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Scientific Review

State of the Art in Burn Treatment

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Abstract. Optimal treatment of burn victims requires deep understanding of the profound pathophysiological changes occurring locally and systemically after injury. Accurate estimation of burn size and depth, as well as early resuscitation, is essential. Good burn care includes also cleansing, debridement, and prevention of sepsis. Wound healing is of major importance to the survival and clinical outcome of burn patients. An ideal therapy would not only promote rapid healing but would also act as an antiscarring therapy. The present article is a literature review of the most up-to-date modalities applied to burn treatment without overlooking the numerous controversies that still persist.

Burn injuries produce overwhelming physiologic and psychologic challenges—more than any other type of injury. In the United States alone, over two million cases of burn injuries per year are brought to medical attention. Many of these injuries are minor; however, about 20,000 patients (1%) sustain severe burns, enough to require admission to a specialized burn unit. These patients develop various complications requiring special care and management [1, 2] such as dealing with fluid shifts, monitoring electrolyte imbalances, proper wound care, respiratory support, treating infections, and sometimes treating sepsis and multiple organ failure syndrome. The occurrence and subsequent management of problems is variable depending on their time of onset following the burn injury.

The physiologic responses to major burn trauma are numerous and include a fall in arterial pressure paralleled by an increase in pulse rate and a progressive decrease in cardiac output and cardiac stroke volume. Early compensatory mechanisms include circulatory reflexes attempting to restore normal vascular functional balance. Metabolic responses are complex and include metabolic acidosis, hyperventilation, and respiratory alkalosis; as the cellular energy levels fall after burn injury, sodium and potassium adenosine triphosphatase is altered, resting cell membrane potential decreases, and cellular accumulation of sodium, calcium, and water is paralleled by a loss of cellular potassium. Immunologic responses include altered macrophage function and altered immunity at both the cellular and humoral levels, and hematologic responses to burn trauma include altered coagulation [3, 4].

Great improvements have been achieved over the past few decades to reduce morbidity and mortality related to burn injuries. Increasingly aggressive surgical approaches with early tangential excision and wound closure probably represent the most significant change in recent years, leading to improvement in mortality rates of burn victims at a substantially lower cost [5–9]. By shortening the hospital stay, early burn wound closure reduces the infective complications. Faster healing decreases the severity of hypertrophic scarring, joint contractures, and stiffness and promotes quicker rehabilitation [6]. However, if health care providers are inadequately prepared, the provision of medical care to burn victims can induce further trauma and result in less than optimal outcome. Irrespective of the burn etiology (flame, contact, scald, chemical, radiation), an accurate assessment of the burn pathology is a crucial early step in treatment planning.

Initial estimation of the surface area burned as well as the depth of injury at the time of a patient's admission to a burn care facility is essential. Though total body surface area (TBSA) burned is more important during the initial assessment than depth (superficial dermal, deep dermal, full thickness), the two parameters are the most important predictors of clinical outcome. The percentage of TBSA (% TBSA) affected is used to calculate the patient's fluid and nutritional needs, which can be enormous for those with severe burns. Burn depth, on the other hand, dictates subsequent local and surgical treatment of burn wounds. Therefore proper and effective evaluation and management of the wound is central to successful management of burns of any size or depth. If a careful initial evaluation is made, basic principles of management are applied, and the response to therapy is regularly monitored, reliably excellent results can be expected for the large majority of patients [10].

Estimation of Burn Extent

Accurate assessment of TBSA is one of the most important aspects of initial care in the emergency department. It determines whether transfer to a specialist burn unit will be required, as well as the magnitude of initial fluid resuscitation [10]. It is also important for determining fluid requirements, calculating nutri-

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tional requirements, and estimating outcomes [11, 12]. The initial assessment is also used as a benchmark against which to monitor patients' progress and as a research tool to compare effects of different treatments. In almost 80% of cases, however, estimation of the surface area involved is made by the most inexperienced staff [13, 14].

Estimates of the relative surface area of various parts of the body as % TBSA were first made in 1924 by Berkow [15]. At present, different methods exist to calculate the extent or size of a burn injury that may be used sometimes in combination [14]. The "Rule of Nines" and the Lund and Browder charts are the two most commonly used methods [10, 12, 16]. The Rule of Nines provides a quick estimate of burn size; however, it usually overestimates the burn size and does not allow for body proportion differences with age. It does not reflect accurately the surface area of children less than 15 years of age, underestimating the head surface area and overestimating the extremities in children. The Rule of Nines is, nevertheless, simple and rapid to apply. The Lund and Browder chart, which accounts for variations in relative surface areas with age and sex, provides a more accurate estimate of the burned surface area [17, 18]. The method, however, is more time consuming to calculate, requiring a table for all ages. Irrespective of the method used, irregularly shaped burns and burns on the trunk and thighs are usually expressed with greater variability than less irregularly shaped burns or burns on more defined anatomical parts of the body. The incidence of error is greater in smaller burns [19]. Burns around 20% TBSA are most accurately assessed, with smaller burns being overestimated and larger burns underestimated [20]. With both Lund and Browder and "Rule of Nines" diagrams, variability in burn size estimation increases initially with burn size, plateaus in large burns, and then decreases slightly in extensive burns. More burn experience, however, leads to less variability in burn area chart drawing estimates [15].

A less frequently used method is the patient's own hand as a complementary, readily available template for estimation of burn area or other areas of disease or injury [16, 21]. The surface area of the palm has been estimated to be 1% of the body surface area [11, 13, 21]. Early twentieth century literature suggests that rather the palmar surface of the entire hand approximates 1% of the TBSA [11]. This is true only in children. In adults, the entire palmar surface corresponds to 0.78% $\pm$ 0.08% of the body surface area. The percentage varies somewhat with age and reaches a maximum of 0.87% $\pm$ 0.06% in young children [21]. Moreover, mean hand palmar surface area (HSA) diminishes significantly as body mass index (BMI) increases in both sexes ($p < 0.001$). This effect is more pronounced in women, particularly those with a BMI greater than 31 kg/m^2, in whom the HSA represents only 0.64% [13]. In general, the 1% estimate of HSA will lead to an overestimation of the burn wound size, particularly in adults [13, 21]. It has been suggested recently that a simple product of length multiplied by width of the hand will closely approximate the hand palmar surface area as determined by planimetry. This would allow a more accurate estimation than the 1% estimate [21].

Errors in estimation of burn size are commonplace. Initial estimates of the area involved in a burn injury by inexperienced clinicians are frequently excessive [13]. Outside reports of burn size and depth are notoriously unreliable, especially from referring physicians with little experience with burns. There appears, however, to be little variance in estimation of burn size made by experienced burn nurses and physicians [16]. In general, burn size is estimated correctly only one third of the time. Overestimation at the transferring emergency room is almost 100%. This incorrect burn size estimation seems related to reliance on guesswork [22]. Significant burn size overestimation ($p < 0.01$) and large interrater variability can potentially have serious effects on calculation of initial fluid resuscitation, nutritional requirements, prognosis, and comparisons of treatment protocols among burn centers [19, 22]. On average, inaccuracies in fluid resuscitation result in patients receiving twice the appropriate volume of fluid for the burn size [20].

There are some problems in accuracy that are common to all chart estimations methods. A patient's body rarely mirrors the idealized forms on the standard charts. Most significantly, all the charts require the representation of a three-dimensional (3-D) burn on a two-dimensional (2-D) drawing [15, 23]. Lateral burns are not well represented on such drawings and are often underestimated, though they may constitute the majority of the burned area [15]. Moreover, reliance on 2-D charts results in wide variation in assessment of identical injuries by different professionals. An easy-to-use, 3-D computer graphics program is bringing a new level of accuracy, consistency, and standardization to the evaluation of burn patients, which should result in more precise treatment plans and better evaluation of new therapies. The software, developed by a team of researchers at the University of Chicago Hospitals' Burn Center, replaces the standard two-dimensional hand-drawn charts of a patient's wounds with a morphable 3-D computer (American College of Surgeons Clinical Congress, Chicago, October 12-17, 1997). Using a mouse or graphic tablet, instead of pencil on paper, the nurse or physician can adjust the diagram to match the contours of the patient's body, chart the extent and depth of the burn wounds as seen from any angle, and compute the percentage of total body surface area burned as well as fluid and nutritional requirements. The computer program is more accurate and far more consistent than the standard system for determining burn surface area, especially for moderate burns, where precise information can make the most difference. Studies comparing the computer-assisted assessment with standard burn assessment found that the computer is much more reliable and consistent, particularly for larger burns. The average error for physician estimate was 42% compared to 29.6% calculated by a computer-assisted program. Additional benefits of computer assistance include a permanent record of injury, burn wound trend analysis, and meaningful statistics involving morbidity, mortality, and comparative treatment protocols among burn centers [24].

Surface Area Graphic Evaluation (SAGE) is a new computer-based modality for estimation of burn wound extent. SAGE 2 is the Palm OS version of the SAGE burn diagramming program and is gaining quick popularity. This version provides a quick way to diagram a patient's burn injuries and automatically generates burn area estimates, as well as Parkland fluid resuscitation estimates for burns over 20% TBSA. It calculates age-adjusted estimates for TBSA, partial, deep, and user-selected palettes, in percentages and cm^2 for 33 body areas. The SAGE 2 program is accessible via the Internet [http://www.sagediagram.com/]. It takes, however, some practice before users become comfortable with drawing diagrams.

The calculation of burn size, even using the most sophisticated systems, contains a certain amount of subjective evaluator bias

[15]. Though computerized mapping devices may lead to more accurate estimates and provide permanent easily accessible documentation [25, 26], these remain estimates after all. The cost of such systems must be weighed against the value of the product (precise burn area estimates and resuscitation fluid volumes calculations) and the use for which the data are obtained [15]. It must be stressed that fluid volumes calculations are used only to initiate fluid therapy and as a guide to fluid replacement, not the absolute rule. Consequently, exact burn area is really not extraordinarily clinically relevant, but is more necessary from an epidemiological perspective [15].

Assessment of Burn Wound Depth

Four levels of burn injuries based on clinical assessment and estimation of outcome are generally recognized. These are superficial epidermal first-degree burns, superficial partial-thickness second-degree dermal burns, deep partial-thickness second-degree dermal burns, and full-thickness subdermal third-degree burns [27, 28]. Superficial epidermal as well as partial-thickness dermal burns heal spontaneously, whereas treatment of deep second-degree and third-degree burns requires early surgical excision and grafting [29, 30]. Determination of depth of injury in burn, wounds is therefore a key decision for proper burn management [27, 28, 30–35] and methods for objective and reproducible measurements are of great clinical interest [36].

Burn wound depth is difficult to determine. Clinical assessment is usually accurate for very deep and very shallow burns that do not constitute a real challenge to clinical judgment. Accurate assessment of depth immediately after injury for partial-thickness burns, however, has always been difficult [37]. Even for experienced investigators, the exact differentiation between superficial and deep dermal burns is not always possible and usually is highly inaccurate [36, 38, 39]. It is difficult to distinguish superficial partial-thickness burns, which will heal well without surgical intervention, from deeper burns requiring excision and grafting on clinical grounds alone [28, 32].

There are many possible causes for inaccuracy in the visual assessment of burns, one of which is that burns are dynamic wounds and are in a state of change for up to 72 hours after injury and may be influenced by resuscitation conditions [28, 33, 40–43]. Thermal trauma causes two different types of injury within the burn wound. First an immediate and irreversible injury, and second a delayed and potentially reversible injury in the area bordering the center site, which is called the *zone of stasis* and is characterized by established edema, which in turn is surrounded by an area of inflammation and active edema formation due to vasodilatation and increased microvascular permeability [28]. Excessive local edema accumulation is followed by a reduction in perfusion, leading to more local tissue ischemia. Twelve to 24 hours postburn, local microcirculation is compromised to the worst extent [41]. Depth of superficial burns seems to remain stable with time, whereas that of deep dermal burns increases with time [33], and burn wounds continue to demarcate for several days [44].

Clinical Evaluation

Measurement of burn wound depth remains an inexact science relying on clinical subjective evaluation and serial examinations [27, 29, 30, 33, 45, 46]. The first diagnosis is usually performed by an emergency surgeon more or less experienced in burn-medicine. A more reliable clinical assessment can be performed after initial wound cleansing and debridement and this also is dependent on subjective visual impression. Clinical assessment relies on burn wound appearance, blanching, capillary return, presence and degree of fixed capillary staining, and evaluation of retained light touch and pinprick sensation [32, 33]. This requires sufficient clinical experience which greatly influences the results. It is also subject to variation between examiners [47]. Often the true extent of the damage is only realized after a few days or at the time of the first operation [34]. Visual and tactile assessments are still in widespread use; however, they are in general highly inaccurate, even if done by experienced clinicians [28, 29, 31, 48–50]. None of the clinical modalities used to assess depth have been demonstrated to be 100% reliable at predicting the length of time to final wound healing and some studies report only 50-65% accuracy rates in determining those partial thickness burns that will not heal within 3 weeks [33, 51]. Clinical assessment alone is accurate only about 64%-70% in diagnosing burns of intermediate depth [32]. The most common error as reported by Niazi, is overestimation of burn depth [46, 49]. Sometimes misleading results can be deduced [28]. Conversely, clinical judgment often vacillates between inability to determine depth, expected healing, and need for surgical excision [46].

Digital Imaging

Photographic images have been used extensively in teaching about and researching burn therapy. The advance from analogue to digital imaging opens new horizons for telemedicine in burn diagnosis and management. It allows the remote transmission of the clinical information contained in the digital image of a burn as non-compressed images in original bitmap (BMP) format (mean size: 1500 Kb) or compressed images with a Q index of 50 (30 Kb files). There is good agreement between the diagnoses of burn depth made using the digital images and those made in person [52]. Accuracy of burn depth estimation from transmitted images is 90% when compared with clinical diagnoses [53]. Burn depth assessment by digital imagery does not, however, offer any real advantage over direct clinical examination. The modality cannot be adopted as an innovative method to improve diagnostic accuracy.

Biopsy

Histologic assessment of burn wound biopsies has been considered the gold standard of burn depth assessment with which the accuracies of other techniques are compared [28, 32, 46]. However, widespread clinical use of the technique has been precluded because of its invasive nature, need for multiple biopsies of different areas of the wound, added scarring, sampling error, delay in diagnosis, and the need for an experienced pathologist [32, 33]. Moreover, no agreed technique for the accurate histological assessment of burn depth is yet available [33]. Some investigators have stressed the importance of assessing the precise level of vascular patency in punch biopsies whereas others attempted to measure the extent of interstitial and cellular destruction [33, 54, 55]. Either way, the analysis is that of structure rather than

function of cells and may be inaccurate if the samples are taken too soon after injury [28].

Microvascular damage is one of the most obvious signs of damage in burns and has been considered to be highly valuable in the prediction of outcome of partial-thickness injuries. The examination of vascular patency appears to be a much more sensitive indicator of tissue damage than the observation of parenchymal changes, such as denatured collagen, which is found only after a much greater dermal insult [33]. Nevertheless, assessment of vascular patency in biopsy specimens remains highly subjective. It is also very difficult to determine the point that exactly corresponds to the final extent of tissue damage. Even in small tissue samples of burn biopsies, there is considerable variation in the depth of vessel patency. Not infrequently, blocked veins are located adjacent to patent arteries. Great care must then be taken in interpreting histological findings. Multiple measurements within the same specimen must be made; the depth of the most superficial patent and deepest blocked vessels should be determined, taking the mean value as the final figure for burn depth. To compensate for the variations in tissue shrinkage that occur during tissue processing and fixation, expressing the burn depth as a percentage of the total dermal thickness might be a realistic approach [33]. Nevertheless, burn biopsies and their microscopical examination provide only a snapshot view of the level of microvascular injury and the degree of tissue viability [33, 56] that remains highly subjective as it is determined by the personal experience of individual pathologists.

Efforts to increase objectivity of burn depth determination have been widespread and include both invasive and noninvasive techniques [27, 46].

Measurement of Tissue Perfusion

It has often been suggested that the blood flow in injured tissues indicates tissue damage extent. Burns of specific depth have distinctive perfusion patterns. Significant differences in average perfusion are observed between deep burns requiring surgical treatment and more superficial ones with regenerative capabilities [41, 57]. Superficial second-degree burns have perfusion values greater than those of normal skin, whereas perfusion in deep second- and third-degree burns is compromised [29]. Based on the assumption that assessment of skin perfusion can help to make accurate and correct burn depth estimation [41], several measuring modalities have been developed.

Laser Doppler Flowmetry (LDF) and Laser Doppler Perfusion Monitoring (LDPM). Light waves undergo a frequency shift when reflected by moving objects such as red blood cells, whereas light reflected by stationary structures remains unchanged in frequency [30, 32, 41]. In 1975, Stern introduced LDF for measuring cutaneous microcirculation. Clinical use of this technique was first described in 1984 by Micheels et al. [204]. The first biomedical laser Doppler instruments for the assessment of tissue perfusion used fiber-optic probes to deliver and receive reflected light at a specific point on the tissue surface and measures perfusion through 1 mm of tissues [41, 57, 58]. Although LDF instruments provided useful dynamic measurements of tissue perfusion, allowing cutaneous perfusion to be determined on a flux scale [30], they are limited to measuring a small fraction of the microvasculature, which is usually heterogeneous [57]. Because of this spatial heterogeneity, several measurements are needed to achieve an acceptable variance [46]. The technique also measures perfusion in only one single spot [29]. Mapping of the whole burn area would be a very lengthy procedure [46]. Only part of the total burn can thus be assessed, with the consequent risk of erroneous diagnosis as a result of sampling error [30]. Another disadvantage of this technique is that it requires contact between a probe and the skin, with the possibility of direct and cross-contamination. Pressure exerted for application of the probe also leads to alterations in local microcirculation and may be painful [30, 41]. As the flux scale is arbitrary, the absolute value used to determine the difference between superficial and deeper burns has varied greatly between studies, creating much confusion [30]. Moreover, flux alone is not a reliable indicator of the potential for healing. To achieve acceptable burn depth estimation with this technique, heating the low flux areas is required to distinguish deep burns from normal skin. The response of burn wounds to heat stress enables the study not only of the blood flow, but also the capability of the burn wound vasculature to dilate in response to heating and the study of flow motion pattern (vasomotion), which becomes more obvious at higher temperatures [50]. On the whole, the technique is not practical for routine clinical application [46].

Laser Doppler Imaging (LDI) and Laser Doppler Perfusion Imaging (LDPI). Recently, biomedical laser instruments have expanded to include a new method for measuring tissue blood flow called LDI, combining laser Doppler and scanning techniques and avoiding the disadvantages associated with LDF as the whole burn may be sampled with a non-contact device [29, 30, 57, 59]. Two-dimensional blood flow images obtained provide color-coded maps of tissue perfusion that can be analyzed to measure blood flow to a specific area [57]. The technique, however, is not without inherent limitations and technical difficulties including instrumental as well as pathophysiological causes such as the type of equipment, scanning distance, curvature of tissues and appearance of the wound [29].

At present there are two laser Doppler perfusion imagers available for clinical applications [57]. The Lisca PIM 1.0 imager (Lisca Development AB, Linkopen, Sweden) and the Moor LDI (Moor Instruments, Devon, UK) [29, 57]. Although these instruments are based on the same principles of photon-tissue interaction, they use different methods of light delivery, detection, and signal analysis that influence their measured response to blood flow changes [57, 60]. The Moor instrument uses two lasers and can perform perfusion scans at red and NIR (near infra-red) wavelengths. The deeper penetrating NIR wavelengths impinge upon deeper lying vascular structures and produce significantly different perfusion maps than red wavelengths, providing more information on tissue vascular structure [57]. The Lisca imager requires longer scan times and does not have a filter over the photo-detector, hence measurements must be performed in a dark room to eliminate artifact from ambient room lighting [57].

As an optical technique, LDI depends on laser wavelength, laser power, scanning distance, curvature of tissue, appearance of the wound, and tissue optical properties [29, 41, 57, 60]. Optical properties vary from tissue to tissue and from region to region within heterogeneous tissues; they are not only specific to the tissue of interest but can change during tissue injury and healing [57]. Burn wound infection can adversely affect LDI determination of burn depth [46]. The correlation between perfusion values and depth of burns is not straightforward. It may be difficult to

determine which level of perfusion level corresponds to which depth of burn [29]. Though the perfusion maps generated may be very useful, the assignment of color palettes to the perfusion levels is highly subjective and can vary between instruments and laser wavelengths [57]. Digital photography, sequential perfusion imaging, as well as imaging following heat provocation for a period of 15 minutes are a valuable if not essential addition to LDI [29, 30]. The use of digital photography results in an accurate match of the perfusion data and the burn itself, and it enables visual assessment and assessment by perfusion data at the same time [29]. However, because of current software limitations, claims of LDI superiority at identifying burn wounds requiring surgery are limited to (unambiguous) visual interpretation of the actual scans [46]. The qualitative nature of the investigation requires informed interpretation of the scan for reliable diagnosis of burn depth, similar to the process of radiologic interpretation of a computed tomography or an ultrasound [30]. Nevertheless, the accuracy of assessment by LDI can reach 99% if infected wounds are excluded [46]. By calculating the average perfusion, it is possible to discriminate between wounds that would or would not heal spontaneously within 3 weeks. The second measurement on day 4 seems to be more useful for this purpose [41].

Indocyanine Green (ICG) Video Angiography. ICG video angiography is an improvement of the earlier technique of ICG fluorescence angiography, which produces normal light ICG still images and provides superior visualization of tissue perfusion [27, 32, 61]. In both techniques a single dose of ICG is injected intravenously (ICG-PulsionR, Pulsion Medical Systems, Munich, Germany or Cardio Green, Becton Dickinson Microbiology Systems, Cockeysville, MD) [27, 32]. ICG is a water-soluble tricarbocyanine dye that has been used for over 40 years to measure cardiac output, hepatic function, and blood flow, and ophthalmic angiography [32]. ICG is relatively safe, with few reported adverse reactions, and is efficiently removed by the liver and excreted into bile [30]. Perfusion of burn wounds is assessed using the technique of laser-fluorescence-videography (IC-VIEW, Pulsion Medical Systems) consisting of a digital video camcoder and a NIR light [27]. Acquired digital video shows the uptake, steady-state distribution, and clearance of the dye. Using the well-perfused non-burned skin as reference, the ICG-real time videos can be interpreted qualitatively as well as quantitatively according to the staining intensities. It is an objective method for the observation of dynamic changes in burn wound perfusion. The method can be a practical, accurate, and effective adjunct to clinical methods for determining burn depth and burn wound progression [27]. However, the most commonly used ointments and dressings have a massive influence on ICG video-angiography and its measurements. They induce decreases in absorption of up to 63% $\pm$ 36% and thereby falsely report deeper burn wounds. In clinical practice, all dressings, ointments, and blood should be completely removed at least 10 minutes prior to measurement by ICG video-angiography to gain exact and reproducible results [36].

Fluorescein Fluorescence. Fluorescein was first reported in 1943 in determination of burn depth. Intravenously injected fluorescein fluoresces at 560 nm (yellow) when illuminated with a 360-400 nm ultraviolet (UV) light source. Because of the shallow penetration of UV light, the technique can evaluate only the superficial cutaneous circulation and is unable to distinguish between superficial and deep partial-thickness burns. Viable cutaneous tissues covered by eschar cannot be detected. Unlike ICG, fluoresein does not bind readily to serum proteins and is more likely to leak from normal vasculature [32]. Repeat doses are not possible until the dye is completely excreted.

Non-Fluorescent Vital Dyes. Evans blue, patent blue V, and bromophenol blue have all been investigated. Although effective in mapping surface necrosis, these techniques are incapable of differentiating accurately full-thickness and partial-thickness burns [32].

Photo-Optical Measurement

Reflection-Optical Multispectral Imaging. The burning process and the subsequent local responses cause changes in the optical properties of the skin. Remission-optical measurement exploits the different spectral back-scattering effects of burned skin at different burn degrees [34]. Light back scattering is influenced by the eschar thickness and the volume fraction and oxygen saturation of blood in the dermis [32, 62]. Multispectral analysis of reflected red, green, and infra-red light as a means of evaluating burn depth was first suggested by Anselmo and Zawaki [205]. The burn area is illuminated by white light generated by a halogen lamp, and the remission intensities are recorded by a multispectral camera. To obtain a high reliability on the determination of burn degrees, at least four characteristic wavelength bands are necessary [34]. An industrial prototype is at present in use at selected burn centers. Validity of the technique still remains to be determined.

Fiber-Optic Confocal Imaging (FOCI). Confocal scanning microscopy is a well known imaging technique for optically sectioning living tissues without physical dissection. The optical sectioning capability of FOCI enables noninvasive subsurface fluorescence microscopy of skin in vivo. In the experimental animal, damaged skin at the burn site was found to fluoresce when illuminated with blue light (488 nm). When imaged with FOCI, the intensity of the burn-associated autofluorescence correlates with the severity of the burn [37]. Spectroscopic and microscopic characteristics of human skin autofluorescence indicate that the major fluorophores at wavelengths of 350 to 470 nm are located in the dermis [63]. Denatured collagen at the burn site could possibly be the source of autofluorescence; however, no identifiable cellular structure could be seen in the autofluorescence induced by heat [37]. In comparison, Masson trichrome stain revealed collagen damage at layers deeper than those at which autofluorescence can be detected by FOCI [37]. Multiple factors confound interpretation of the depth of detection of the autofluorescence signal. These include optical aberration and distortion of distance. FOCI should not be considered in absolute units of depth of damaged tissues. It may be used to provide an index of burn depth [37]; however, its clinical value has not yet been demonstrated.

Polarization-Sensitive Optical Coherence Tomography (PS-OCT). PS-OCT can measure the reduction in birefringence of collagen denatured by thermal injury using depth resolved changes in the polarization state of light propagated in, and reflected from, the burned tissues. In an experimental animal

model, the calculated Stokes vectors for each point in the PS-OCT images and the reduction in the rate of phase retardation between two orthogonal polarizations of light showed a consistent trend with burn exposure time. A correlation between birefringence and actual burn depth determined by histological analysis was established. PS-OCT has potential use for noninvasive assessment of burn depth. It might eventually provide the physician with a quantitative estimate of actual burn depth [35, 64]. However, the validity of this new modality has not yet been demonstrated clinically.

Mueller-Matrix Optical Coherence Tomography (OCT). Conventional polarization-sensitive (PS) Mueller-matrix optical coherence tomography (OCT) can be calculated with a single incident polarization state. However, whenever the retarder is nonlinear but rather elliptical, two incident polarization states are needed. PS multichannel Mueller-matrix OCT offers simultaneously comprehensive polarization-contrast mechanisms, including the amplitude of birefringence, the orientation of birefringence, and the diattenuation in addition to the polarization-independent intensity contrast. In the experimental animal, it was demonstrated that Mueller optical coherence tomography (OCT) provides complementary structural and functional information on biological samples. It was revealed also that polarization contrast is more sensitive to thermal degeneration of biological tissue than amplitude-based contrast. Thus, Mueller OCT has significant potential for application in the noninvasive assessment of burn depth [65].

Thermography

Numerous investigations have demonstrated that full-thickness burns are colder than partial-thickness burns. Thermography of burn wounds was first reported by Lawson et al. [206]. Though 90% accuracy in diagnosis of burn depth has been reported based on the detection of 1°C temperature variations, false full-thickness results can result from evaporative heat loss from the burn wound [32]. An infrared transparent, water-impermeable membrane may be used as a wound cover to abolish evaporative cooling artifacts. Moreover, for valuable assessment of burn depth, thermography should be performed within 3 days following the injury [66], which may not be clinically practical.

Radioisotopes and Nuclear Magnetic Resonance

Radioactive phosphorus has been used to map burns in the experimental animal. Radioactive isotopes, however, are considered unsuitable for routine clinical diagnosis [32]. Nuclear magnetic resonance (NMR) has also been investigated; however, practical considerations have limited further exploration of this technique [32].

Ultrasound

Mixed results about the usefulness of pulse-echo sonography in burn depth determination have been reported [32, 67]. The requirement of a contact probe has further hindered the applicability of this technique. A new and unique noncontact ultrasonographic method to estimate burn depth has been recently developed [48]. With the probe held 1 inch from the skin, it is possible, to visualize the epidermis, dermis, and dermal-fat interface. The destruction of the dermal-fat interface is interpreted as a deep burn. It seems that noncontact ultrasonography may allow for the rapid evaluation of burn depth with high accuracy, without contacting the patient, and without causing pain or discomfort [48]; however, its relevance and value in a clinical setting still needs to be tested.

Estimation of Burn Injury Severity

Over the past few decades, interest in developing quantitative measures of patient illness status has been gaining momentum in response to both internal hospital needs and external pressures [68]. Injury severity scoring systems have become essential to both triage and systemic assessment of treatment outcome of victims of injury [69]. Hospitals need to define their patient population and to evaluate the costs and outcomes of care. They also face external pressures for care evaluation and cost containment, for which quantitative measures are useful [68]. An injury severity scoring system for burn patients has considerable practical value for physicians, nurses, and emergency medical technicians in regional emergency medical systems. It is statistically derived from injury-related predictors and outcome measures and has considerable practical value for health professionals in emergency medical systems. First, it can be used to recognize high-risk patients and predict the outcome of injury. Also, it can provide an indication as to the setting in which the patient should be treated. Lastly, it can be used in an audit of burn care in a regional system [68–71].

Factors most consistently cited by researchers that have the most influence upon burn victim morbidity and mortality include five severity variables: age, sex, percentage of TBSA burned, presence of full-thickness burn, and presence of inhalation injury [69]. Other researchers have identified other risk factors related to four main topics: depth (percent of partial-thickness and full-thickness burns), extension (% TBSA burned and injury to dorsogluteal area), morbidity on admission (preexisting illness and complications caused by inadequate transfer), and presence of inhalation injury (IHT) [72–74]. These are pretreatment variables that are routinely collected at the time of patient admission or transfer [69]. Others have included length of hospital stay, number of transfusions required, and number of operative procedures in the regression analysis to produce equations useful for the prediction of morbidity parameters [71]. Though these equations may be useful tools in medical audit and in assessing improvements in burn care, they are of no value as an admission severity grading tool to aid in patient management.

Although there is considerable agreement in defining risk factors, the question of how to combine them to produce an accurate, yet simple, scoring system has been problematic [70]. Specifically, there has been lack of agreement about whether the area of full thickness burned (FTB) should be included in addition to the area of total burn (TB) particularly because FTB is difficult to estimate at the time of initial assessment [68]. Since the pioneering work of Bull, a number of attempts have been made to develop models that allow estimation of the probability of a burned patient's survival [207]. Early efforts characteristically used probit analysis to obtain tabled probabilities. Later attempts have used multivariable logistic models and discriminant analysis [68, 71]. On the whole, results of multiple regression analysis

showed that TBSA burned is the best single predictor of survival, together with age [73–75]. In general, the cut-off point 20% TBSA score seems to provide maximum separation between survivors and fatalities [75]. Combined use of TBSA with type and severity of burn, age, sex, etc. does not seem to appreciably affect the final risk assessment. However, chronic alcohol abuse and smoking, inhalation injury, and pre-existing cardiac and neurologic conditions may have a significant impact in borderline groups with an Abbreviated Burn Severity Index (ABSI) score of 7–10, where these risk factors cause "mortality-shifting" [74].

The Baux rule is a simple scoring system based on predictor variables that are available without the use of laboratory tests or special instrumentation. It is a simple rule of thumb to calculate the Burn Index (BI) (Baux Index) that adds the age of the patient to the percentage of body surface area burned. This rule assumes that TBSA burns exceeding 75% indicate a dismal prognosis [70, 76]. Stern and Waisbren have modified the Baux rule by excluding patients younger than 20 years of age. In this modified Baux rule, the burn victim has a less than 50% chance of survival if the sum of the age and TBSA exceeds 95 [70, 77]. Both the Baux and modified Baux rules represent simplistic approaches, yet they may have great clinical utility [70]. The ABSI is a more recent index based on five variables: age, sex, FTB, TBSA burned, and inhalation injury [69, 70, 76, 78]. It is as simple and as easy to use as the clinical rule of thumb of Baux, yet it is more accurate and specific in describing outcomes for the victims of burn injury [68]. This index, however, does not take into account preexisting diseases that have a significant influence on outcome [73]. Moreover it is an ideal prediction score system, especially with respect to scientific evaluation [76]. The DEMI score (Depth Extension Morbidity Inhalation) has been proposed lately as a simple and accurate tool to predict mortality risk in burned children. Only four items are included in the logistic regression formula: TBSA burnt, inadequate transfer, inhalation syndrome, and dorso-gluteal burn [72]. It is claimed that the DEMI score is highly specific and sensitive. More complex computation formulas have also been reported to estimate the risk of death of burn patients from the patient's age, sex, size of burn (TBSA with or without FTB), perineum involvement, and time from burn to admission [68]. Coefficients for each risk factor have been proposed. Computations can easily be performed on a calculator, however, the mathematical formula is complex and may not be easily grasped and memorized. A computer program to aid in the computation has also been described [79].

It has been suggested also that net fluid retention is an accurate measure of the extent of burn injury, and is approximately equivalent to body surface area as a predictor of mortality [80, 81]. Even fluid retention was claimed to be a better predictor of mortality than any individual component of the ABSI. 230 cc/kg lean body weight of retained fluid in the initial 48 hours post-burn constitutes an excellent discriminant value for separating burn victim survivors from nonsurvivors in the clinical setting. This value represents a relatively easy and statistically accurate tool for predicting the probability of survival [80].

Acute Resuscitation Following Burn Injury and Fluid Replacement

The high survival rates of modern burn care can be largely attributed to application of prompt and aggressive resuscitation using intravenous infusion fluids [82, 83]. Fluid replacement within the first 24 hours after burn injury usually follows well-established guidelines and formulas. There is, however, no unanimity in the literature regarding the type of fluids to be used or the rates at which they may be infused. Many formulas have been described to provide adequate fluid replacement for resuscitation; however, we know of no definitive studies comparing the effectiveness of these many different formulations [82]. Some prefer the use of colloids and crystalloids while others use crystalloid solutions only. Most crystalloid favoring formulas, including the Parkland's formula, suggest the infusion of 3–4 ml/kg% of TBSA (calculations made up to 50% TBSA maximum) during the first 24 hours (84, 85) half of the calculated volume to be given within the first 6 hours following injury. Infusion of large crystalloid volumes should be, however, done with extreme care in elderly patients, who may develop congestive heart failure and lung, edema. The benefits of using colloid solutions such as albumin within 24 hours of the burn injury remains controversial (85, 86), nevertheless, most agree that colloids should not be infused within the first 6–12 hours. Some invetigators have demonstrated that the use of albumin within the early resuscitating phase increases mortality and that prolonged hypoalbuminemia does not decrease survival (85). Those who favor the use of colloids argue that these solutions may limit the total volume of fluid infused almost by half (Brook Army formula, Evans formula) and hence minimize the cardiac and electrolyte abnormalities that can arise from the infusion of large volumes (85, 87, 88). Gueugniaud et al. suggested the following formula: 2 ml/kg/% TBSA of lactated Ringer's solution for the first 6 hours followed by 1 ml/kg/% TBSA crystalloid with 1 ml/kg/% TBSA colloid over the next 18 hours [208]. It is important to note that all these formulas for fluid resuscitation in burn patients are only guidelines and that the exact amount of infusion needed can be determined only by monitoring the vital signs and urinary output in these patients.

Most of the world's population, however, does not have access to prompt advanced medical care and IV therapy. Even when they do, available resources and facilities are very often overwhelmed during situations of mass casualties. There is a need therefore to have better means applicable in most locations and at a large scale for initial resuscitation of burn shock [82]. Despite a fairly extensive literature on enteral resuscitation of burn shock, most present-day clinicians are unaware of its utility as an option, except in minor burns where resuscitation is not critical [82]. Recently, Brown et al. have suggested considering enteral resuscitation on larger burns up to 40% TBSA alone or used in conjunction with IV fluids [89].

Enteral resuscitation has been evaluated in animal experiments as well as in reasonably large -scale trials of burn injured children and adults, [82, 89–91]. These studies appear to have established the effectiveness and value of enteral resuscitation using balanced saline solutions. Recent research has established significant clinical advantages of early intestinal or enteral resuscitation, even if it can only be performed in hospitals. While early enteral feeding has been traditionally avoided in burn patients, it has recently been demonstrated to be safe and effective for nutrition when started immediately with hospital care of patients suffering from large burns [82, 92] or used before, during, and immediately after surgery of burned patients [82, 91]. Enteral resuscitation appears to be much more effective when started within the first hour after injury [82]. It increases intestinal blood flow and results in better

maintenance of gut barrier integrity. This may reduce the risk of sepsis and multi-organ failure [93]. Added benefits from early enteral resuscitation may be obtained, particularly when specific nutrients are included in the formulation. Clearly, the dangers of enteral resuscitation must be weighed against the benefits [82].

The disappearance of enteral resuscitation from contemporary medical consciousness is largely due the development of plastic IV catheters and the rise of critical care medicine and trauma specialists, with a focus on advanced team care of individual patients. The option of enteral resuscitation as a clinical treatment for burns has been bypassed and its utility is largely forgotten [82]. Many studies have been conducted on the development of better IV solutions to treat circulatory shock. However, the hypothesis that enteral resuscitation could play a role in initial resuscitation has not yet been tested against modern resuscitative regimens. The optimal composition of oral replacement fluid has not been determined and will likely vary for different indications. At present no definitive best enteral solution is identified. Different oral hydration strategies for astronauts have been tested to expand blood volume and prevent orthostatic hypotension prior to shuttle reentry during descent. Oral rehydration with salt water or with water and salt tablets was shown to expand blood volume. Greenleaf et al. [94] concluded that cation content is more important than osmotic content for plasma volume expansion. This led to the development of AstroAde, a high-sodium oral hydration solution effective at expanding plasma volume of dehydrated subjects during exercise [95]. It seems logical that a solution similar to Ringer's lactate or hypertonic lactated saline solution (HLSS) with the addition of glucose with an osmolarity range of 260-330 mOsm/l could rapidly be absorbed by the gastrointestinal mucosa and provide the large volume and Na necessary for burn resuscitation. Although such a formulation appears logical, it will require substantial research to define the optimal solution for enteral burn resuscitation [82].

While considerable controversy regarding the volume and rate of fluid resuscitation persists, the standard treatment approach to major burn trauma remains intravenous infusion of solutions, mainly crystalloids, to correct hypovolemia and improve peripheral tissue perfusion. Despite the aggressive use of fluid resuscitation, two primary concerns exist. The first concern is that tissue ischemia and oxygen debt persist despite large resuscitation volumes. A second important concern is that volume resuscitation and the re-introduction of molecular oxygen into previously ischemic tissue contributes to the production of excess oxygen derived free radicals that, in turn, produces additional tissue damage [96]. Free radicals that are produced during fluid resuscitation after burn trauma alter numerous components of cells including nucleic acids, lipids, and proteins. While persistent hypoperfusion will result in cell death over time, volume resuscitation to correct the perfusion deficits exacerbates the ischemia-mediated injury, a term that has been described as the "oxygen paradox." Tissue reperfusion results in the production of hydrogen peroxide and superoxide, clearly recognized deleterious free radicals. In addition to xanthine oxidase-related free radical generation in burn trauma, adherent-activated neutrophils produce additional free radicals. Free radicals may directly impair some aspect of cell membrane or intracellular organelle function, or they may initiate an inflammatory signaling cascade that results in the production of numerous mediators of cell injury. Antioxidant strategies designed to inhibit free radical formation, to scavenge the burst of free radicals, or to interrupt some aspect of the resulting inflammatory cascade have been shown to reduce tissue injury, to improve organ function, and to improve outcome [96–100]. Data collectively support the hypothesis that cellular oxidative stress is a critical step in burn-mediated injury, and suggest that antioxidant strategies designed to either inhibit free radical formation or to scavage free radicals may provide organ protection in patients with burn injury [96–98]. Oral antioxidant therapy in burn trauma (ascorbic acid, glutathione, and *N*-acetyl-L-cysteine) has been shown to effectively prevent the burn-sepsis mediated mortality and to attenuate the change in cellular energetics as well as the burn-sepsis mediated change in tissue antioxidant levels [96, 98–100]. The clinical relevance of this finding remains to be determined.

Local Burn Wound Therapy

Despite major advances in therapy, infection remains the leading cause of morbidity and mortality from extensive burn injury [101]. Infected wounds not only heal more slowly but also may lead to systemic infections and prevent adequate skin grafting [102, 103]. Improper burn treatment (lack of proper wound care, edema formation, lack of resuscitation) may actually increase the size and/or depth of the wound [102]. The development of several topical antibacterial agents has decreased wound-related infections and morbidity in burn wounds when used appropriately [102, 104]. Important qualities for topical agents used in burn wound care included a broad spectrum of antibacterial activity, low development of resistance, limited adverse effects, and decreased risk of systemic toxicity [104]. A wide variety of agents are available for treatment of burn wounds, including ointments, creams, and biological and nonbiological dressings [104].

For centuries silver has been known to have bactericidal properties. As early as 1000 B.C., the antimicrobial properties of silver in rendering water potable were appreciated [104, 105]. Today, silver has reemerged as a viable treatment option for infections caused by burns. The inhibitory action of silver can be attributed to its strong interaction with thiol groups present in cell respiratory enzymes in the bacterial cell. Additionally, silver has been shown to interact with structural proteins and preferentially bind with DNA bases to inhibit replication [104, 105]. Several products have incorporated silver for use as a topical antibacterial agent, such as silver nitrate, silver sulphadiazine—SSD (Flammazine—Smith & Nephew Healthcare Limited, Hull, Canada) [106], Silvadene, Juplar (Gulf Pharmaceutical Industries, UAE), silver sulphadiazine chlorhexidine (Silverex—Motiff Laboratories Pvt. Ltd. Kare Health specialities, Verna, Goa), SSD with cerium nitrate (Flammacerium-Solvay, Brussels, Belgium), and silver sulphadiazine-impregnated lipidocolloid wound dressing Urgotul SSD (Laboratoires Urgo, Chenove, France) [105-108]. In contrast to these silver agents, newly developed products such as Acticoat (Westaim Biomedical Inc., Fort Saskatchewan, Alberta, Canada) and Silverlon (Argentum Medical, L.L.C., Lakemont, GA) have a more controlled and prolonged release of nanocrystalline silver to the wound area. This mode of silver delivery allows the dressings to be changed with less frequency, thereby reducing risk of nosocomial infection, cost of care, further tissue damage, and patient discomfort [104, 109–111]. Irrespective of the source of silver, whether released from solutions, creams, and ointments or nanocrystalline silver released from commercially available new

dressings, silver is highly toxic to both keratinocytes and fibroblasts [112]. Fibroblasts appear to be more sensitive to silver than keratinocytes. Consideration of the cytotoxic effects of silver and silver-based products should be taken when deciding on dressings for specific wound-care strategies. This is particularly important when using keratinocyte culture, in situ, which is playing an increasing role in contemporary wound and burn care [112].

Though burn injuries have traditionally been considered as special types of wounds requiring specialized management protocols, healing of burn wounds does not differ in any way from healing of any other type of wound. It is only logical that general principles of wound healing apply to burn wounds as well [113]. There is more recent evidence in the literature that good hydration is the single most important external factor responsible for optimal wound healing [114]. Despite all the documented benefits, applying the moist healing principles to large surface areas, in particular to large burns, is hindered by the major technical handicap of creating and maintaining a sealed moist environment over these areas [115–117]. The use of occlusiye dressings should be reserved only for the treatment of small, superficial wounds. Many types of these dressings are available commercially.

Widely used topical antimicrobial agents for local burn wound management have been developed mainly to reduce the incidence of burn wound sepsis and its associated morbidity and mortality. This goal has been largely attained, however, without much concern for burn wound healing. On the contrary, reduction in burn wound sepsis resulted in net prolongation of healing time because of delayed eschar separation and digestion of the necrotic cutaneous layers by released bacterial enzymes. A recently described moisture retentive ointment (Moist Exposed Therapy-MEBO-Julphar, Gulf Pharmaceutical industries, UAE) has the peculiar advantage of addressing both issues of burn wound sepsis and burn wound healing at the same time. It acts like other ointments as an effective antibacterial agent, and at the same time it acts like an occlusive or semiocclusive dressing, promoting rapid autolytic debridement and optimal moist wound healing [113, 115]. The moisture-retetentive ointment also promotes earlier functional recovery of regenerating keratinocytes and improves scar quality [118–120].

The recent surge in biotechnology has resulted in the development of new products particularly useful in the management of partial-thickness burns. Carboxymethylcellulose-based hydrofiber dressing (Aquace-ConvaTec/Bristol-Myers Squibb Storefront, Princeton, NJ), compared to earlier experience with allograft skin, is a safe, suitable, and easy-to-use material for treatment of partial-thickness burns [121]. Beta glucan collagen matrix (BGC), which combines the carbohydrate beta-glucan with collagen, has been used as the primary wound dressing and as a temporary coverage for partial-thickness burns with reported good results. BGC markedly simplifies wound care for the patient and family and seems to significantly decrease postinjury pain, improves healing, and yields better scar appearance [122]. Arginine-glycine-aspartic acid (RGD) peptide matrix promotes dermal healing by providing a molecular scaffold that facilitates cell ingrowth and establishment of normal tissue architecture. With RGD peptide matrix application under synthetic occlusive dressing, the incidence of healing of partial-thickness burns is nearly threefold higher than similar burns treated with silver sulfadiazine [123].

Topical diphenylhydantion is a cheap and easy-to-use medicament, effective in suppressing burn wound bacteria and relieving pain, thereby promoting healing: it may be advocated for the purpose in resource-scarce environments [108]. Deflamol (Sopharma AD, Bulgarian Pharmaceutical Group Ltd, Sofia, Bulgaria), povidone-iodine (Isobetadine cream—Asta Medica, Brussels, Belgium) [103], as well as a multitude of other antisceptic preparations have been used for topical burn wound treatment. Suitable indications for preference of each of the topical agents can be found: however, the agent that most effectively suppresses the emergence of burn wound sepsis, while at the same time promoting healing, should ultimately be chosen.

Burn Wound Excision and Reconstruction

Methods for handling burn wounds have changed in recent decades. Increasingly aggressive early tangential excision of the burn tissue and early wound closure primarily by skin grafts is being applied and probably is the most significant change in recent years, leading to improvement in mortality rates at a substantially lower cost [124–128]. By shortening hospital stay, early wound closure would reduce pain associated with local burn wound care, number of operative procedures, and infective complications. It also decreases the severity of hypertrophic scarring, joint contractures and stiffness, and promotes quicker rehabilitation [124, 127].

Reduction of Blood Loss

Significant blood loss continues to plague burn surgery. Blood loss from early tangential excision of the burn wounds as well as from skin graft donor sites may be considerable, necessitating blood transfusion [129-135]. Although various techniques to reduce intraoperative blood loss have been described, there is an absence of uniformity and consistency in their application. Furthermore, it is unclear whether these techniques compromise intraoperative tissue assessment and wound outcome [132]. Adrenaline-thrombin solution (1 ml of 1:1,000 adrenaline, thrombin 10,000 units, in 1 of normal saline) has been traditionally used topically to reduce blood loss [129, 131]. Topical thrombin (soaks or 1000 U/ml spray) significantly reduces blood loss (43.5%) with no adverse effects on the rate of wound healing or the scar quality [136]. The routine use of local epinephrine in combination with topical thrombin, however, may not be necessary during total wound excision in pediatric patients, as it may not result in further reduction of blood loss [133].

The tumescent technique of presurgical subdermal infiltration of both the burn wound and split skin graft donor sites with a 1:500,000 adrenaline solution has been employed to decrease intraoperative blood loss without compromising burn depth assessment or impairing graft survival [129, 130]. The subdermal epinephrine/saline injection creates a smooth, tense surface, which assists in debridement and donor harvest. Within certain limitations, it is safe, inexpensive, and easy to use with no clinically detectable arrythmias or changes in heart rate or blood pressure [129]. The modified tumescent surgical technique: subcutaneous injection of adrenaline (1) ppm in warm saline solution) combined with pneumatic tourniquets in extremities and saline-adrenaline soaked nonadherent pads significantly reduces the intraoperative blood loss and total blood transfusion requirements [131]. Subcutaneous and topical adrenaline appears

to be safe and produces minimal acute cardiovascular effects [137].

Continuous tourniquet application during tangential excision on the extremities in burn patients is highly effective in reducing operational blood loss and the need for blood transfusion, and in shortening operative time [134, 138]; adequacy of excision has been questioned under these conditions. Tourniquet use without exsanguinating the limb prior to tourniquet inflation may improve visualization of bleeding points and subsequent engraftment [134]. Tourniquet application, however, is not possible for burns on the trunk or face. Nor is it possible for burns or skin graft donor sites high on the thigh or arm.

Fibrin glue (fibrin sealant—FS) from pooled human plasma has been used in Europe for many years. It eliminates the need for topical bovine thrombin (TBT) and epinephrine. FS does not have an adverse impact on the surgical outcome [135, 139]. Because of the risk of hepatitis and now of acquired immunodeficiency syndrome, this compound has not been approved by the U.S. Food and Drug Administration for use in the United States [140]. Autologous fibrin glue, on the other hand may be a viable alternative to standard hemostatic techniques in burn patients. It reduces alloantigen exposure, blood-borne viral infection risk and the need for blood transfusion. It does not seem, however, to have an additional benefit over thrombin application [140].

The application of a strict and comprehensive intraoperative blood conservation strategy during burn excision and grafting, including donor site and burn wound adrenaline tumescence, donor site and excised wound topical adrenaline and thrombin, and limb tourniquets followed by tight wrapping with a thin plastic film and a pressure bandage for approximately 10 minutes after deflating the tourniquet, is probably the right approach and should be adopted. A profound reduction in blood loss and transfusion requirements, without compromising wound outcome, is to be expected from this strategy [132, 141].

Skin Graft—Autograft, Allograft, and Xenograft

Autografts from uninjured skin remains the mainstay of treatment for many patients. Severe burn patients, however, invariably lack adequate skin donor sites [142-147]. An additional limitation of this modality is the creation of additional wounds that further increase the TBSA affected. Autografts can be meshed, expanding the coverage area up to four times the donor site. The disadvantage of this technique is that the recipient area heals with an irregular meshed pattern [147]. The interest in the production of fresh and long-term stored viable skin grafts has been increasing continuously [148]. Experience leads to the suggestion that there is a clinical need for human cadaver allograft skin (HCAS) to be used both for research purposes and as a means of providing immediate coverage of excised burn wounds as a temporary biologic dressing when limited available skin donor sites or the overall patient condition do not permit immediate grafting with autologous skin [147, 149, 150]. On the other hand, HCAS can also be used as a dressing to cover widely meshed autografts in large burns [151]. Allograft dermis has been shown to be incorporated over time without rejection. Dermal allografts have been prepared in a lyophilized form and have been useful with the epidermal autografts [147]. Xenografts have been used for hundreds of years as temporary replacement for skin loss. Donor species include frog, lizard, rabbit, dog, and pig. Although these grafts provide a biologically active dermal matrix, the immunologic disparities prevent engraftment and predetermine rejection over time [147]. It must be stressed that xenografts and allografts are only a mean of temporary burn wound cover. True closure is achieved only with living autografts or isografts (identical twins) [148]. One exception, though, is human skin allografts in patients taking the usual dosages of immunosuppressants for renal transplantation. In such category of patients, skin allografts seem to survive indefinitely with minimal repopulation of skin allografts by autogenous keratinocytes and fibroblasts. In case of discontinuation of immunosuppression, the skin allograft does not reject acutely. It persists clinically and the allograft cells are destroyed and replaced slowly with autogenous cells [143].

Serious problems, however, are associated with HCAS, including limited supply, variable and occasionally poor quality, inconvenience of harvesting skin in the mortuary, and ultimate immune rejection [149, 151]. Despite strict adherence to American Association of Tissue Banks (AATB) protocols or other similar protocols, the availability of cadaveric allografts is also limited by potential pathogenic microbial and viral contamination [151, 152]. Cell viability in allograft skin is also an essential consideration to ensure a supply of good quality material for clinical repair of wounds [153]. Initially, cadaveric skin was stored frozen at -28°C providing graft material with good cellular viability comparable to that of fresh skin stored at 4°C for 4 days [153, 154]. At present it is exceptionally used as fresh. To avoid the frequent problems encountered in the use of fresh cadaver skin and the expense of cryopreservation, HCSA is now mostly processed and preserved by glycerolization [154]. Low-cost glycerol-preserved allograft (GPA) skin banks have gained rapid popularity [146, 151]. GPA is mainly used as a temporary cover on freshly excised wounds and as an overlay on widely expanded autografts. It is also used to improve the quality of the wound bed prior to autografting with cultured keratinocyte sheets [146, 147].

Keratinocyte Culture

The culture and transplantation of keratinocytes are considered a major and important advance in the treatment of severe burns [155]. Cell therapy is an emerging therapeutic strategy aimed at replacing or repairing severely damaged tissues with cultured cells [156]. Epidermal regeneration obtained with autologous cultured keratinocytes (cultured epithelial autografts — CEAs) can be lifesaving for patients suffering from massive full-thickness burns [157, 158]. In 1975, serial subculture of human keratinocytes was first described. Clinical application of this discovery was made possible after the preparation of these cells into epithelial sheets. In 1981, the earliest application of cultured autologous epithelia was made for the treatment of extensive third-degree burns. Cultured epithelia avoid the mesh aspect obtained with a split-thickness autografts as well as the discomfort for the patient of skin graft harvesting [159]. Although the most important advantage is the large surface area obtained from a relatively small biopsy of healthy skin from the patient, a major disadvantage is the delay, which is too long to provide cultured keratinocyte sheets for practical clinical use [159]. Fragility and difficult handling of the grafts, an unpredictable "take" and extremely high costs are other disadvantages [157]. Another factor affecting success of the technique is also the enzymatic detachment of the confluent multilayered keratinocyte sheet from the irradiated

fibroblast feeder layer, which is a critical step of the classical culture method. This leads to a temporary loss of $\alpha 6\beta 4$-Integrins essential for cellular adhesion [147, 156]. Storage and preservation of viable sheets have also been a major handicap [160]. Widespread use of cultured autografts has been primarily hampered by poor long term clinical results that have been consistently reported by different burn units treating deep burns, even when cells were applied on properly prepared wound beds [147, 156, 161]. Though CEAs represent the common standard in clinically applied engineered skin substitutes, they are unsuitable as a permanent skin substitute in burn patients [147, 156]. Dermal substitution in association with CEAs is required to enhance results [156]. Nevertheless, deep second-degree burns remain an application of choice for the cultured epithelia, as the presence of the dermis limits retractions responsible for functional complications usually observed in third-degree burns where dermis is absent [159].

Allogenic cultured epidermis, obtained more quickly from donor skin, has been described recently in the treatment of leg ulcers, repair of skin donor site harvested for split-thickness autografts and in second-degree burns. With due attention to safety and security, a bank of allogenic keratinocytes has also been created. Use of allogenic keratinocytes in the first phase of treatment of extensive deep second-degree burns while awaiting autologous cultured keratinocytes has been described with encouraging results [159]. The cost effectiveness of this modality as compared to GPAs still needs to be determined.

Tissue Engineering—Bilayered Substitutes

The technological progression in skin substitutes has been from autologous grafts to bioengineered grafts [147]. Which is the best technique to deliver cultivated autologous keratinocytes with optimal growth potential after the shortest cultivation period possible, and how can this transplantation technique be combined with a dermal substitute [156] remain unresolved questions. New keratinocyte culture technologies based on the original technique described by Rheinwald and Green, and/or new "delivery systems" have been developed to overcome the numerous problems encountered with regular keratinocyte cultures, particularly when using mass-produced complex media [162]. Success of cell therapy with high keratinocyte "take," and with reproducible, and permanent clinical results, requires cultivation and transplantation of stem cells, subconfluent noncontact-inhibited cells with higher proliferative and wound healing capacity, rather than confluent cell layers where cellular differentiation has been stimulated [156, 161]. Practical and safe transplantation necessitates "easy to handle" scaffolds that could be fabricated as carriers. Moreover, a satisfactory long-term result requires the transfer of not only epithelial cells but also of dermal elements. Practical application of these principles led to the revolutionary construction in vitro before grafting of artificial bilayered skin composed of cultured auto-keratinocytes on allo-dermis cultured with fibroblasts in a specially designed scaffold [147, 155, 156, 163]. To circumvent the enzymatic step during the cultivation procedure and to simplify handling, keratinocyte cultivation is combined with various natural or synthetic carrier materials like polyurethane membrane, silicon-collagen membranes, hyaluronic acid-based membranes, collagen sponges, and fibrin glue [156]. The new keratinocyte culture system on a dermal equivalent or substitute suitable for skin wound closure thus obtained is flexible and has good mechanical properties resulting in good graft take without rejection [155, 164]. Transplantation of cultured autologous keratinocytes as a single-cell suspension in a fibrin glue matrix combined with allogenic skin grafting is also being investigated [156, 157].

Apligraf (Organogenesis, Canton, MD) (type 1 bovine collagen with cultured human fibroblasts and keratinocytes) and Epicell CEA (Genzyme, Boston, MD) (auto keratinocytes and fibroblasts cultured separately then combined on a collagen-glycosaminoglycan matrix) are commercially available products [147]. Though results are encouraging, their use is still limited to certain selected conditions.

Cultured Dermal Substitutes

Allogeneic cultured dermal substitute (CDS) (artificial skin) can be prepared by culturing fibroblasts on a two-layered spongy matrix of hyaluronic acid (HA) and atelo-collagen (Col) [165–167]. CDS can be cryopreserved and transported to other hospitals in a frozen state, retaining its ability to release essential cytokines for wound healing, particularly vascular endothelial growth factor (VEGF) [168]. Cryopreserved allogeneic CDS functions as an excellent cell therapy for intractable skin ulcers as well as for burn injuries and other skin defects [166, 167]. However, the wound surface must be checked rigorously for the occurrence of infection during the healing process to guarantee a favorable outcome [165]. Taking into account the manufacturing cost, coupled with the potency of VEGF release, a two-layered sponge of HA and Col with a weight ratio of 5/2 is very promising for commercial application [168].

Various artificial skin substitutes are available commercially. They are effective for management of contractures, chronic wounds, and chronic skin illnesses. Because they can be used in conjunction with autologous cultured epithelium applications, they may decrease or avoid the risk of donor area morbidity, which is more difficult to treat in children [169]. Decellularization of porcine skin to produce an acellular dermal matrix (ADM) for possible biomedical applications has also been described [170]. The practical application of ADM in the management of burn wounds still needs to be clarified.

Autologous CDS, on the other hand, allows quick wound bed preparation that would take a thin split-thickness autologous skin graft. Clinical trials with this therapeutic modality have yielded extremely promising results without development of severe contractures over a period of several months that are expected following very thin split-thickness skin grafts. The application of autologous CDS is promising for the treatment of extensive burn scar contractures, particularly in children [171].

Dermal Substitutes

Integra (Johnson and Johnson, Hamburg, Germany) is a tissue-engineered dermal template covered by a silicone epidermal substitute. The take rate and the rate of infection are essential for its successful use. It has been suggested that fibrin glue and negative-pressure therapy could shorten the period from coverage to its integration [172], following which autologous epidermis could be applied. An ingenous novel micrografting technique taking advantage of this technology has been described for the

treatment of a head and neck full-thickness burn injury. The burn wound is reconstructed with a tissue-engineered dermal template followed by early implantation of microdissected hair follicles through the silicone epidermis. The treatment results in complete reepithelialization and a hair-bearing scalp without the need for a split-thickness skin graft [173].

Alloderm (Life Cell Corporation, Woodlands, TX) (cryopreserved acellular cadaveric dermal matrix) serves as a scaffold for the ingrowth of cells and blood vessels and is usually combined with autografting. Dermagraft (Advanced Tissue Sciences, La Jolla, CA) (neonatal foreskin fibroblasts cultured on a polyglactin mesh) is usually combined with autograft. Terumo (Terumo, Tokyo, Japan) (bovine collagen analog) and Pelnac (Kowa Company, Tokyo, Japan) (silicone epidermis and collagen matrix dermis) are also combined with thin epidermal autografts to close difficult wounds and ulcers [147]. The application of these products in burn treatment is still limited to specific indications, though results are rather encouraging.

Bilaminar Skin Substitutes—Dressings

The simple objective of these products is to replicate the function of skin as closely as possible while healing takes place. These products are easy to use, readily available, and technologically simple. They are theoretically valuable in the management of second-degree burns; however, they may be responsible partially for some morbidity [147]. Biobrane (Dow Hickam, Sugarland, TX) (outer silicone film and inner layer of nylon and collagen) has a shelf life of 3 years. Transcyte (Smith & Nephew, Cargo, FL) (similar to Biobrane with added biologic layer derived from neonatal fibroblasts) is stored at −80°C. Laserskin (FIDIS Advanced Biopolymer, Abano Terme, Italy) (sheet of benzyl esterified hyaluronic acid perforated by laser then seeded with nonproliferating fibroblasts) can be used as an engineered bilayered graft or as a matrix for application of cultured autologous keratinocytes [147]. Cultured cellular sheets composed of mixture of autologous or allogenic keratinocytes (KC) and fibroblasts (FB) seeded on a polyurethane membrane (mixed culture sheet) have been useful for the treatment of STSG (split-thickness skin graft) donor wounds to accelerate the epithelialization process [143]. This may be extremely useful in extensive burn injuries where available donor skin is so limited that skin grafts need to be harvested repeatedly from the same area of unburned skin [143].

Graft Adherence and Fixation

The most important factor influencing the ultimate success and graft viability is adherence of the biological graft to the wound surface [174]. This goal is even more challenging in anatomically mobile regions [175]. Rapid and sustained adherence, the ability to resist shear stress, and a void-free surface-to-surface contact are critical [176]. Staples are ideally suited for skin-graft fixation. Metal staples are very convenient, not expensive, and are widely used, however, they ultimately need to be removed. Absorbable staples have recently been developed (Auto Suture Multifire Graftac-S) [177]. They have not yet gained widespread popularity, probably because of their elevated cost. To secure good graft adherence to the bed, the tie-over bolster dressing is the most commonly used method. However, it requires surgical skill and experience to make a skin graft adhere closely, particularly when the site has a complicated curved surface [178]. A range of "contact media," including Hypafix (Smith & Nephew Healthcare Limited, Hull, Canada), Elastofix (BSN medical GmbH & Co. KG, Hamburg, Germany), silicone gel, and elastomer products have been used to secure graft adherence. Hypafix has been adapted for use in all sites to become the standard technique in the management of pediatric burns in some centers. The technique is versatile, safe, simple, reliable and inexpensive, and, has proven to be effective, requiring repeat grafts in only 2% of cases [179].

Fibrin bonding of skin grafts to wounds is an essential part of the graft-adherence process [180]. Fibrin glue is presented as a new valid alternative to secure skin graft adherence, particularly for facial burns, hand burns, and difficult graft sites. Independent of fibrinogen concentration, a thin layer of fibrin tissue adhesive, when applied between two opposing surfaces, does not interfere with and may support the healing process, contrary to a thick layer of adhesive which inhibits skin graft healing [181]. An autologous technique, which eliminates the danger of multidonor preparations such as the risk of transmissible viral diseases (AIDS, hepatitis) has been developed. Neither sutures nor pressure dressings are required [182]. As an adjunct in skin grafting, fibrin glue may offer certain advantages that are not achieved by suturing alone. It has been demonstrated that graft sites treated with fibrin glue contracted less. The mechanism by which fibrin glue inhibits wound contraction may be related to increased adherence of grafts to the underlying wound bed [183]. Octyl-2-cyanoacrylate is a medical-grade tissue adhesive intended to be a rapid, painless, suture-free method for closure of simple lacerations and surgical wounds [184]. It is not, however, suitable for skin graft fixation. The product may be applied only on the skin surface; when applied in the wound, it delays healing.

Unlike existing techniques that apply pressure on the skin graft, negative pressure dressing applies pressure to the space between the skin graft and the grafting site to remove hematomas and pull the whole skin graft onto the grafting site with uniform force of adhesion [178]. Its has been extremely valuable to improve the take of dermal substitutes [172, 185] and may as well be adapted for skin graft fixation at specific locations.

Currently used mechanical and adhesive fixation aids to achieve graft adherence are not free of problems. Photochemical tissue bonding (PTB) is an emerging laser technique with numerous applications in surgical specialties. In a recently published experimental animal study, ex vivo skin grafts were treated with a photosensitizing dye, rose bengal (RB), then irradiated with argon laser. Enhanced skin graft adherence by forming dermal-dermal bonding was observed. The increase in adherence was a function of the concentration of RB and the laser fluence. The results also suggested that PTB is a potentially safe procedure because it is nonthermal in nature and does not significantly affect skin cell viability [176]. It could have clinical applications in the future.

Future Prospects of Burn Therapy

The goal of an ideal therapy that would not only promote the rapid healing process but would also act as an antiscarring therapy [186] remains a source of inspiration and is a major driving force for developing better therapeutic modalities and patient care. There are currently very few effective small molecule treatments

available for the promotion of dermal healing. The healing effects of antibiotic treatments are to resolve infection of the wounded area and cannot strictly be classified as substances to promote accelerated healing [186]. As our knowledge of the basic mechanisms of wound healing and the body's response to injury is expanding to the bio-molecular level, new prospects for therapy are emerging. It is not science fiction any more to imagine that the effect of "positive" growth hormones and cytokines may be enhanced and that of "negative" factors suppressed through molecular or genetic manipulation. Data suggest, for example, that the presence of the EGF (epidermal growth factor) receptor is a common denominator in the wound-healing process after burn injury. When coupled with the clinical evidence of acceleration of reepithelialization following exogenous application of EOF, findings suggest an endogenous growth factor-mediated pathway during wound repair may be amenable to exogenous manipulation [187]. Moreover, fluids that accumulate at wound sites may be an important reservoir of growth factors that promote the normal wound-healing response [188]. Data support a role for platelet-derived growth factor (PDGF) and heparin-binding epidermal growth factor-like growth factor (HB-EGF) in burn wound healing and suggest that the response to injury includes deposition of HB-EGF and PDGF into blister fluid and a redistribution of HB-EGF in the surface epithelium near the wound site [188, 189]. As promising as it may be, research into this domain has yet to overcome numerous obstacles, one of which is our incomplete understanding of the intricate mechanisms involved.

Major thermal injury is a particularly severe form of trauma that is characterized by high cardiac output, increased oxygen consumption, and protein and fat wasting. This vulnerable hypermetabolic state compromises the immune system and attenuates wound healing. Moreover, it causes tissue damage by membrane destabilization and energy depletion at the cellular level, resulting in tissue necrosis [190, 191]. The response to thermal trauma is a complex and intricate cascade of events that contributes to pathology and loss of function, as well as to recovery. The different signaling and effector pathways of recovery work in concert with overlapping and cross-reacting elements. A logical therapeutic approach to promote recovery after burn trauma would therefore be to block the immediate triggering of the inflammatory cascades that result in prolonged metabolic imbalances. A second component of the therapy would be to enhance wound healing, several molecular elements of which are regulated in part by components of the inflammatory cascade [190].

Over the last few years, numerous growth factors have been shown to accelerate cell proliferation in vitro and to promote burn wound healing in animal models [186, 192, 193]. Despite the vast interest in growth factor and cytokine biology and their potential for wound healing, clinical trials have, in most cases, been disappointing [186]. Local application of these factors as proteins (transforming growth factor-TGFβ, heparin-binding epidermal growth factor-like growth factor-HB-EGF,...) has been shown to be ineffective and of little clinical value because of enzymes and proteases locally present in the wound, and because of lack of adequate receptors [190, 192, 194]. Systemic administration of growth factors is another proposed therapeutic modality. Insulin-like growth factor-I (IGF-I) has been shown to improve metabolic rate, gut mucosal function, and protein loss after a burn injury. It mediates the actions of growth hormone in the hypermetabolic state by attenuating lean body mass loss, improving the immune response, attenuating the acute phase response, and enhancing wound healing. Large amounts of systemic IGF-I needed for the desired therapeutic effects, however, result in serious side effects, such as hypoglycemia, mental status changes, edema, fatigue, and headache. These adverse side effects limit the therapeutic utility of IGF-I in the treatment of burns [190].

Gene therapy is emerging as an effective therapeutic approach to improve clinical outcomes after thermal injury [195]. Skin is an especially attractive target for genetic manipulation because it is readily accessible and easily monitored for both the presence and the expression of inserted genes [186, 196]. Particle-mediated gene transfer in thermally injured skin is feasible and may provide a means of introducing biologic agents into injured tissue capable of enhancing bacterial clearance and improving wound healing [196]. Gene therapy to the skin, or to any other organ, is dependent, however, on a number of factors. All gene delivery systems have the following aims. First, they should be able to accept a suitable therapeutic gene and not be restricted by the size of the gene. Second, therapeutic genes need to be expressed at the correct level for the right amount of time. Finally, the therapeutic gene must be in a delivery vehicle that is taken up by cells [186].

Gene transfection is a promising therapeutic approach. There are, however, several obstacles to overcome before this approach can be effective. Major obstacles are the selection of an appropriate vector for gene delivery as well as an appropriate delivery mechanism. Viruses have been used as delivery vectors [190, 195]. The most common viruses used for transfection have been the retroviruses, adenoviruses, and adeno-associated viruses [190, 197, 198]. Viral infection-associated toxicity, immunologic compromise, and possible mutagenic or carcinogenic effects, however, make this approach potentially dangerous [197]. The use of naked non-encapsulated DNA or plasmid DNA constructs alone, without viral genes, have been used topically or delivered with a pneumatic "gene gun." Both have proven to be inefficient, perhaps due to the fragility of the naked DNA constructs in the extracellular environment and the traumatic consequences of gene gun discharges on cellular integrity. Non-viral liposomal cDNA genes are stable complexes [190, 194]. Their use, however, has been limited because of their low in vivo transfection efficiencies [190]. Modification of the standard liposomal structure to a cationic structure and the inclusion of cholesterol, together with the use of cytomegalovirus promoters in the cDNA constructs used for gene transfer, have nevertheless increased the efficacy and transgenic expression levels [190, 199].

Bombardment by gene gun at various helium pressures (200-600 psi) is one route into the skin [186, 196]. Other direct physical "injection" techniques include subcutaneous injection at the burn wound margin [200], the use of microseeding, microfabricated needles, and puncture-mediated DNA transfer [186, 197, 198, 201, 202]. Transport of macromolecules, as yet untested with DNA, has been achieved using depth-targeted pulsed electric fields and ultrasound. The development of gene-activated matrices (biodegradable polymers incorporating a therapeutic gene) takes the possibilities for wound treatment beyond gene therapy and into the realm of tissue engineering [186]. Nucleic acid vaccine [203] is yet another promising modality to promote wound healing following burn injury. As we enter the new millennium,

gene therapy will play a major role in the treatment of diseases and their sequelae, wherever topical delivery of DNA is feasible and whenever development of therapeutic gene cassettes and delivery vehicles is economically viable [186].

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