the bacterial contamination of wounds is an important global health-care
issue that is bound to grow as the population ages. Bacterial wound infections increase morbidity and mortality and are of considerable socioeconomic impact. The risk of wound infection increases as disturbances in local conditions favor bacterial growth rather than host defense. This can lead to impaired wound healing, resulting on the one hand in rising treatment costs and on the other hand in a traumatic and potentially life-threatening condition for the patient. The current clinical gold standard for treating wound infection is antibiotic therapy. However,
HOST DEFENSE PEPTIDES
Parallel evolution of hosts and pathogens has led to a diversity of multifaceted survival mechanisms that are either encoded in the germ line (innate immunity) or acquired through adaptation to specific antigens (adaptive immunity). Among the numerous defense mechanisms, HDPs are important evolutionarily conserved elements of innate immunity. They are found in organisms as diverse as insects and humans. Salient examples include the cecropins (insects), magainins (amphibians), and defensins (mammals). They are generally small peptides (12–50 amino acids) that contain at least two positively charged (arginine or lysine) residues. Predominantly membrane active, these peptides exhibit a high positive net charge and can adopt amphipathic conformations. This amphipathic design, consisting of spatially separated hydrophobic and charged regions, permits intercalation of the peptide into microbial membranes. Their unique mechanism of action and ability (in many cases) to kill organisms make them attractive prototypes for antibacterial drug development. Recent developments highlight the importance of vertebrate epithelial cells as sites of antibiotic peptide production and suggest a critical role for this system in human health and disease. Several reviews on HDPs have already been published.3,10,11

The importance of this innate system as a check on infection is evident when one considers that most bacteria have generation times of 20–30 minutes, whereas the mounting of a specific adaptive immune response may take days or weeks. Although many cationic peptides demonstrate direct antimicrobial activity against bacteria, fungi, eukaryotic parasites, and viruses, it has also been established that many also have a key modulatory role in the innate immune response and form an important link between the innate and adaptive immune responses.13 Owing to their multiple functions, they are considered promising agents for new therapeutic approaches in infectious diseases and wound healing.3,4,6,10,11,14-18

The emergence of bacterial resistance to antibiotics increased the urgency of exploring alternative means of combating pathogenic assault. HDPs are rapidly emerging as attractive candidates for antimicrobial treatment. Different therapies that effect wound repair have been proposed over the past few decades. Although HDP therapy was initially focused on antimicrobial activity, it can be applied equally well to the local, temporary treatment of acquired diseases, including impaired wound healing and tissue repair.

Thomas-Virnig et al. elegantly investigated the potential therapeutic role of genetically engineered NIKS skin substitute tissue expressing hCAP-18 (NIKShCAP-18), which significantly counteracted bacterial infection by a multidrug-resistant nosocomial bacterial strain in a mouse model of infected third-degree cutaneous burn wounds.9 Cathelicidins are a family of HDPs found in most mammalian species. They consist of an N-terminal cathelin domain, which is highly conserved throughout species, and a variable C-terminal peptide. This propeptide is activated extracellularly after proteolytic cleavage.

The only known member of this family thus far is hCAP-18, an 18-kDa human cationic antimicrobial protein that is produced mainly by leukocytes and epithelial and mucosal cells. Its cationic C-terminal 37–amino acid domain, LL-37, displays broad antimicrobial activity mediated through direct interaction with and disruption of the microbial cell membrane. The enzyme responsible for cleavage of the proprotein in neutrophils is serine proteinase 3. In skin, the serine proteases kallikrein 5 and 7 were recently reported to mediate alternative processing of hCAP-18 generating several novel peptide fragments, suggesting that peptide profiles may differ among tissues and biological conditions.19 This opens a potential new area of research, in that their functional profile may differ. Interestingly, both the proprotein, hCAP-18, and the mature processed form of LL-37 are constitutively produced in sweat.19 The unprocessed proprotein does not have antimicrobial activity20 until it is cleaved by proteinase 3.

In addition to being antimicrobial, LL-37 is implicated in diverse biological processes, including chemotaxis, cytokine production, histamine release, and angiogenesis.13,14,21,22 Furthermore, hCAP-18/LL-37 is highly expressed during normal wound healing23,24 displays growth factor–like properties enhancing the in vitro proliferation and migration of human keratinocytes, and is involved in re-epithelialization of wounds.24,25 LL-37 is upregulated at the messenger RNA and protein levels upon exposure to bacteria or by sterile incision.26 Expression is also increased by various growth factors that are involved in wound healing, such as insulin-like growth factor. Consistent with this, LL-37 has been demonstrated to be involved in the re-epithelialization of skin wounds. Using a noninflammatory ex vivo wound healing model of organ-cultured human skin, up-regulation of LL-37 expression has been demonstrated in the wound area, and antibodies specific to LL-37 have been shown to inhibit the re-epithelialization process in a concentration-dependent manner.27 Because LL-37 is probably not found at concentrations sufficient for antimicrobial activity,28 it is possible that one of the primary functions of LL-37 in the skin is to promote re-epithelialization. None of the clinically used antibiotics demonstrates these interesting features.

As a result of the alarming increase of multidrug-resistant bacteria, the new experimental approach of Thomas-Virnig et al. with hCAP-18/LL-37 has considerable clinical relevance. The investigators demonstrated a two-log reduction in the growth of a clinical isolate of multidrug-resistant Acinetobacter baumannii. This well-conducted study provides strong evidence that the antimicrobial peptide hCAP-18/LL-37 may reduce the risk of infection from pathogenic bacteria, including multidrug-resistant nosocomial strains, and thereby actively enhance
wound healing. Although this study did not show evidence of better wound healing in addition to reduced bacterial growth, as Thomas-Virnig et al. note, HDPs may also act to amplify the endogenous innate immune response to invading microbes. Moreover, there is evidence that HDPs such as hCAP-18/LL-37 exhibit wound healing properties distinct from their antimicrobial function, such as the stimulation of keratinocyte growth and/or migration and the stimulation of wound vascularization.30 This situation not only enables testing of important skin features, such as epidermal thickness, epidermal kinetics, and gene transfer efficiencies between animal and human tissue, have limited the use of HDP therapy. Thomas-Virnig et al. nicely circumvented this problem by using a human-skin substitute grafted onto immunodeficient mice (athymic nude mice). Their approach is particularly attractive because the regenerated human skin on these mice could preserve clinical, histological, molecular, and functional properties of the donor skin (either normal or diseased).

For cutaneous HDP therapy aimed at the treatment of genetic skin disorders such as psoriasis, retention of pathological defects in vivo provides a valuable system for testing the efficacy and safety of strategies. It interesting to note that keratinocyte gene transfer has also been explored as a treatment modality for nondermatological conditions. Considerable progress has recently been made toward the treatment of systemic diseases caused by insufficient amounts of a protein in the circulation, using the epidermis as a “bioreactor” for producing therapeutic protein.

The concept of delivery of HDPs by a skin substitute is a new development. Combinations of HDPs or their sequential use may be the future answer to accelerating wound healing processes, and with the advent of their delivery by a skin substitute, these HDPs may become increasingly important. Developments in matrix components and tissue engineering technology may offer promise for the future. The development of slow-release matrices may prolong the delivery of their potential antimicrobial and wound healing properties.

Viral vectors are the original and most established technology for gene delivery. A wide range of applications have been developed, and many virus-mediated gene transfer models are successful. The production of viral vectors, however, is costly and time-consuming, transfection efficacy is variable, and the risk of local or systemic infections that may lead to fatal outcomes remains a concern. Thomas-Virnig and colleagues’ nonviral approach using sustained delivery of hCAP-18 to the wound bed from temporarily applied human-skin-substitute tissue should also circumvent viral gene transfer issues such as the preexisting immunity exhibited by much of the human population toward adenoviruses. Other positive attributes of their approach include elimination of the risk of viral infection and avoidance of the higher cost of vector production.31

The transient nature of gene expression is also a benefit in wound healing applications. On the negative side, some nonviral gene transfer methods tend to be nonspecific and highly variable within the levels of gene expression reported.

THE USE OF SKIN SUBSTITUTES TO DELIVER HDPs

The problem then will be establishing which of the many HDPs identified as having potential therapeutic benefit should be the ones of choice. This question highlights the need for a database of HDP functions in wound healing to facilitate further study. The numerous therapeutic options for delivery of HDPs by a bioengineered skin substitute include anti-infection, anti-endotoxin, immunomodulation, chemotaxis, cell proliferation and differentiation, wound healing, angiogenesis, influencing initiation and polarization of adaptive immunity, induction of gene expression, and enhancement of protein secretion functions.

Recent advances in cell biology have identified a variety of molecules—specifically, growth factors and their receptors—that are critically involved in directing cell functions during soft-tissue organogenesis. The study by Thomas-Virnig et al. indicates that delivery of HDPs via a skin substitute is a promising therapeutic tool for modifying this repair microenvironment. Skin with inherently poor or pathologically altered healing potential would thus benefit from a therapeutic bioengineered skin approach that could augment healing capacity while minimizing infection.
A PARADIGM SHIFT

HDP research is becoming an area of great importance. Delivery of HDPs by a bioengineered skin substitute has evolved from a purely experimental scientific endeavor to a clinically pertinent treatment for the skin. In wound healing, there remain challenges in the selection of optimal target cells, development of sequential therapeutic methods, and identification of factors that may be detrimental to the introduction of a genetically modified skin substitute.

It is probable that the only way to answer many questions that still arise about therapeutic delivery of HDPs by a skin substitute will be through clinical trials. The following steps have been achieved/developed: (i) culture and targeting of human epidermal stem cells, (ii) surgical techniques that allow the grafting of large skin areas without scarring, and (iii) sustained transgene expression in vivo in preclinical models. Now is the time to validate these steps in a clinical context. Implementation of a phase I/II clinical trial of HDP therapy in selected individuals suffering from psoriasis may shed light on important issues, mainly, the persistence of transgene expression at therapeutic levels and host tolerance to the gene product. The focus in the development of HDPs for clinical applications has been on small HDPs containing the biologically active core of the endogenous molecules, thereby limiting related toxicity components, improving efficacy, and lowering the cost of goods. Only a modest number of HDPs have been tested in clinical-efficacy trials, and they demonstrated potential in both preclinical and clinical trials.24-27

Thomas-Virnig and co-workers’ study indicates that delivery of HDPs via skin substitute is a promising therapeutic tool for modifying the repair microenvironment and may lead to a paradigm shift in the management of skin pathologies. Future basic and clinical research will tell if and when this new powerful “biological weapon” will become part of the health professionals’ armamentarium. Further research of HDPs will improve our knowledge of their involvement in the recognition and neutralization of pathogens, which will support the development of new anti-infective therapeutic approaches. HDP immunomodulating functions will become clearer with additional research on structure–function analyses to elucidate their mechanisms of action. Most important, future research must take advantage of and build on the diverse nature of HDPs and adhere to physiologically relevant conditions, ultimately validating, in vivo, their beneficial functions.

REFERENCES