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# Burn Wound Healing and Tissue Engineering

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## 8.1 Definition

Wound healing is a complex and dynamic process that starts after injury and continues for months to years as the scar undergoes remodelling. For the purpose of this paper wound healing refers to the initial phase of healing that ends with wound closure or re-epithelialization and restoration of the epidermal barrier, either by secondary spontaneous healing, or by primary closure as with a skin graft. According to the FDA Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds - Developing Products for Treatment, complete wound closure is defined as skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits 2 weeks apart.[1]

## 8.2 Indications for clinical care and research

Wound closure after burns is one of the most important determinants of survival and long-term outcomes such as function and aesthetics. Early wound closure reduces the risk of infection and fluid losses and has been shown to reduce mortality, length of hospital stay, and subsequent hypertrophic scarring.[2–5] Thus, early closure of burns is a cornerstone of comprehensive burn care. Despite its importance, validated methods to objectively measure wound closure are lacking, making any comparisons of novel therapies difficult.

It is understood generally that wound healing and long-term outcomes are determined by burn depth. While partial thickness burns can heal spontaneously with minimal scarring, deep partial thickness and full thickness burns require more than three weeks to close and are often associated with significant scarring and functional limitations unless excised and grafted within the first few days of injury.[6] Thus, accurate diagnosis of burn depth is necessary in order to achieve optimal outcomes. It is also known that burn injury may continue to progress over the first few days leading to the conversion of superficial burns to deep burns. Better understanding of the mechanisms leading to burn wound conversion is likely to lead to novel therapies that limit burn wound progression leading ultimately to better healing.

Because burns are characterized by the presence of various amounts of non-viable, necrotic tissue, removal of the eschar and proper wound bed preparation are necessary, regardless of

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whether wound closure is to be achieved by primary or secondary closure. For these reasons, research in wound healing is necessary to identify the optimal approach to wound preparation and closure.

## 8.3 Priorities in wound healing research

Priorities in wound healing research were established at the State of the Science conference of the American Burn Association (ABA) in 2006[7], and included: 1. Development and validation of standardized tools to assess wound healing; 2. Defining, grading, and understanding the pathophysiology of hypertrophic scarring and pruritus; 3. Development of innovative treatment modalities for wound healing and scarring; and, 4. Optimization of wound healing. Those priorities were complemented by the ABA in 2012 with metrics for evaluation of burn wound healing, burn wound infection, wound closure, and identification of gaps in knowledge to promote opportunities for research [8].

To advance the current state of the science, members of the committee agreed upon the following priorities in wound healing research over the next ten years:

- Development and validation of inexpensive, simple and reliable methods to determine burn depth
- Better understanding of the pathophysiology of burn wound conversion and discovery of methods to prevent or reduce burn injury progression
- Need for alternative methods to achieve eschar removal while preserving viable tissue, and the development of tools to assist the surgeon in determining the endpoint for eschar removal in addition to bleeding of viable tissue
- Development of novel methods for early diagnosis of burn wound infection and better therapies to address biofilms, such as novel topical antimicrobial or antiseptic agents effective against multi-drug resistant organisms
- Development and validation of non-invasive and objective tools to measure wound closure
- Development of methods to accelerate wound closure and promote durable wound closure with less breakdown after closure
- Development of advanced skin substitutes, which contain all elements of native skin (i.e., epidermal barrier, basement membrane, dermis, hair follicles, sweat and sebaceous glands, nerves, vessels, pigment, immune cells) [9]

## 4.0 State of the Science

The current paper is not intended to be a comprehensive review article on all the topics identified as priorities for research. Rather, this section will summarize some of the past findings emphasizing the most recent promising developments in each respective area.

### 4.1 Determination of Burn Depth

Accurate determination of burn depth is one of the most important decisions in burn care. It not only predicts healing but also helps determine optimal therapy. Despite its critical importance, in most cases, clinical assessment is used for determining burn depth. Unfortunately, even when performed by an experienced burn clinician, the accuracy of clinical assessment is less than 75%[10,11]. As a result, a large number of methods have been developed and evaluated for increasing the accuracy of burn depth determination including radioactive isotopes, vital dyes, thermography, photometry, MRI, and ultrasound.[12] Of all methods, scanning laser Doppler imaging (LDI) is the most commonly used to help determine burn depth.[13] While some studies suggest an accuracy of up to 96–100% 2 days earlier than clinical assessment, others have found lower accuracy and called for greater validity and reproducibility.[14] A recent systematic review of 26 studies confirmed that LDI was more accurate and feasible to use than laser Doppler flowmetry.[15] However, due to several limitations, such as large size, high cost and long image acquisition times, LDI has not been widely adopted outside of research settings. Fast LDI, using a faster laser Doppler Line Scanner (LDLS) that scans 300 $cm^2$ in 4 s significantly reduces scanning time increasing its feasibility, especially in children. A recent analysis of 596 burn areas from 204 patients demonstrated an accuracy of 94.2% for the new scanner compared with 94.1% for the original LDI scanner.[16] Its smaller size and mobility were additional benefits of the new scanner. In this study, as in many other LDI studies, scans were performed between 2–5 days after injury. However, by 5 days after injury, clinical accuracy is greatly improved. Thus, there is still a need for reliable and simple methods that allows accurate depth determination as early as possible after injury.

Advancements in infrared thermography have also renewed the interest in this modality for diagnosing burn depth. A recent clinical study of 39 burns found that the overall accuracy of static thermography (using a hand held, commercially available camera) in predicting burn depth was considerably higher than that of clinical assessment; 87.2% (95% CI: 71.8–95.2) vs. 54.1% (95% CI: 37.1–70.2), as early as 48 hours after injury.[17] Active dynamic thermography, which measures the ability of tissue to conduct heat transfer relative to its surroundings, has also been explored in animal models with promising results.[18] Another promising modality is dermoscopy, which allows visualization of the microanatomy of the skin’s vasculature using a small, hand-held device. A study of 30 burns, which were assessed between 4–166 h after injury, demonstrated an accuracy of 96.7% at distinguishing between superficial and deep dermal burns.[19] A study comparing infrared thermography, spectrophotometric intra-cutaneous analysis and LDI in adult patients with burns demonstrated that both thermography and spectrophotometry were less expensive, easier to use and more acceptable to patients than LDI.[20]

Other novel modalities that have been recently evaluated for burn depth diagnosis include video-microscopy, optical coherence tomography (OCT), photoacoustic imaging and hyperspectral imaging.[21–25]

Attempts to enhance the accuracy of burn depth estimation have also combined several methods of assessment. For example, dual imaging with optical coherence tomography (OCT) and pulse speckle imaging (PSI) has been evaluated in a porcine model at 1 h, 24 h,

and 48 h after injury. The combined performance provided an overall Receiver Operating Characteristic-Area Under the Curve of 0.87.[26]

While there have been major technological advances in the area of burn depth diagnosis, an ideal or optimal method, which is non-invasive, simple, rapid, cost effective, and accurate as early as possible after injury remains elusive. Further research in this area is encouraged to attain this goal. For a more in depth review of recent developments in methods to diagnose burn depth the reader is referred to Paul et al.[27]

### 4.2 Limiting Burn Injury Progression and Conversion

Burn injury progression, or the conversion of superficial burns to deep burns, is characteristic of many burns leading to worse outcomes. While incompletely understood, a number of mechanisms likely play a role in its pathophysiology.[28,29] Injury progression occurs as a result of reduced dermal microcirculation, free radical generation, and the release of a large number of cytokines and wound modulators. Increased vascular permeability leads to tissue edema and together with hypercoagulability and vessel thrombosis further impair dermal perfusion. Based on these observations a large number of potential therapies have been evaluated in an attempt to limit inflammation and oxidative stress and improve dermal circulation thereby reducing burn injury progression and conversion. A comprehensive review of the many therapies evaluated thus far is beyond the scope of this paper, and is presented in greater detail in a review by Shupp et al.[29] However, to date, none of these therapies have been approved for use in limiting burn progression. This is not surprizing since the pathophysiology of injury progression is multifactorial and may vary from patient to patient, and burn to burn. Thus, multimodal approaches using more than one agent or therapeutic interventions, specifically tailored to the individual patient and burn, may be more effective than currently available unimodal approaches. However, better understanding of the underlying pathophysiology as well as the contributions of individual interactions between the genetic makeup of the burned patient and environmental insult may open up new avenues for exploration and development.

### 4.3 Eschar Removal

In 1970 Janzekovic reintroduced the concept of early excision and immediate grafting of deep burns.[30] Since this monumental publication, early excision of the burn eschar has become the standard of care for deep burns[31]. While the critical importance of early eschar removal and wound coverage is well accepted, the exact method and timing remain the subjects of debate. While very effective, in its current form, tangential excision is traumatic, requires specialized personnel and equipment and is often delayed several days until accurate burn determination can be made, especially in mixed and indeterminate depth burns. Furthermore, the extent of excision is based on visual presence of bleeding of the viable wound bed, which may not always be accurate. A histological study of excised burn eschar suggests that not uncommonly, normal viable tissue is sacrificed while non-viable eschar may remain[32]. Thus, additional methods that enhance surgical assessment of the endpoint of excision beyond visualization of bleeding may further improve the selectivity and specificity of this time honoured procedure. Alternative methods of eschar removal that

are less traumatic and more selective than excision include laser debridement and rapid enzymatic debridement.

An observational study has suggested that topical application of methylene blue to burns with mixed depth may help to distinguish between viable and nonviable tissue facilitating more precise and complete wound debridement.[33] A hydrosurgery system has also been advocated for burn wound debridement, with the suggestion that enhanced preservation of dermal tissue might reduce subsequent scarring. Thirty-one children underwent conventional debridement, and 30 underwent hydromechanical debridement. There was a significant difference in the amount of viable dermal preservation between the two groups ($p=0.02$), with more viable tissue lost in the conventional group (median 325 μm) versus the hydrosurgery group (median 35 μm).[34] However, there was no difference in graft take and 3–6 month scarring. Thus further refinements or alternative methods are required in order to improve upon conventional surgical excision.

Use of laser for burn eschar debridement has also been reported, but mostly in experimental animal models of burns[35–37]. A study of $CO_2$ laser ablation in full thickness porcine burns demonstrated that long-term scarring, based on Vancouver scar scale assessments and histologic evaluation, was equivalent at 6 months in laser-ablated and sharply excised burns[36]. While use in humans is limited,[38] a pilot study in 21 children with full thickness burns demonstrated the feasibility of laser vaporization of burn eschars in patients with successful immediate autografting[39]. Since then, major refinements and advances in laser technology have occurred that will likely further enhance this methodology for eschar removal.

While enzymatic debridement is not new,[40] a bromelain based enzymatic debridement agent has recently been approved by the European Medicines Agency (EMA) and is currently undergoing an FDA regulated phase 3 clinical trial. With this agent, rapid debridement is achieved after a single 4-hr application in most cases. A recent RCT included 182 patients aged 4–55 years with deep partial and full thickness burns covering 5–30% of their total body surface area (TBSA). The enzymatic agent significantly reduced the time from injury to complete debridement (2.2 vs. 8.7 days, $P<0.0001$), need for surgery (24.5% vs. 70.0%, $P<0.0001$), the area of burns excised (13.1% vs. 56.7%, $P<0.0001$) and the need for autografting (17.9% vs. 34.1%, $P=0.01$) compared with standard surgical or non-surgical treatment[41]. Furthermore, scar quality and quality of life scores were similar in both study groups as were the rates of adverse events.

### 4.4 Management of Microbial Contamination and Wound Infection

Loss of the *stratum corneum* of the epidermis provides an entry point for environmental microorganisms which persists until the outermost layer of the skin is restored. In partial thickness burns, this period may range from a few days to a few weeks during which microbial organisms may proliferate, form biofilms, impede wound healing, or invade open wounds. As discussed above, early removal of the burn eschar and frequent cleansing of the wound will slow microbial progression. Topical application of antimicrobial agents, such as bacitracin, nystatin and silver sulfadiazine [42,43] have been shown to promote restoration of the *stratum corneum*, to initiate the long-term closure of the wound, and to support

resolution of the inflammatory phase of healing. More recently, dressings that release elemental silver have been shown to be effective in reduction of wound contamination on partial thickness burns, and to require less nursing care than administration of traditional topical antimicrobials [44].

Current management of full-thickness wounds includes early excision, or enzymatic debridement, both of which require a temporary coverage of open wounds with either cadaver allograft, or an acellular skin substitute with an artificial barrier, such as silicone or polyurethane [45–47]. In full-thickness burns of large TBSA, wounds may remain open and at risk for wound infection for months. In addition to the environmental sources of wound organisms, the gut and the lungs also become sources of wild-type and antibiotic-resistant organisms. Due to the ubiquitous nature of microorganisms in the hospital environment, prophylactic administration of highly-effective topical agents, such as 5% v/v mafenide acetate solution have been used for extended periods until autografting can be completed [48]. However, 5% mafenide acetate solution has been shown to be cytotoxic to tissue engineered skin substitutes [49]. Tissue-engineered grafts containing cultured keratinocytes and/or fibroblasts have been used with mixtures of antimicrobial agents that cover common types of Gram-negative, Gram-positive and fungal organisms without toxicity to the transplanted cells, or to the angiogenic response of the ingrowing vasculature [50,51]. Although these alternative approaches may help to control microbial overgrowth in open burn wounds, risks of complications remain until the wounds are closed.

Among the greatest challenges of microbial management of burn wounds are multi-drug-resistant organisms which may lead to invasive wound infection, and potentially lethal complications, such as pneumonia, sepsis and fasciitis. Several species of bacteria, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococcus* (VRE), Carbapenem-Resistant *Enterobacteriaceae spp.* (CRE), *Pseudomonas spp.*, non-albicans *Candida spp.*, and *Aspergillus spp.*, have been associated with increased mortality after burns [52,53]. Most of these strains have nosocomial origins, can spread within burn units, and be very difficult to eradicate. Although a new generation of antimicrobial agents is being developed [52,54], it is often necessary to use agents with high chemical toxicity, such as chlorhexidine gluconate, which may impede wound healing while controlling wound infection.

Ultimately, wound closure with autologous epithelium restores both a source of innate immunity, such as defensins and cathelicidins [55,56] in the epidermis, and a full complement of immune effector cells in the dermal tissue. Together with improved nutrition to maintain the cellular immunity [57,58], accomplishment of wound closure with autologous epidermis remains a definitive factor to long-term control of microbial contamination and infection of burn wounds.

### 4.5 Measuring Wound Closure and Scar

Simultaneous with these novel advances, an array of non-invasive, biophysical instruments has become available to provide absolute, objective measures of cutaneous qualities including color, shape/surface texture, visco-elastic pliability, blood flow, surface hydration and water vapor transpiration. With regard to wound closure, skin surface hydration

measured by electrical capacitance has been reported after treatment of excised, full-thickness burns with meshed and expanded skin autograft, or engineered skin substitutes.[59,60] Because a dry epithelial surface is easily determined by an experienced clinician, direct tracing of wounds, or scaled photography, followed by image analysis has also been used reliably and accurately to quantify burn wound closure. [61,62] Most of the non-invasive instruments have been validated for clinical use in the EU, but not in the US. [63] An important source of error in most assessments with instruments is the measurement of individual points within the wound, rather than the entire wound as a heterogeneous field. Therefore, use of a uniform sampling pattern is critical to validity of instrumental measures. Instruments, such as the scanning LDI described above, overcome this limitation by assessment of the entire wound field. These kinds of instruments complement traditional ordinal scoring of scars by a trained assessor as with the Vancouver Scar Scale [64], by the patient in the Patient-Observer Self-Assessment Score (POSAS), or by the University of North Carolina “4P” assessment of pruritus, pain, parasthesias and pliability [65,66] scales of scar assessment.

### 4.6 Accelerating Wound Healing and Durable Wound Closure

For wounds that are judged not to require grafting, negative pressure wound therapy (NPWT) has been reported to facilitate epithelial closure[67]. NPWT has also been reported to promote engraftment of split-thickness skin grafts by improved immobilization.[68] Despite numerous anecdotal reports of the benefits of NPWT in treatment of burns, there are not yet sufficient well-controlled, prospective, randomized clinical trials to validate any putative benefits of NPWT in burns.

### 4.7 Skin Substitutes and Tissue Engineering

In addition to the potential benefits of devices to promote, revise and assess healing wounds, the fields of tissue engineering and regenerative medicine have begun to offer cellular therapies for burns, other cutaneous wounds, and most of the tissues in the body. Since 2006, advanced models of engineered skin substitutes have been described that consist of allogeneic cells to provide temporary protection of wounds, and to promote epithelial closure with autologous epidermal keratinocytes in the wound, or dermal-epidermal autografts [69,70]. An initial model of gene therapy with an engineered skin model secretes elevated levels of the native antimicrobial peptide, cathelicidin, and has been cleared for clinical trial[56]. Engineered skin substitutes with autologous keratinocytes are capable of providing sufficient epidermal cell populations to cover burns up to 99% TBSA, and may be applied as keratinocyte sheets, sprays of cell suspensions, or bi-layered compositions with both epidermal keratinocytes and dermal fibroblasts [71]. These kinds of cell therapies for skin wounds have been shown to provide definitive wound closure that enables survival after life-threatening burns [70]. Some of these models are applied as stratified epithelial sheets which suppress granulation tissue and scar. Most, if not all of these cell therapies leverage the biological capabilities of exponential cell replication to generate large cell populations to conserve donor tissue for expansion far beyond conventional standards for meshed split-thickness autografts. Conservation of donor skin also increases availability of sheet autograft for early grafting of critical areas, such as the face, hands, feet and perineum.

Despite these important advances with cell therapies, current models are based upon post-natal wound healing physiology which does not stimulate regeneration of native microvasculature, sensory nerves, pigmentation, sweat glands, sebaceous glands, hair follicles, or native dermal extracellular matrix. These structures form only during fetal development, but are required to restore complete anatomy and physiology of the skin to an uninjured condition. This complete restoration to the uninjured condition distinguishes ***tissue engineering*** based on mechanisms of wound healing from ***regenerative medicine*** based on mechanisms of embryonic and fetal development. [9] Because of the categorical importance of true regeneration of uninjured tissues of all types, there has been explosive growth in the fields of regenerative medicine, stem cell biology and gene therapy. Due to the relatively rapid expansion of knowledge in the normal development and biology of human skin, it may be predicted with confidence that discoveries of basic science knowledge will be translated into new therapies with capabilities to minimize morbidity, and restore a more normal quality of life.

Concurrently with the development of advanced cell therapies, regulatory standards for determination of safety and efficacy have also continued to advance[72]. Among the several criteria for evaluation, determination of medical risk is a fundamental factor. Because most of the novel compositions for transplantation of cells and degradable scaffolds have no precedent, by definition, they fall by default into the highest category of regulatory risk, which is Class 3 for devices. A high risk category places the highest burden of proof on the developers and sponsors of the therapies, and leads to very long and costly studies to satisfy the regulatory statutes for safety and efficacy. It may be anticipated that as precedents for cellular therapies become established, and consensus designs emerge, that time and resources to obtain regulatory permissions for marketing may decrease.

## 5.0 Summary of Roundtable Discussion

While the group participants agreed that much progress has been made since the last State of the Science conference, greater advancements in burn depth diagnosis, wound bed preparation, microbial management, wound closure and the development and use of skin substitutes are still needed greatly. Of particular importance was the perspective of the burn survivors. According to these key stakeholders, two of the most important and unmet needs of burn survivors are finding more effective ways to relieve burn itching, and avoiding recurrent and problematic skin breakdown, after initial wound closure. The group acknowledged that little was known regarding the frequency, causes, and consequences of recurrent skin breakdown. More effective management of this lack of durability of skin closure certainly will require better understanding of the mechanisms leading to skin breakdown. However, more durable methods of wound closure are clearly needed.

Recognizing that burn depth is a dynamic and progressive process as well as a major determinant of outcome, better understanding of the mechanisms leading to the conversion of superficial burns to deep burns is needed in order to develop novel methods to reduce burn wound progression. This will probably require multi-pronged approaches using various therapeutic “cocktails” that combine multiple agents targeting different pathways. In addition, objective, simple, inexpensive, non-invasive methods that allow accurate burn

depth diagnosis, as early as possible after injury, should also be a major research focus in the years to come.

The major importance of early and selective burn debridement and eschar removal, regardless of method of wound closure, was discussed. Surgical methods of removal of the eschar (such as tangential excision) remain the standard of care for deep partial thickness and full thickness burns. However, this method is somewhat crude sometimes sacrificing viable tissue while leaving non-viable tissue behind. Furthermore, the endpoint for surgical excision is somewhat subjective and operator dependent. Thus better methods for removing the eschar and determining the endpoint for debridement are needed. Less invasive methods such as hydrotherapy, laser ablation, and rapid enzymatic debridement will need to continue to be developed and evolve.

Microbial contamination of burn wounds, local infection and sepsis continue to be major problems leading to considerable morbidity and mortality. The emergence of numerous, multi-drug resistant organisms has further complicated matters. The development of methods that allow early detection of wound contamination and infection, before the patient becomes septic, were also noted to be an important area for future research. The development of novel topical and systemic antimicrobial agents effective against the emerging multi-drug resistant “superbugs” will require greater cooperation and collaboration between industry and academia. Furthermore, the central role of biofilm formation was recognized and will need to be addressed if effective yet nontoxic therapies are to be developed successfully.

Wound closure continues to be one of the most important outcomes of burn care. Wound closure not only reduces evaporative water losses and protects the patients from contamination and infection, but it is also associated with subsequent scarring. While a number of objective methods of measuring wound closure have been proposed, clinical assessment remains the most commonly used method to verify closure. More objective methods that are simple, inexpensive, and non-invasive are clearly needed. Additional development of methods aimed at accelerating closure of the burn and donor sites is also needed.

With regard to skin substitutes, the group acknowledged the major advances made over the last decade. Considerable regulatory and scientific barriers still remain, which have slowed down development. To address the regulatory delays, the group expressed strong interest in development of a collaborative dialogue with the FDA to facilitate evaluation and future availability of novel burn therapies. The need for skin substitutes that contain all of the essential elements of the skin including an epidermal barrier, a durable yet elastic collagen based dermal matrix, pigment cells, blood vessels, nerves, hair follicles, sebaceous glands, sweat glands and immune cells was reemphasized. Availability of a multi-layered, multi-component, non-immunogenic, off-the-shelve, universal skin substitute remains one of the most important objectives of burn wound management.

Together, the participants in the roundtable discussion on burn wound healing and tissue engineering identified these several objectives as areas in which active research may

improve outcomes for burn patients during the next ten years and beyond. With a perspective on the momentum of burn research since the 2006 State of the Science conference to the present, it can be predicted with confidence that most, if not all, of the topics discussed here will be addressed, and that new knowledge will generate additional reductions in mortality and morbidity from burn injuries in the years ahead.

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