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Wound Coverage Technologies in Burn Care: Novel Techniques

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Abstract

Improvements in burn wound care have vastly decreased morbidity and mortality in severely burned patients. Development of new therapeutic approaches to increase wound repair has the potential to reduce infection, graft rejection, and hypertrophic scarring. The incorporation of tissue engineering techniques, along with the use of exogenous proteins, genes, or stem cells to enhance wound healing, heralds new treatment regimens based on the modification of already existing biological activity. Refinements to surgical techniques have enabled the creation of protocols for full facial transplantation. With new technologies and advances such as these, care of the severely burned will undergo massive changes over the next decade. This review centers on new developments that have recently shown great promise in the investigational arena.

Keywords

technologies; development; future of burn care; wound coverage; gene therapy; stem cells; tissue engineering

INTRODUCTION

Over the past several decades, the incorporation of early excision followed by autografting has improved burn patient outcome by removing a major source of inflammation (the eschar) while covering the wound. Early excision of the burn wound eschar and subsequent coverage prevents fluid and protein loss, hypothermia, and the risk of infection. Development of dressings that are biosynthetic (Biobrane[®], AWBAT[®], Suprathel[®]), biological (Amnion), dermal analogues (Integra[™], Alloderm[®]), or derived from the patient

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(cultured endothelial autograft) have provided coverage options beyond simple meshed grafts, and these dressings prevent infection while making the wound environment optimal for healing (reviewed in Jeschke *et al.*, this issue). Although these additions to the burn and plastic surgeon's armamentarium have enabled faster wound coverage and rapid repair, burn wound care continues to evolve, as seen by experimentation with full facial transplants, use of stem cells to improve wound repair, and replacement of injured tissue with engineered tissue (Summary in Table 1). Here, we review some of the experimental techniques that have met with early success, including autograft fixation with fibrin sealant,^{1, 2} facial transplants,³ tissue engineering,⁴ stem cells, and gene therapy.⁵

In general as indicated in Table 1, there have been several novel developments in the area of dermal and epidermal substitutes and we believe that these tissue engineering approaches hold a lot of promise to modify and improve burn care during the next decade.

FIBRIN SEALANT FOR GRAFT FIXATION

Refinement of burn wound management has greatly improved patient outcome by significantly reducing morbidity and mortality.⁶⁻⁸ Full-thickness wounds are covered by meshed split-thickness skin grafts following excision.⁹⁻¹² Autograft skin is usually affixed to the wound bed by sutures or skin staples. However, these methods are not optimal. Attachment via sutures can be time consuming, and both methods require anesthesia during lengthy removal procedures.^{13, 14} Achieving full contact between the graft and the underlying wound surface is often difficult with extensively grafted wound areas, as seen in patients with massive burns. A newer autograft fixation method that has been successfully applied is the use of fibrin sealant.^{2, 15-19} Originally developed as a hemostatic agent to enhance clotting, fibrin sealant is a mixture of fibrinogen and thrombin that is both non-toxic and bio-degradable. The slow-clotting version (Artiss®, Baxter Inc., Deerfield, IL, USA), which has reduced thrombin content, can be successfully applied as a thin layer directly to the wound with a spray applicator. Use of fibrin sealant enables omission of bolster dressings following surgery. Seroma and hematoma formation is greatly reduced due to improved contact between the graft and the wound bed. As a result, the fibrin sealant becomes a matrix that supports vascularization, and collagen-producing fibroblasts move across the scaffold to re-epithelialize the wound. The rapid wound coverage achieved through this method provides a barrier against infection.^{20, 21} Application of fibrin sealant significantly decreases the length of surgical procedures, especially when grafts must be applied to the face or other areas where standard fixation methods are difficult to use. The formation of hypertrophic scarring may also be reduced as a result of better re-vascularization, graft adherence, and subsequent acceleration of wound closure. Side effects are rare, with the most commonly reported adverse events being an allergic reaction to the fibrin sealant proteins and air or gas embolism after application. One disadvantage of fibrin sealant is its high cost. This makes it a less frequent choice of most burn surgeons, who use it only when treating burns on the face, hands, or other locations requiring a sheet graft.

FACIAL TRANSPLANTATION

One of the greatest technical challenges in treating burn wounds arises from severe facial burns. Significant deformities can result from the injury as well as poorly applied wound coverage. Conventional treatment modalities are insufficient for addressing the aesthetic and functional outcomes needed to effectively treat these difficult injuries. Following the lead of a surgical team in Amiens, France in 2005,²² several groups in Europe, China, and the United States have been able to meet this complex clinical challenge with the use of composite tissue allotransplantation. Although restoration of function is the ultimate goal, this has been a slowly developing approach that is only used under extreme circumstances.

During reconstructive interventions, healthy donor facial tissue is transplanted onto the injured area to improve cosmesis and restore function. The technical challenges are immense and despite successful transplantation of approximately 20 patients, the process cannot be called routine, by no means. Despite technical issues, there is the immunologic issue with intense immune therapy to prevent tissue rejection. The strategy used for immunotherapy is based on organ transplantation.^{3, 23}

The psychosocial and ethical issues associated with this new treatment present unique challenges, which need to be addressed by a dedicated team.^{24, 25} The conventional treatment modalities for severe facial burns offer little improvement in function and appearance, and they often leave patients significantly debilitated. As a result, these patients frequently become isolated, both socially and personally, and many suffer from psychological disorders and phobias. These patients require multiple reconstructive procedures with minimal normal tissue (secondary to burns in other areas) available to use during these procedures. Facial transplantation in such patients can offer the possibility of improved quality of life.

Since the initial face transplant in 2005, there have been 12 additional full transplant procedures. The procedures will eventually reduce the knowledge gap, as the teams performing these new reconstructive procedures have shared both their successful and unsuccessful strategies. Facial transplantation poses significant challenges, which when overcome, provide a promising treatment modality for the severe facial burn injury.²⁶ We have recently published an in-depth review in which we discuss pros and cons of this emerging approach.²⁷

TISSUE ENGINEERING WITH STEM CELLS

Human skin provides important protection from the environment that is critical for survival. Despite the success of wound healing following skin grafting, the resulting skin contains scar and frequently lacks the flexibility and elasticity of normal skin. Recent advances in stem cell isolation techniques have enabled the application of stem cells for several uses. Stem cells can be applied directly to the injured area to augment wound closure. A second application of a multi-layer skin substitute that more closely resembles the dermal and epidermal structure of normal skin can allow the full repair or replacement of damaged tissue. Modifications to dermal analogs and cultured epidermal autograft have led to the development of combination products that are dermal and epidermal skin replacements.²⁸ Fibroblasts and keratinocytes are obtained from the patient, processed, and then directly re-applied to the patient or cultured *ex vivo* and layered onto collagen-glycosaminoglycan substrates.^{29, 30} Further culture and processing at an air-liquid interface provides nutrients, which are delivered to the dermal layer by the liquid medium. It also enables contact of the epidermal layer to air, enabling the keratinocyte layers to stratify and cornify.^{31, 32} Dermal fibroblasts proliferate into the collagen matrix, subsequently degrading it while generating new autologous dermal matrix. Formation of collagen fibers and the basal membrane takes place at the dermal-epidermal junction.³³ As a result, the dermal-epidermal junction is strengthened, decreasing the risk of epidermolysis and blistering frequently encountered with application of cultured epithelial autografts and split-thickness grafting.

New techniques have been used to further improve these engineered skin replacements. The addition of melanocytes can decrease hypopigmentation and achieve better cosmesis with closer color matching.³⁴ Vascular endothelial growth factors and angiogenic cytokines have also been introduced to counteract the absence of a vascular plexus, shorten healing time, and prevent graft loss.^{35, 36}

Stem cells represent a new hope in the management of burns. These cells have been shown to improve the wound repair process, both locally and systemically, through several different mechanisms. In humans, stem cells are found in many tissues including bone marrow, muscle, umbilical cord, amnion, adipose tissue, and skin as well as in the blastocystic mass of embryos.³⁷ Ethical concerns regarding the destruction of embryos to obtain embryonic stem cells have driven advances in isolating and characterizing stem cells from other tissues without risk to the donor. A classification of stem cells and their origin are shown in Table 1.

The inherent “stemness” of stem cells makes these cells a promising tool for healing burn wounds. Given the clonality and pluripotency of stem cells, they can be used to regenerate dermis and accelerate re-epithelialization.³⁸ Dermal regeneration and re-epithelialization can be expedited by clonal amplification of these pluripotent cells, which can be transplanted without rejection issues. Stem cells are considered to be “immune privileged” because they lack immunogenic surface markers.³⁹

Stem cells present in the bone marrow migrate to affected tissue after injury and aid in healing and regeneration processes.^{40–42} While the cells are blood-borne and after they have reached the affected tissue, they control inflammation by decreasing pro-inflammatory cytokine release and increasing secretion of anti-inflammatory cytokines such as IL-10.⁴³

Human embryonic stem cells can be differentiated into keratinocytes *in vitro* and then stratified into an epithelium that resembles human epidermis.⁴⁴ This graft can then be applied to open wounds on burn patients as a temporary skin substitute until autograft or another permanent wound cover becomes available. However, this approach is still in the early stages of experimental clinical application. Until recently, the majority of stem cell studies had been performed with human embryonic stem cells. Recent work by Natesan and colleagues⁴ demonstrated that adipose-derived stem cells from burn patients can be used to create a multi-layer skin substitute, though improvements on the initial product are under development. As adipose-derived stem cells are routinely removed and discarded from patients with extensive burn injuries, this approach may enable the creation of custom replacement skin from the patient’s own cells.

SPRAY-ON SKIN: KERATINOCYTE SUSPENSION

Wood et al.⁴⁵ reviewed the literature related to the use of cultured epithelial autograft for extensive burns and concluded that its application ranges from useful to non-beneficial given its difficult handling and fragility as well as the lack of standardized application at burn centers. Because of these limitations, a technique involving delivery of a keratinocyte suspension to the wound through an aerosol spray has been developed.

Using a porcine model, Reid and colleagues⁴⁶ showed that wound contraction after healing was significantly lower in wounds receiving both a split-thickness skin graft and sprayed keratinocytes than in wounds receiving only a split-thickness skin graft. James et al.⁴⁷ later showed in a clinical trial that wound contraction and healing time may be reduced by spraying cultured autologous keratinocytes over meshed autograft. Also, the use of sprayed keratinocytes may overcome some of the limitations of cultured epithelial autograft sheets. In this series, split-thickness skin was obtained from unburned areas, submerged in a 2% dispase solution, and placed in an orbital shaking incubator to separate the epidermal layer. This layer was then placed in a trypsin solution under an incubator to generate a cell suspension that was then cultured in growth medium. The cells were expanded during a 3-week period to a concentration of 10^7 cells per ml, and 5×10^5 cells per cm^2 were subsequently aerosolized and applied to the wound.

One of the major drawbacks of this technique is the delay of application while the cell expansion takes place. Zweifel and coworkers⁴⁸ described a three-patient case report in which a non-cultured autologous keratinocyte suspension was delivered to split- and full-thickness burn wounds via an aerosol spray at 2 days after admission. The results suggested that this approach decreased healing time and hypertrophic scarring.

Spray-On Skin: ReCell

Recent developments have enabled the isolation of fibroblasts, melanocytes, Langerhans cells, and keratinocytes from split-thickness biopsies. The cells are immediately isolated and reapplied using a proprietary spraying device. According to the manufacturer of ReCell, up to 80 times the surface area of the original donor biopsy can be covered with the isolate. ReCell has been tested clinically and shown to improve wound healing, decrease scarring, and support repigmentation of the injured area per the manufacturer.

Gene Therapy and Growth Factors

During the early days of gene therapy, gene insertion directly into the tissue or cells in a patient was only condoned to correct a metabolic function absent due to a congenital defect or malignancy in late-stages.⁴⁹ Skin, the most easily accessed organ that can also be continuously monitored, provides an environment ideal for gene transfer aimed at improving wound repair defects. With development of improved administration and localization protocols, introduction of exogenous genes into skin cells has expanded rapidly both *in vitro* and *in vivo*.

One of the oldest gene transfer methods depends on the utilization of viruses. Viral vectors are useful for transferring genes due to innate viral ability to insert foreign genes into target cells for replication and expression. A myriad of viruses have been used to achieve transfer into skin cells including lentiviruses, herpes simplex viruses, retroviruses, adenoviruses, and adeno-associated viruses.^{50–64} Viral vector production is time consuming and expensive, and the pitfalls—variable transfection and the increased risk of infection—decrease the clinical utility of this methodology. In 1995, Hengge directly injected naked IL-8-encoding DNA.⁶⁵ Significant dermal neutrophil recruitment was achieved with this approach. However, this transfer method is inefficient, and much of the DNA is degraded without reaching the nucleus.⁶⁶

In 1998, Eriksson pioneered the “microseeding” technique, which involves direct injection of naked DNA into target cells using a modified tattooing machine.⁶⁷ Transfection was only found superficially, with little detection of DNA in deeper tissue, and it lasted 1 to 2 weeks. This technique was followed by development of the “gene gun.” The gene gun propels tiny (1–5 µm) tungsten- or gold-coated particles, which serve as carriers for plasmid DNA, at a high rate of speed into skin cells.⁵⁸ Transient gene transfer is achieved, with the highest expression being achieved at 1 to 3 days post injection.^{56, 57} In a porcine model of partial thickness wounding, transfection of epithelial growth factor cDNA into the skin increases re-epithelization and the wound healing rate.⁶⁸ In addition, tensile strength is significantly increased by gene gun particle delivery of PDGF in a rat model of wound healing.⁵⁸ However, the depth of transfection is quite variable, ranging from transfection of the epidermis with standard use of the gene gun⁶⁹ to epidermal and dermal transfection achieved by delivering plasmids at a greater speed.⁷⁰ This second technique results in peak expression of the transfected genes in the muscle and skin at 24 hours post transfection, with minimal tissue damage.

Acceleration of the repair of chronic and diabetic wounds has been successfully achieved with electroporation—the application of an electric field to facilitate uptake of a gene by

cells.^{71, 72} Delivery of tissue growth factor-1 cDNA by electroporation has been shown to increase wound healing in diabetic mice by increasing collagen production, angiogenesis, and the rate of re-epithelialization in the wound bed.⁷³ Delivery of keratinocyte growth factor (KGF) plasmid DNA via electroporation significantly improves wound repair over that seen in untreated controls; however, transfection of naked KGF plasmid DNA yields comparable results.⁷⁴ These experiments show that the technique has merit and does not result in greater tissue damage, suggesting that it may have greater applicability for delivering large molecules to the cell.

An alternative approach to enhancing gene delivery is the use of cationic liposomes to encapsulate the gene to be delivered. These positively charged, synthetic vesicles surround negatively charged DNA, chaperoning it through the cell membrane and preventing degradation within the wound environment. The liposome-DNA construct attaches to the negatively charged cellular membrane, and the DNA enters the cell via endocytosis.^{75, 76} Gene delivery using this method can be achieved following topical application or injection. The LacZ gene has been successfully transfected into murine hair follicles, dermis, and epidermis⁷⁷ following topical application of the cationic liposome/DNA complex. Gene expression can be detected 6 hours following application, persists for 48 hours at maximal levels, and can still be detected at 7 days after transfection. Cationic liposomes have been used successfully to transfect growth factors under a variety of conditions.^{58, 78, 79} Both subcutaneous injection and topical application of fibroblast growth factor-1 increase tensile strength of injured skin in diabetic mice.⁷⁹

Transfections performed in the above-mentioned manner can have systemic as well as local effects. IGF-1 cDNA/liposomal complex was injected adjacent to thermally injured rat skin, and a high transfection level was achieved in the skin, with 70–90% of endothelial cells, macrophages (including multi-nucleate giant cells), and myofibroblasts expressing the transfected gene.^{80, 81} In addition, proliferation of basal skin cells was increased, suggesting that biologically active IGF-1 was produced.⁸¹ Re-epithelialization was greatly increased (>15%) over rates achieved with naked or liposomal-encapsulated IGF-1 protein. We have found that rats with the transfected gene also experience significant increases in body mass and muscle protein. Despite systemic improvements, gene delivery was localized, and there was no evidence of the expression of the control gene (β-galactosidase) or IGF-1 gene in the blood, spleen, kidney, or liver following dermal injection.⁷⁶ Transfection of liposome-encased PDGF cDNA into a thermally injured pig was shown to increase mRNA and protein production by 2 days post transfection.⁵ Re-epithelialization of the wound and graft adhesion were increased within 9 days following transfection. Both epidermal and dermal repair were greater with PDGF cDNA transfection, suggesting that similar approaches hold clinical benefit.

The overall utility of gene therapy using a single growth factor gene to promote wound healing may be limited. As wound healing progresses, each phase requires a different combination of growth factors for optimal results. For example, PDGF and IGF-1 have synergistic effects in healing partial-thickness wounds.⁸² Similarly, the combination of FGF-2 and PDGF improves DNA synthesis to a greater degree than application of a single growth factor.⁸³ We have found that transfection of a combination of KGF and IGF-1 cDNA is more efficacious in wound healing than transfection of either cDNA alone.⁸⁴ Sequential provision of growth factors in timed manner to enhance each phase of wound repair will be the next step in refining this method.

Additional methods of delivering genes include diethylaminoethyl-dextran,⁸⁵ microbubble-enhanced ultrasound,⁸⁶ biomaterials,⁸⁷ and calcium phosphate transfection.⁸⁸ Gene delivery and expression can be maintained for prolonged times using gel⁸⁹ or slow-release⁹⁰

matrices. Great strides in controlling gene expression in the individual cell have been made using a “genetic switch” to activate or deactivate gene expression.⁹¹ The maintenance of a wet wound environment using the wound chamber technique reduces inflammation and improves wound healing, and it also may improve the efficiency of gene delivery by providing a liquid interface.⁹² Although these techniques are largely experimental, they may have great clinical applicability following further testing and refinement. Improving the healing process in severely burned patients will enable reduction of hypertrophic scar development as well.

Beyond the mentioned growth factors, there are currently various trials that investigate cytokines and/or growth factors administered as protein or as genes, e.g. VEGF, angiogenin, TGF- β 2, TIMP-1, and TIMP-2. These factors are given either topically or systemically as genes, particles or proteins, and their efficacy is yet to be determined. An extensive review of current gene and stem cell therapy can be found in two recent publications.^{93, 94}

CONCLUSION

Progress in the acute treatment of burn patients within the last decades has been a success story, with significant improvements being seen in ICU mortality and the long-term survival of severely burned patients. This progress has given rise to a new set of challenges for burn researchers, specifically reducing scarring, improving skin graft quality, and creating pluristratified dermal and epidermal constructs for covering excised burn wounds. Therefore, continuous and critical re-evaluation of all aforementioned aspects of temporary and definitive burn wound coverage is imperative. The design of new molecular methodologies and animal models for the study of pathophysiological mechanisms underlying poor wound healing will enable manipulation of disease pathology with the goal of improving patient outcome. The successful integration of future technology into clinically applicable products will depend on the conduct of tightly controlled multicenter clinical investigations of new skin constructs and most importantly, an integration of all these efforts with multidisciplinary stem cell research.

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Table 1**Engineered skin substitutes**

Model	Description	Indications
<i>Acellular</i>		
Biobrane® (Bertek Pharmaceuticals, Morgantown, WV, USA)	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, USA)	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, USA)	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
Matriderm® (Dr. Suwelack Skin & Health Care AG, Germany)	Non-cross linked bovine collagen and elastin matrix that allows cellular ingrowth and neovascularization	Template for dermal reconstruction in the treatment of full thickness burns
<i>Cellular-Allogeneic</i>		
Dermagraft® (Advanced Biohealing, Westport, CT, USA)	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® (Organogenesis/ Novartis, Canton, MA, USA)	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 epidermolysis bullosa
OrCel™ (Forticell Bioscience, Englewood Cliffs, NJ, USA)	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 epidermolysis bullosa
<i>Cellular-Autologous</i>		
Epicerl® (Genzyme Biosurgery, Cambridge, MA, USA)	Autologous keratinocytes cultured from patient skin biopsy, transplanted as epidermal sheet using petrolatum gauze support	Permanent wound closure in patients with burns covering greater than 30% of the total body surface area and in patients with congenital nevus
Epidex® (Modex Therapeutiques, Lausanne, Switzerland)	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)	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, USA)	Bilayer; autologous keratinocytes and fibroblasts cultured from patient skin biopsy, combined with degradable bovine collagen matrix	Permanent wound closure in patients with burns covering greater than 50% of the total body surface area; also used in patients with congenital nevus and chronic wound

Table 2

Review of stem cell nomenclature

Cell	Source	Potency	Advantages	Disadvantages	Examples of Utility
Embryonic stem cells	Inner cell mass of blastocyst	Pluripotent	Pluripotent Clonogenic	Teratogenic Ethical controversy	Knockout mouse
Umbilical cord blood stem cells	Umbilical cord blood	Pluripotent	Pluripotent Non-immunogenic Clonogenic	Limited supply with low yield	Bone marrow transplantation
Mesenchymal stem cells	Bone marrow stroma, blood	Multipotent	Autologous Accessible Clonogenic	Require time to culture Harvest invasive Limited supply	Parkinson's, myocardial remodeling, wound healing
Adipose-derived stem cells	Adipose tissue	Multipotent	Non-immunogenic Abundant supply Accessible Clonogenic	Processing required	Wound healing, tissue engineering
Resident progenitor cells	Numerous tissues/organs	Unipotent	Accessible Potential for transdifferentiation	Limited potency and clonogenicity	Re-epithelialization of wounds from hair follicular cells

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