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Engineered skin substitutes: practices and potentials

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Abstract Wound healing can be problematic in several clinical settings because of massive tissue injury (burns), wound healing deficiencies (chronic wounds), or congenital conditions and diseases. Engineered skin substitutes have been developed to address the medical need for wound coverage and tissue repair. Currently, no engineered skin substitute can replace all of the functions of intact human skin. A variety of biologic dressings and skin substitutes have however contributed to improved outcomes for patients suffering from acute and chronic wounds. These include acellular biomaterials and composite cultured skin analogs containing allogeneic or autologous cultured skin cells.

Introduction

Wound coverage can be problematic in several different clinical settings. In one extreme example, massive burn injuries can require replacement of skin covering nearly the entire body surface area. Burns are an important medical problem in the United States, where greater than 1 million burn injuries occur each year.[1] Fires and burns result in 45,000 hospitalizations and 4500 deaths annually. Many advances in burn care have however caused a decline in burn mortality rates. In 1952, only half of all pediatric patients with greater than 50% total body surface area (TBSA) burns survived. Currently, most survive a 50% TBSA burn, and half of children who receive 98% TBSA burns survive.[2] Advances in burn care contributing to the decline in mortality include early excision, improved fluid resuscitation, infection control, nutritional support, and aggressive physical therapy.[2-4] Because most patients survive the initial resuscitation phase, even after very severe burns affecting a large percentage of TBSA, wound management is critical for recovery. Autografting with split-thickness skin, either meshed or unmeshed, has been considered the preferred treatment for coverage of excised burn wounds, but donor sites for autograft are limited in patients with very large burns. In these patients, wound coverage requires repeated harvesting of available donor sites, which is associated with pain and scarring at the donor site and lengthy hospital stays.

Chronic wounds represent a different kind of challenge for wound healing. These wounds do not usually involve a large surface area, but they have a high incidence in the general population and thus have enormous medical and economic impacts. The most common chronic wounds include pressure ulcers and leg ulcers.[5] In the United States alone, these wounds are estimated to affect more than 2 million people[6] with total treatment costs as high as $1 billion annually.[5] These figures may be expected to rise as the average age of the population increases. Pressure ulcers, characterized by tissue ischemia and necrosis,[7] are common among patients in long-term care settings, but patients hospitalized for short-term care or in home settings are also at risk if mobility is impaired.[5] Leg ulcers can have a variety

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of etiologies. Venous ulcers are the most common, often resulting from dysfunction of valves in veins of the lower leg that normally prevent the backflow of venous blood. Venous congestion leads to leakage of blood and macromolecules into the dermis, which can act as physical barriers to diffusion of oxygen and nutrients from the vasculature into the skin.[7] Arterial insufficiency and diabetes also contribute to the development of leg ulcers. Arterial blockage can lead to tissue ischemia, causing ulcers or necrosis. Patients with diabetes are prone to leg ulcers because of several aspects of their disease, including neuropathy, poor circulation, and reduced response to infection. Diabetic foot ulcers can lead to complications that result in as many as 50,000 amputations annually in the United States,[6] accounting for 45% to 70% of all lower-extremity amputations performed.[5] Historically, treatment of relatively small chronic wounds has included the use of topical agents and occlusive dressings, and grafting of split- or full-thickness skin.[8] Skin grafts can provide timely wound coverage, but may lead to painful donor sites which are slow to heal and may be unsuccessful because of underlying deficiencies in wound healing.

Though they affect a relatively small fraction of the population, congenital skin conditions and diseases represent significant challenges for wound coverage. For example, giant congenital nevi can cover more than 50% TBSA and when untreated can significantly increase a patient's lifetime risk for development of melanoma.[9] Traditional treatment has involved serial excision and skin autografting, but donor site morbidity, including hypertrophic scarring, can result. Another example is epidermolysis bullosa (EB), an inherited mechanobullous disorder that is characterized by erosions and blistering of the epidermis.[10] The genetic causes are heterogeneous but primarily affect proteins of the basement membrane zone of the skin, leading to mechanical fragility. Wounds in patients with EB are difficult to avoid and tend to heal slowly or progress to chronic wounds. Because EB results from genetic mutations in proteins of the patient's own skin, autografting is not a viable treatment. Clinical management has focused on protection from damage and topical therapy, including sterile dressings, antibiotics, and analgesics.[11,12] Currently, treatment is supportive at best, and there is no known cure.

There are clearly many medical needs for safe and effective therapeutic options for wound coverage in these diverse patient populations. Skin substitutes have been developed in response to these needs, providing new alternatives for temporary coverage and permanent wound closure with stable skin tissue.

Available skin substitutes

Human skin performs a wide range of protective, perceptive, and regulatory functions, but its role in providing a protective barrier is most critical for survival. The barrier function of skin is performed by the epidermis, which is comprised mainly of keratinocytes. The keratinocytes form a stratified epithelium, with proliferating basal cells at the innermost layer and the keratinized, relatively impermeable outer stratum corneum layer at the surface. Other cells of the epidermis include Langerhans' cells, which function in immune regulation, and melanocytes, which produce skin pigment. Beneath the epidermal layer, the dermis provides structural integrity, elasticity, and a vascular plexus to nourish the skin. The cellular components of dermis include fibroblasts, endothelial cells, smooth muscle cells, and mast cells, but the bulk of dermis is made of extracellular matrix. Various skin appendages, such as hair follicles and sweat glands, span the dermal and epidermal layers. Ideally, skin substitutes for wound healing would replace all of the structures and functions of native skin. Unfortunately, there are currently no engineered skin substitutes that can completely duplicate the complexity of human skin. There are, however, several skin substitutes that have been useful for replacement or reconstruction of one or both layers of the skin, facilitating wound healing in several different clinical settings (Table 1). These skin substitutes can act as temporary wound covers or permanent skin replacements, depending on their design and composition.

The most basic of the skin substitutes are synthetic, acellular materials designed to act primarily as barriers to fluid loss and microbial contamination. Two examples that have been widely used for coverage of excised burn wounds are Biobrane[13-16] and Integra.[17-23] Biobrane is a synthetic dressing composed of a trifilament nylon fabric partially imbedded in a silicon film. Collagen-derived peptides are chemically bound to the nylon/silicone matrix, providing a flexible and adherent surface for wound coverage. The silicone surface is semipermeable and controls water vapor loss from the wound. Biobrane is intended as a temporary wound dressing and is removed either when the wound is healed or when autograft skin is available. A related product, Biobrane-L, is made using a monofilament nylon and is designed to be less adherent than Biobrane. Clinical studies have demonstrated that Biobrane is as effective as frozen human allograft for temporary coverage of excised full-thickness burns[14] and was associated with improved healing and decreased hospitalization times in pediatric patients with second-degree burns.[15] Integra Dermal Regeneration Template is another widely used option for coverage of excised burn wounds, which has proved to be particularly valuable in patients with large burns and limited autograft donor sites.[19,21] Integra consists of 2 layers: a dermal substitute made of porous bovine collagen and chondroitin-6-sulfate glycosaminoglycan, and an epidermal substitute made of a synthetic silicone polymer. The dermal layer serves as a matrix for infiltration by fibroblasts and other cells from the wound bed. As the collagen matrix is populated by these cells, it is gradually degraded and replaced by newly synthesized collagen. The silicone layer provides a func-

Table 1 Engineered skin substitutes

Model	Description	Indication(s)/Use(s)
Acellular		
Biobrane (Bertek Pharmaceuticals, Morgantown, WV)[13-16]	Very thin semipermeable silicone membrane bonded to nylon fabric	Temporary adherent wound covering for partial-thickness excised burns and donor sites
Integra (Integra Life Sciences, Plainsboro, NJ)[17-23]	Bilayer structure; biodegradable dermal layer made of porous bovine collagen-chondroitin-6-sulfate matrix; temporary epidermal layer made of synthetic silicone polymer	Grafting of deep partial- or full-thickness burns; epidermal layer removed when donor sites available for autografting
Alloderm (LifeCell Corporation, Branchburg, NJ)[24-27]	Structurally intact allogeneic acellular dermis; freeze-dried after cells were removed with detergent treatment; rehydrated before grafting	Dermal template for grafting to burns and other wounds; repair of soft tissue defects
Cellular-allogeneic		
TransCyte (Smith and Nephew, Largo, FL)[28-30]	Allogeneic neonatal foreskin fibroblasts seeded on nylon mesh; freeze-dried to kill cells and preserve dermal matrix	Temporary covering for excised deep partial- and full-thickness burns before autografting
Dermagraft (Smith and Nephew, Largo, FL)[31-33]	Cryopreserved allogeneic neonatal foreskin fibroblasts seeded on bioabsorbable polyglactin mesh scaffold; cells are metabolically active at grafting	Treatment of full-thickness chronic diabetic foot ulcers
Apligraf (Graftskin) (Organogenesis/ Novartis, Canton, MA)[11,12,34-40]	Bilayer; allogeneic neonatal foreskin fibroblasts and keratinocytes in bovine collagen gel	Treatment of chronic foot ulcers and venous leg ulcers; also used for burn wounds and EB
OrCel (Ortec International, New York, NY)[41,42]	Bilayer; allogeneic neonatal foreskin fibroblasts and keratinocytes cultured in bovine collagen sponge	Treatment of split-thickness donor sites in patients with burn and surgical wounds in EB
Cellular-autologous		
Epicel (Genzyme Biosurgery, Cambridge, MA)[43-45]	Autologous keratinocytes cultured from patient skin biopsy, transplanted as epidermal sheet using petrolatum gauze support	Permanent wound closure in patients with burn with greater than 30% TBSA injury and in patients with congenital nevus
Epidex (Modex Therapeutiques, Lausanne, Switzerland)[46,47]	Autologous keratinocytes isolated from outer root sheath of scalp hair follicles; supplied as epidermal sheet discs with a silicone membrane support	Treatment of chronic leg ulcers
TranCell* (CellTran Limited, Sheffield, UK)[48]	Autologous keratinocytes cultured from patient skin biopsy, grown on acrylic acid polymer-coated surface; transplanted as epidermal sheets	Treatment of chronic diabetic foot ulcers
Cultured skin substitute* (University of Cincinnati/Shriners Hospitals, Cincinnati, OH)[49-53]	Bilayer; autologous keratinocytes and fibroblasts cultured from patient skin biopsy, combined with degradable bovine collagen matrix	Permanent wound closure in patients with burn with greater than 50% TBSA injury; also used in patients with congenital nevus and chronic wound

This list gives examples of several types of skin substitutes currently available and is not intended to be all-inclusive. Asterisk denotes products that are not commercially available but are currently in clinical trials.

tional barrier that is removed upon vascularization of the dermis, to be replaced by a thin layer of autograft.

Alloderm[24-27] is similar to Integra in that it is intended to provide a matrix for dermal tissue remodeling, but it is not a synthetic material. Alloderm is composed of human allograft skin that has been screened for absence of transmissible pathogens and then processed to remove epidermal components and all dermal cells. Dermal cells are removed by detergent treatment followed by freeze-drying, which preserves the matrix in a structural form similar to normal human dermis. Because the allogeneic cells have been removed, Alloderm is not rejected by the immune system and can be grafted like a dermal autograft and covered with a thin autograft.[25] Preclinical studies

suggest that this material might also be useful for repair of soft tissue defects, as in abdominal wall reconstruction.[26]

Several allogeneic skin substitutes are available, all of which act as temporary wound coverings for various types of wounds. These products are distinguished by the absence or presence of viable cells and the composition of the scaffold material used. TransCyte is very similar in composition to Biobrane, and like Biobrane it is used as a temporary covering for excised burns awaiting placement of autograft.[28] It is made from a nylon mesh that is seeded with allogeneic fibroblasts that are cultured from newborn human foreskin. The fibroblasts secrete extracellular matrix components and growth factors that can aid in the healing process. Before grafting, the cells in TransCyte are destroyed to reduce the risk of immune response. This is accomplished using a freezing process intended to preserve the tissue matrix and growth factors, offering a prospective benefit for wound healing over strictly synthetic materials.[28] Like TransCyte, Dermagraft is prepared using human neonatal fibroblasts, but in this skin substitute the fibroblasts are cryopreserved to maintain cell viability and the matrix is made from a bioabsorbable polyglactin mesh.[31-33] Dermagraft is indicated for use in the treatment of full-thickness foot ulcers.[33] It functions by providing a dermal matrix that facilitates re-epithelialization by the patient's own keratinocytes.

An additional layer of complexity is found in Apligraf, which contains both allogeneic fibroblasts and keratinocytes derived from neonatal foreskin.[34] The matrix used for cell growth and differentiation is a gel derived from bovine collagen. Apligraf has been useful in the treatment of venous leg ulcers and diabetic foot ulcers, increasing the percentage of wounds healed and decreasing the time required for wound closure.[34-38] A more recent application of Apligraf has been in the treatment of pediatric patients with various forms of EB.[11,12,39,40] Acute rejection reactions were not observed in these patients, and they reported faster and less painful healing compared to standard dressings.[11,12] Although greater than one quarter of treated wounds experienced reblistering after Apligraf treatment and healing, this was attributed to the patient's own skin cells replacing the cells in the tissue-engineered skin.[12] Because EB is caused by a genetic defect, it would be expected to affect all of the patient's cells. Interestingly, in one study, the patients reported that in wounds that experienced reblistering, the duration and severity of the blister were less than usual.[12]

OrCel is similar in composition to Apligraf in that it contains both fibroblasts and keratinocytes from neonatal foreskin, but the matrix used is a type I collagen sponge.[41,42] It is designed for grafting to partial-thickness wounds to provide a favorable matrix for host cell migration. OrCel has been indicated for use in the treatment of donor sites in patients with burn, and surgical wounds and donor sites in patients with EB. In a clinical study that directly compared OrCel with the acellular material Biobrane-L for the treatment of split-thickness donor site wounds, OrCel-treated sites had faster rates of healing and reduced scarring.[42] The improved healing with OrCel was attributed to the presence of the collagen sponge, in combination with cytokines and growth factors produced by the viable allogeneic cells.[42]

The models described above can be considered biologic dressings because the components are intended for temporary wound coverage. A beneficial characteristic shared by all of these models is that they can be ready to use when needed. Eventual replacement by patient-derived cells is however required, either by regrafting or overlaying with a split-thickness autograft, as in large wounds, or by gradual replacement by ingrowth of autologous keratinocytes, as in small wounds. For a skin substitute to be suitable for permanent wound closure, autologous cells must be used. The use of autologous cells can impart a significant delay in emergent treatment because time is required to culture cells from a biopsy of the patient's skin. The length of the delay will be inversely proportional to the size of the biopsy and the efficiency of cell expansion in culture and directly proportional to the amount of material required for wound coverage. During the preparation of autologous skin substitutes, other temporary skin substitutes can be used for wound coverage. For example, Integra can be grafted for temporary coverage and preparation of a vascularized wound bed before grafting with skin substitutes containing autologous keratinocytes and fibroblasts.[22,54] The benefit of autologous skin substitutes is that once they have engrafted, permanent wound closure is accomplished and physiologic stability is restored. Thus, despite the necessary delay because of preparation, they can theoretically reduce the number of procedures required for wound coverage and decrease hospitalization time for patients with large skin injuries. A further benefit is the reduction in donor site utilization when autologous skin substitutes are used. Keratinocytes and fibroblasts can be cultured from relatively small split-thickness skin biopsies and the cell numbers expanded exponentially in just a few weeks of in vitro culture.[55-58] Hypothetically, a biopsy representing less than 2% TBSA could be sufficient to cover the entire body with autologous cultured skin in about a month.[59]

For one innovative grafting product, autologous skin substitutes can be prepared without collection of a skin biopsy. EpiDex is a skin substitute that uses autologous keratinocytes cultured from the outer root sheath of anagen hair follicles.[46,47] This product has been shown to improve healing of chronic leg ulcers that are relatively small.[47] Because keratinocytes found in the follicle have a high capacity for proliferation, sufficient populations of cells can be obtained from roughly 100 scalp hairs for preparation of EpiDex and application within 5 to 6 weeks.[47] EpiDex is composed of cultured autologous keratinocytes transplanted with a supportive silicone membrane. Discs with a diameter of 1 cm are placed within the wound margins, and the silicone backing is

removed at the first dressing change. Because the grafts are small and circular, they may not provide complete coverage and multiple grafting procedures may be required for definitive wound closure.

For larger wounds, such as giant nevi or burn wounds, a skin biopsy is generally required for the preparation of sufficient quantities of cultured cells for wound coverage with autologous skin substitutes. Currently, Epicel is the only autologous cultured skin product commercially available in the United States. Also referred to as cultured epidermal autografts (CEA), Epicel is indicated for patients with full-thickness burns covering greater than 30% TBSA and in patients with giant congenital nevus.[43-45] Cultured epidermal autografts are sheets of autologous keratinocytes attached to a supportive petrolatum gauze backing that is removed approximately 1 week after grafting. Epicel CEA has proven to be extremely valuable in patients with very large (>60% TBSA) burns where the availability and/or quality of donor sites is poor.[45] In one study of 30 extensively burned patients, permanent coverage of a mean TBSA of 26% was obtained, similar to the area covered by conventional autograft.[45] This represented an average take rate of CEA of approximately 69% of the area treated, which is considered relatively high. This was attributed in part to great care taken in the selection of antimicrobial agents, which can be toxic to cultured keratinocytes,[60,61] and to the gentle handling of the fragile CEA grafts.[45] Mechanical fragility was considered among the major disadvantages of Epicel CEA, which was greatest during the period of maturation of the dermal-epidermal junction. This was evident clinically as blisters that formed because of even small amounts of friction in the first several months after graft application.[45] Other disadvantages of CEA included hyperkeratosis, contracture, and scarring, though hypertrophic scarring appeared to be reduced compared to meshed expanded skin autograft.[45] The high cost and labor-intensive procedures required for Epicel, both in preparation of the CEA and in the care required after grafting, were cited by the authors of this study as a disadvantage,[45] but this is not specific to Epicel. Compared to conventional treatments, virtually all skin substitutes are expensive, but there may in fact be ultimate reductions in costs if the number of procedures, length of hospitalization, amount of physical therapy, and number of reconstructive procedures can all be reduced.

The major disadvantage that is specific to CEA is mechanical fragility, which results from the absence of an integrated dermal component at the time of grafting. Favorable clinical results have been obtained with cultured skin substitutes (CSS) composed of collagen-glycosaminoglycan substrates containing autologous fibroblasts and keratinocytes, providing permanent replacement of both dermal and epidermal layers in a single grafting procedure.[49,50,54,59] Though this material is not yet commercially available, clinical studies have demonstrated its utility in treating burns of greater than 50% TBSA and giant congenital nevi.[51,54,59] Cultured skin substitutes are prepared using patient-derived fibroblasts and keratinocytes that are isolated from a small split-thickness skin biopsy using standard techniques.[57,58,62] The amount of skin used for cell culture is determined by the surgeon's estimate of the area to be covered by CSS, factoring in the amount of donor sites available for autografting. For preparation of CSS, fibroblasts and keratinocytes are serially inoculated onto collagen-based substrates at high cell densities.[52,53,63] Culture at the air-liquid interface for 7 to 14 days provides a liquid to gas transition, with nutrient medium contacting the dermal substitute and air contacting the epidermal substitute, resulting in stratification and cornification of the keratinocyte layer.[64-67] In the dermal layer, fibroblasts fill the biopolymer substrate, begin to degrade it, and generate new extracellular matrix (Fig. 1). At the dermal-epidermal junction, evidence of basement membrane formation in vitro has been demonstrated.[68] Thus, the blistering encountered with CEA is not a clinical complication after grafting of CSS because the maturation of the dermal-epidermal junction is

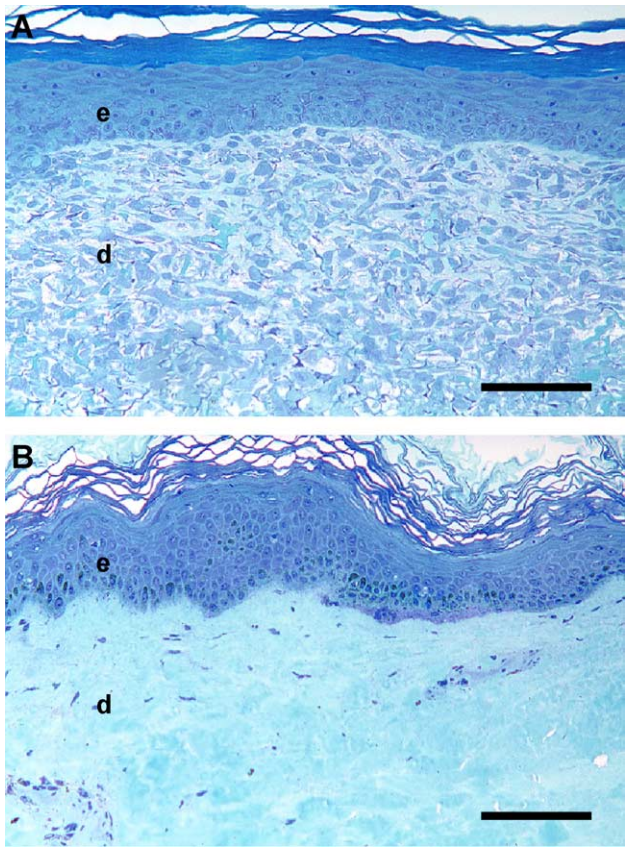

Fig. 1 Cultured skin substitutes compared with normal human skin. A, CSS at day 14 of in vitro incubation. B, Human breast skin. Both CSS and human skin display a stratified epidermis (e) with a cornified surface. The dermis (d) of the CSS has a higher cell density than human skin, whereas human dermis is predominantly extracellular matrix. Sections were stained with toluidine blue. Scale bars, 0.1 mm.

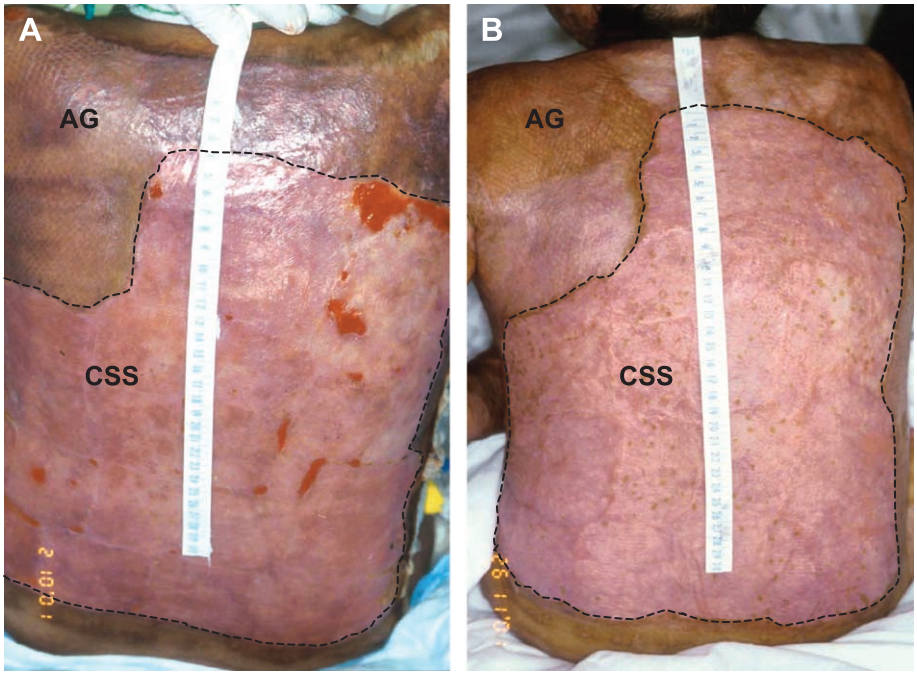

Fig. 2 Burn wounds healed with CSS. Shown is the back of a pediatric patient grafted with CSS (outlined by dashed lines) and split-thickness skin autograft (AG). A, At 2 weeks after grafting, the borders of some of the CSS were discernable but the wound was mostly closed. B, At 10 weeks after grafting, the healed CSS was pliable and hypopigmented, though some pigmented foci were observed.

accomplished before grafting. After healing, CSS resemble split-thickness autograft and provide a satisfactory cosmetic outcome (Fig. 2). Shown in Fig. 2 is an example of the clinical results obtained with CSS. This 6-year-old boy sustained a 77% TBSA burn, and approximately 17% TBSA was treated with CSS. At approximately 10 weeks after grafting, the healed CSS was soft and pliable, and regrafting was not required. Cultured skin substitutes have been demonstrated to reduce donor site utilization for closure of large excised burns and congenital giant nevus, with a 60- to 70-fold expansion of donor skin for grafting.[51,59] This cultured skin substitute has also been used as an adjunctive treatment of chronic wounds, but for those patients, allogeneic fibroblasts and keratinocytes from screened human cadaveric donors were used.[50]

On the horizon

Despite favorable results with skin substitutes, limitations in anatomy remain which can influence engraftment and functional and cosmetic outcome. Because skin substitutes currently available contain at most only 2 cell types, fibroblasts and keratinocytes, they cannot replace all of the functions of native skin. Recent and ongoing studies are addressing the preparation of engineered skin containing additional cell types to increase homology to native human skin and improve functional outcome. For example, incorporation of endothelial cells has been studied to initiate angiogenesis in engineered skin grafts in vitro. Because cultured skin lacks a vascular plexus, it is vascularized more slowly than split-thickness skin autograft after grafting. This can contribute to graft failure by increasing the time that grafted cells are deprived of nutrients and by increasing susceptibility to microbial contamination. This has been addressed clinically, in part, by bathing cultured skin grafts with nutrients and antimicrobial dressing fluids for several days after grafting.[69-71] The dressing fluids nourish and protect the grafts until vascularization occurs, usually within 5 days after grafting. In recent studies, human umbilical vein endothelial cells[72,73] or human dermal microvascular endothelial cells[74,75] have been incorporated into cultured skin grafts. Human umbilical vein endothelial cells are readily available but can only be used in allogeneic skin substitutes. For clinical application of autologous endothelialized skin substitutes, use of dermal endothelial cells is optimal, and these cells should be isolated from the same skin biopsy used for preparation of fibroblast and keratinocyte cultures. These criteria have been met in preclinical[74] and clinical[75] studies, but enhanced vascularization because of inclusion of endothelial cells has not yet been demonstrated. A current practical limitation to the inclusion of endothelial cells in CSS is the slower growth in primary culture of human dermal microvascular endothelial cells compared to fibroblasts and keratinocytes, which delayed preparation of endothelialized CSS for grafting to patients.[75] In addition, studies have shown that only a small proportion of human dermal microvascular endothelial cells persist during culture of engineered skin grafts,[74] which has been proposed to be due to apoptosis of the endothelial cells.[73,76,77] Thus, though

the addition of endothelial cells to engineered skin for in vitro angiogenesis is theoretically possible, technical hurdles still need to be overcome.

Another limitation of cultured skin is absent or irregular pigmentation. In intact skin, pigmentation results from the proper distribution and function of epidermal melanocytes. These cells are important both physiologically, to protect skin from damage by ultraviolet irradiation,[78,79] and psychologically, influencing a patient's body image and personal identity. Melanocytes can sometimes unintentionally persist in cultures of epidermal keratinocytes; referred to as passenger melanocytes, these can result in foci of pigmentation after grafting (Fig. 2).[54,67,80,81] In preclinical studies, selective cultivation of human melanocytes and deliberate addition to CSS showed that uniform pigmentation can be achieved,[82] though the intensity of pigment was not regulated. Future models of pigmented skin will benefit from a more thorough understanding of melanocyte function and factors that regulate skin pigmentation.[83,84]

An exciting possibility for development of engineered skin substitutes involves the addition of genetically modified cells. Using the tools of molecular biology, genetic modification of cells within skin substitutes can hypothetically be used to overcome limitations in anatomy and physiology, resulting in skin substitutes with greater homology to native human skin and improved performance. The gene expression profile of keratinocytes can be altered by the transfer of recombinant genes,[85,86] and the genetically modified cells have been shown to retain their ability to differentiate into a stratified epidermis.[85] Genetic modification can be used to ectopically express cytokines not normally expressed in a particular cell type, to compensate for deficiencies of engineered skin compared to native skin. Alternatively, skin substitutes can be genetically engineered to overexpress growth factors that aid in wound healing to enhance their therapeutic value for wound repair. For example, CSS containing keratinocytes genetically modified to overexpress vascular endothelial growth factor, a mitogen for microvascular endothelial cells, showed enhanced vascularization and improved healing after grafting to athymic mice.[87,88] Thus, overexpression of an angiogenic cytokine in genetically modified CSS was able to compensate for the absence of a vascular plexus in grafted cultured skin.

Genetically modified cultured skin grafts could potentially act as vehicles for cutaneous gene therapy. One application of this technology would be the correction of cutaneous defects in genetic diseases, such as EB. Junctional EB (JEB), one of the more severe forms of the disease, can result from mutation of genes encoding subunits of laminin 5, a component of anchoring filaments in the basement membrane zone of skin.[10] Preclinical studies have demonstrated correction of the JEB phenotype by gene transfer of LAMB3, which encodes the beta 3 subunit of laminin 5. Using a retroviral gene transfer vector, the LAMB3 gene was introduced into keratinocytes cultured from patients with laminin 5-dependent JEB.[89] Organotypic cultures prepared with LAMB3-transduced cells showed normal assembly of the dermal-epidermal attachment structures that are missing in the skin of patients with JEB, indicating correction of the mutant phenotype.[90] Animal studies further confirmed the utility of LAMB3 gene transfer for correction of the JEB defect. Cultured skin prepared using keratinocytes derived from a patient with JEB was grafted to mice. Cells without LAMB3 modification showed an abnormal phenotype characteristic of human JEB, whereas the cells transduced with the LAMB3 gene were phenotypically normal.[91] These and other related studies demonstrate the feasibility of combining tissue engineering with gene therapy to treat cutaneous disease.

Another application of genetically modified skin substitutes is the secretion of factors into the bloodstream to treat systemic disorders. Proteins secreted by keratinocytes, whether native or introduced by genetic modification, can reach detectable levels in the serum after grafting.[92,93] Preclinical studies have examined the use of genetically modified keratinocyte grafts for delivery of human growth hormone,[94,95] which could be useful for treatment of growth deficiencies, and human factor IX,[96-98] which is involved in the bleeding disorder hemophilia B. In a recent study, a genetic defect that causes obesity in mice was corrected using grafts of genetically modified keratinocytes. Leptin, a hormone that regulates food intake, is associated with obesity in human beings and is deficient in the genetically obese *ob*/*ob* mutant mouse.[99] Composite cultured skin was prepared using human keratinocytes, genetically modified to overexpress the leptin gene, and fibrin-fibroblast gels, and the grafts were transplanted to *ob*/*ob* mice. Human leptin was detectable in the serum of grafted mice and was correlated with significant reductions in food intake and body weight.[100] This study represented the first clear demonstration that genetically modified human keratinocyte grafts could correct a deficiency of a circulating protein, highlighting the enormous potential genetically engineered skin substitutes.

Conclusions

Technological advances in the fabrication of biomaterials and the culture of skin cells have permitted the production of engineered skin substitutes. The variety of products currently available has contributed to improved treatment of burns, chronic wounds, and congenital skin disorders. Continued research will focus on improving the anatomy and physiology of skin substitutes, working toward better homology to native human skin. In addition, evaluation of the use of genetically modified cells in engineered skin may lead to enhancement of wound healing, and innovative new treatments for cutaneous diseases and perhaps even systemic deficiencies. These efforts will further enhance the utility and versatility of engineered skin substitutes for clinical use.

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