990). Porous dressings allow both delivery of topical solutions and drainage of wound exudate from grafts during the period of engraftment. To avoid mechanical disturbance, the frequency of dressing changes is low (1–3), during the first week, and increases in frequency as the mechanical strength of the 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 a reduction in the requirements for donor skin autograft (Fig. 4). Similar results have been obtained in preliminary studies on the treatment of chronic wounds with skin substitutes containing allogeneic skin cells (BOYCE *et al.*, 1995*a*).

4.3 *Assessment*

After treatment of wounds with engineered skin substitutes, the outcome must be measured to determine whether the benefits of a prospective therapy justify any risks associated with the therapy, and whether associated risks are reduced for the disease being treated. Assessment may range from the level of the individual (e.g. survival) (SAFFLE *et al.*, 1995) to function (e.g. range of motion, return to work) (AMERICAN MEDICAL ASSOCIATION, 1993) and from tissue integrity (epithelial closure, scar formation) (SPANN *et al.*, 1996; ROBSON *et al.*, 1992) to cellular and molecular markers (e.g. cell phenotypes, synthesis of proteins and nucleic acids) (COMPTON *et al.*, 1989). Although specific studies may collect quantitative data for the assessment of outcome, routine practices of surgery and dermatology depend most heavily

Fig. 4 *Outcome at four months after grafting of full-thickness burns with engineered skin substitutes. Healed skin after treatment with CSS is smooth and supple, with formation of linear scars at junctions between grafts. Pigmentation has uneven distribution. Area of wounds healed with CSS was 197 times area of biopsy from which CSS was prepared*

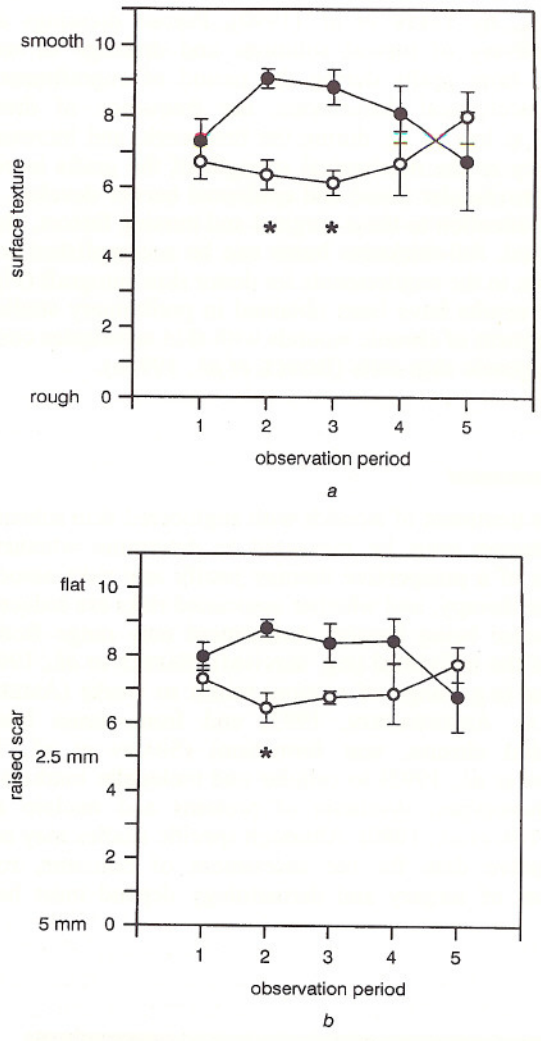

Fig. 5 *Assessment of outcome with engineered skin substitutes. (○) Skin autograft; (●) skin substitute; (*) $p < 0.05$. (a) Ordinal scoring of surface texture shows engineered substitutes have statistically smoother surface texture 2–6 months after grafting (observation periods 2 and 3), and no difference by one year (observation period 5). (b) Ordinal scoring of raised scar showed no statistical differences between skin substitutes and skin autograft by one year after grafting (observation period 5). (Reprinted with permission from* BOYCE *et al. (1995b))*

on the examination of a pathological condition by the trained eye of the clinician. Clinical examination integrates multiple properties in the wounds according to the perceptions of the physician. The Vancouver scale for burn scars provides an ordinal score for skin properties including pigmentation, vascularity, pliability and scar height (SULLIVAN *et al.*, 1990). Similar comparative scales have been developed for engineered skin substitutes that show no statistical difference from skin autograft at one year after grafting (Figs. 5*a* and *b*) (BOYCE *et al.*, 1995*b*). 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 (WILLIAMSON *et al.*, 1995). Nonetheless, most studies with keratinocyte sheets quantify efficacy as 'percentage take' (ODESSEY, 1992). However, RUE *et al.* (1993) have illustrated astutely that superimposition of the negative correlation between wound area and percentage take introduces substantial error into the interpretation of a prospective clinical benefit. Conversely, endpoints without confounding variables such as 'percentage TBSA covered' provide a more accurate measurement for the assessment of the efficacy of engineered skin substitutes.

Alternatively, objectivity can be increased by assessment of wounds with non-invasive instruments that measure biophysical properties in skin, including size and shape, vascular perfusion, epidermal barrier, pliability, colour, heat and surface pH (Table 3). These instruments assess the individual biophysical parameters of skin and can be used to standardise the normal, healthy condition. Depending on the dermatological disease, one or more of the parameters will be outside the normal range. In extreme conditions, such as full-thickness skin wounds, virtually all the biophysical properties of skin are outside the normal range and can easily be distinguished statistically from uninjured skin.

Measurement of surface electrical capacitance with a dermal phase meter confirms the restoration of the definitive property of the epidermal barrier, but it does not predict functional recovery. Nonetheless, this instrument is easily transferred to the clinical assessment of wound closure for comparison of engineered skin and skin autograft (GORETSKY *et al.*, 1995) (Fig. 6), but it does not assess scar formation. Mechanical pliability can also be quantified in healed skin using the dermal torque meter as a parameter of extensibility and recovery (BOYCE *et al.*, 1997*b*) (Fig. 7). Therefore, multiple parameters of skin function must be measured to quantify whether epidermal barrier, blood flow, mechanical strength and pigmentation are statistically distinguishable from uninjured skin. This kind of multi-parameter, quantitative index is required for practice of dermatology in certain European countries (SEIDENSCHNUR, 1995), but is not yet part of skin assessment in the United States.

Development of diagnostic and prognostic guidelines for non-invasive, biophysical instrumentation requires validation of individual endpoints for single assessments, followed by interrelation of individual endpoints according to multi-variate mathematics. Several individual instruments have been validated, including computer-assisted planimeters, laser Doppler flowmeters, dermal phase meters, infrared cameras and instruments for *in situ* measurement of visco-elastic properties. However, integration of multiple instruments to substitute for clinical examination has not yet been completed. Accomplishment of the goal may potentially allow development of absolute standards for wound assessment that can be applied

Table 3 Non-invasive biophysical instruments for assessment of skin conditions

Cutaneous property	Endpoint	Biophysical instrument
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, transepidermal water loss	dermal phase meter, evaporimeter
Visco-elasticity	stress/strain, historesis	cutometer, dermal torque meter
Colour	visible spectrum, grey-scale	chromameter, laser scanner
Heat	temperature	infrared camera
pH	acid mantle of skin surface	surface pH meter

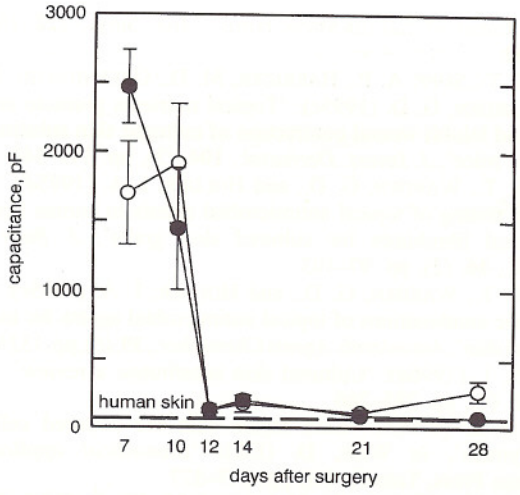

Fig. 6 *Non-invasive biophysical assessment of epidermal barrier in CSS. Surface electrical capacitance of wound closure with (–●–) cultured skin substitutes and (–⊖–) skin autograft shows no statistical differences in time to repair of epidermal barrier. (Reprinted with permission from* GORETSKY *et al. (1995))*

universally. The establishment of standards for assessment of wound healing with non-invasive biophysical instrumentation may proceed by the kind of disciplinary consensus used currently for chronic wounds (LAZARUS *et al.*, 1994).

5 Conclusions and future directions

As the anatomy and physiology of engineered skin substitutes improve, they will become more homologous to native skin graft. 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 have more fidelity to native skin. Better homology of cultured skin substitutes to skin autograft can be expected to reduce stringency for clinical use and to accomplish greater efficacy. After predictable efficacy is shown with autologous cells, successful models will become platforms for testing chimeric grafts and genetically modified cells (EMING *et al.*, 1995; 1996; MORGAN *et al.*, 1987; KRUEGER *et al.*, 1994).

Gene therapy for treatment of local or systemic conditions is feasible with cultured skin substitutes (LU *et al.*, 1996; EMING *et al.*, 1995; KRUEGER *et al.*, 1994; FENJVES *et al.*, 1989; 1994; VOGT *et al.*, 1994). 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 systematically will probably require physiological regulation to be efficacious (BOYCE, 1994).

As the engineering of skin and other tissues makes a transition from research to clinical practice, then members of the clinical community will be compelled to think and act like engineers. Therefore, as with other engineering disciplines, uniform standards for quantitative analysis of cultured skin substitutes must be established for the evaluation of materials' composition and performance. This goal will require interdisciplinary review and development of consensus. For this purpose, instrument-based, non-invasive assessment of outcome will be as important as material analysis.

Instrumental assessments may include measurement of epidermal barrier (BOYCE *et al.*, 1996; GORETSKY *et al.*, 1995), blood flow (ATILES *et al.*, 1995), pigmentation and erythema (KOLLIAS and BAGER, 1988; FEATHER *et al.*, 1989), visco-elastic properties (MATSUZAKI *et al.*, 1995; AGACHE *et al.*, 1980) and surface texture (MCQUISTON and WHITESTONE, 1995). Establishment of these engineering standards will provide a platform from which the 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 (LYMAN, 1968; ANDERSON and GIBBONS, 1974; HILLEMAN, 1990). If past progress in the engineering of cultured skin substitutes is an indication of progress ahead, it is easy to predict the reduction of medical morbidity and mortality through the use of engineered skin substitutes.

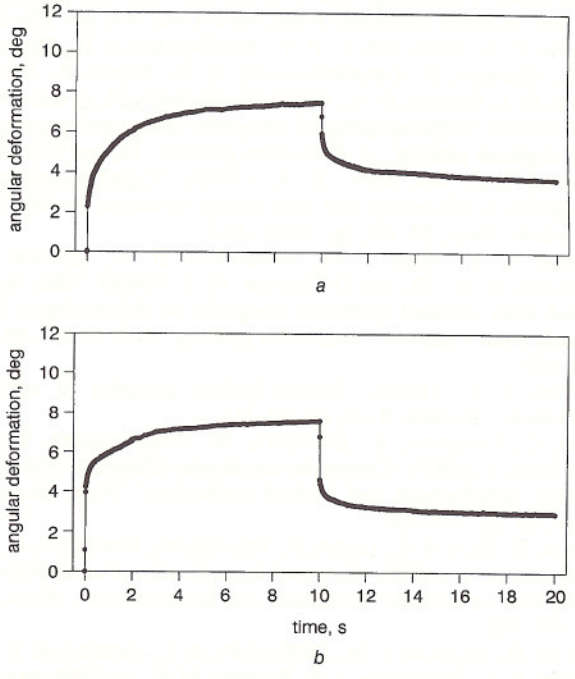

Fig. 7 *Non-invasive biophysical assessment of visco-elastic properties in CSS. Mechanical assessment of healed skin in situ with dermal torque meter shows that skin extensibility (t = 0–10 s) and recovery (t = 11–20 s) of (a) healed CSS at 1 year after grafting is within range of (b) uninjured skin on volar forearm of a healthy volunteer*

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