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What is New in Wound Healing?

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Abstract: Wound biology is complex. Wounds which were until recently seen only as defects in tissues are now increasingly interpreted in cellular and molecular terms. Growth factors, cytokines, proteases and adhesion molecules which participate in wound healing are discussed in this article. From a clinical perspective, conceptual shifts of importance, including moist wound healing, wound bed preparation and wound assessment, are presented. The frontiers of therapeutics employed in wound healing continue to advance with an increasing array of modalities joining the ranks at a regular pace. A range of currently available as well as evolving therapies– physical (topical negative pressure therapy, warming, electrical stimulation), biological (larva therapy, skin substitutes, stem cell therapy, growth factors, gene therapy) and of a miscellaneous variety (hyperbaric oxygen, dressings)– are appraised.

Key Words: Wound healing, Growth factors, Physical therapy, Biological therapy

Introduction

A healing wound is an extremely complex and dynamic tissue which in some ways could be regarded as an organ, albeit a temporary one. Scientific enquiry into the many facets of wound healing is far from complete and consequently the knowledge base is continually being enriched by input, as much from the clinician at the bedside, as the researcher's bench.

Increasing clarity in definitions, a systematic approach to research, an organised and multi-professional approach to management, and a willingness to consider the patient's perspective are some of the important changes that have paralleled technological advances and expertise, which, as ever, have been the prime drivers of progress.

This article provides an overview which reflects on some of the developments in cutaneous wound healing over the last couple of decades that have either changed or are likely to change the way wounds are understood, evaluated and treated. In keeping with the generalist theme of the article, discussion on specialised areas of wound healing like scar management and issues relating to specific problems like pressure ulcers have not been addressed.

The conceptual advances pertaining to wound healing will be outlined first, followed by a discussion on advances in wound assessment. Finally the therapeutic advances will be considered.

Conceptual advances in wound healing

Advances from a molecular perspective

Normal wound healing occurs in recognisable, usually progressive, though overlapping, phases: the haemostatic/inflammatory phase, the proliferative/cellular phase and the remodelling phase. Giant strides have been made in the understanding of wound healing at the molecular level by the discovery of many families of molecules which have provided insights into the multitude of steps and interactions involved in each of these phases.

Wound healing is a collaborative process involving a variety of cells and matrix components which need to interact continually towards a common goal. Growth factors, cytokines, proteases and adhesion molecules, which are discussed here, are but a few examples of substances by which cells conduct a molecular talk and engage in interactive cascades. In addition, it is increasingly becoming clear that important regulatory

roles are played by systems as diverse as the plasmin cascade and nitric oxide.

Growth factors and cytokines

Growth factors and cytokines are 2 distinct categories of signalling proteins that modulate wound healing at a molecular and cellular level. However, in certain instances the distinction may be blurred and some mediators like PDGF, TGF β and TNF α straddle the divide.

Growth factors are constitutively present, usually released by a few selected subsets of cells and have a primarily trophic effects on cells. However, they may indirectly influence inflammatory processes. Platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), connective tissue growth factor (CTGF), and transforming growth factor (TGFβ), along with insulin-like growth factor (IGF) and colony stimulating factors (granulocyte-G-CSF; granulocyte/ macrophage- GM-CSF; macrophage-M-CSF) are amongst the key growth factors in wound healing. A summary of the profiles of a few selected growth factors is given in Table 1.

Table1. Profile of selected growth factors of importance in wound healing.

Growth factor	Physiological effects	Clinical and experimental correlates
TGFβ Transforming growth factor [β1, β2, β3 isoforms]	• Chemotactic to fibroblasts, monocytes, macrophages, lymphocytes • Proliferation of epithelial cells, macrophages, lymphocytes, fibroblasts • Stimulates keratinocyte migration • Induces expression of Pro- MMP-9 in keratinocytes • Induces fibroblasts to secrete TIMPs • Augments cell adhesion to matrix proteins by modulating integrin receptor • Stimulates fibroblasts to contract collagen matrix	Chronic wound fluid contains lower TGFβ when compared to acute wounds While higher concentrations of TGFβ1 and TGFβ2 are associated with hyperfibrotic disorders TGFβ3 has been found to reduce scarring Scarless healing in the embryo has been attributed to an absence of TGFβ
PDGF Platelet derived growth factor	• Neutrophil, monocyte, lymphocyte chemotaxis • Monocyte maturation • Potentiates VEGF production • Stimulates MMP production by fibroblasts • Stimulates myofibroblasts to contract matrix collagen	First growth factor to be licensed for topical therapy
KGF Keratinocyte growth factor [KGF-1, KGF-2]	• Potent mediator of keratinocyte proliferation which under the influence of KGF 2, upregulates the expression of many genes • Stimulates keratinocyte and fibroblast motility	Trials of topical application underway
VEGF Vascular endothelial growth factor	• Potent angiogenic factor	VEGF administration improves granulation-tissue formation Potential being explored in salvage of ischaemic flaps and in tissue expansion
EGF Epidermal growth factor	• Directs epithelialisation in an autocrine fashion • Stimulates fibroblast collagenase secretion • Inhibits foetal wound contraction	Aged dermal fibroblasts have a decreased EGF-receptor expression EGF may contribute to the scarless repair seen in utero

Cytokines are small molecular weight mediators which primarily have a variable effect on inflammatory processes by their influence on the cells of the immune system. They may be released by practically all nucleated cells, are expressed transiently and locally and elicit varying responses in different cells. A summary of the profiles of a few selected cytokines is given in Table 2.

Proteases and their inhibitors

Matrix metalloproteinases (MMPs) are a large family of about 24 mammalian endopeptidases that play a crucial role in wound healing. There are 4 collagenases, 2 gelatinases, 2 matrilysins, 3 stromelysins, 6 membrane type MMPs and a miscellaneous group of 7 enzymes. Remodelling of the extracellular matrix, which is an important step in a number of stages in wound healing like cell migration, angiogenesis and wound contraction, is dependant on the MMPs. The alteration of the matrix architecture may have profound secondary effects on cellular proliferation, morphology and migration. In addition, the MMPs may modulate the wound milieu by their effect on the rate of degradation of growth factors, cytokines and their receptors (1).

Table 2. Profile of selected cytokines in wound healing.

Cytokine	Physiological effects	Clinical and experimental correlates
TNF α Tumour necrosis factor α	• Leucocyte chemotaxis • Monocyte maturation • Macrophage activation • Inhibits fibroplasia • Stimulates IL-6 by fibroblasts • Increases activity of MMP-2 and MMP-9 • Potentiates VEGF production	Elevated levels linked to insufficient collagen deposition and poor healing Levels high in chronic nonhealing wounds
Interleukin-1	• Leucocyte and fibroblast chemotaxis • Macrophage activation • Angiogenic • Stimulates fibroblast MMP production • Stimulates keratinocyte migration	High levels associated with delayed healing
Interleukin-6	• Inhibits extracellular matrix breakdown • Stimulates fibroblasts to secrete TIMP	High levels associated with poor healing Peaks at 24 h
Interleukin-8	• Leukocyte chemotaxis • Enhances epithelialisation • Keratinocyte migration • Upregulates plasminogen activator in keratinocytes • Inhibits fibroblast induced contraction of collagen	Parallels IL-6, peaking at 24 h
Interleukin-4 and Interleukin-10 (The anti-inflammatory interleukins)	• Inhibit leucocyte chemotaxis • Downregulate expression of many pro-inflammatory cytokines	IL-10 exhibits bimodal wound levels peaking at 3 h and 72 h
Interferons (The antifibrogenic cytokines)	• Inhibitory to fibroblasts • Inhibit post-translational changes to collagen • Increase collagenase activity	IFN γ reduces keloid size when administered intradermally

The activity of the MMPs is counterbalanced by tissue inhibitors of MMPs (TIMPs) which bind to the MMPs, inactivating them (2). TIMPs may also independently stimulate or inhibit cellular proliferation and act to inhibit angiogenesis (2,3). Other inhibitors include α_2 macroglobulin, which serves as the predominant antiprotease immediately after wounding.

Realisation of the importance of this protease-antiprotease balance has opened new avenues amenable to intervention in an effort to improve wound outcomes. Examples include *Promogran* ®, a dressing consisting of collagen and oxidised regenerated celulose that seeks to mop up and inactivate the MMPs in the wound, and the possibility of manipulating the protease content in a wound by gene therapy.

Adhesion molecules

Adhesion, at a molecular level between cells and between cells and the matrix, is a vital physical process which initiates and modulates many chemical and biological effects. Adhesion is accomplished by a large family of adhesion molecules which are transmembrane proteins that serve as lines of communication between the internal processes of the cell and its exterior. There are currently 6 recognised superfamilies of adhesion molecules – the immunoglobulin-like superfamily, the cadherins, the integrins, the receptor protein tyrosine phosphatases, the selectins and the hyaluronate receptors. The 3 illustrative examples which follow outline how indispensable these ubiquitous recognition molecules are to the process of wound healing.

Platelet aggregation and leucocyte margination in the capillaries, one of the earliest steps in the inflammatory phase of wound healing, is dependent on the expression of selectins and integrins.

Keratinocyte migration is an important step in re-epithelialisation . Migrating keratinocytes overexpress a set of integrins which engage molecules like fibronectin and vitronectin in the blood clot and matrix to provide them a foothold as they edge forward.

Adhesion molecules may also be shed by endothelial cells at the direction of cytokines released by activated leucocytes. Soluble E selectin and soluble VCAM-1 (vascular cell adhesion molecule-1) shed in this manner adhere to adjacent endothelial cells and exert a direct angiogenic effect (4).

Advances from a clinical perspective_

Moist wound healing

Ever since Winter's studies (5-7) highlighted the importance of a moist wound environment, there has been a growing body of evidence lending it support. The concept of maintaining a moist wound environment has sparked the so-called "dressing revolution" with a wide spectrum of moisture-retaining and semi-occlusive dressings flooding the market.

A moist wound environment is claimed to have the following advantages:

1. It prevents tissue dehydration, which helps to preserve the viability and proliferative potential of the cells. This is associated with earlier epithelialisation.

2. Accelerates angiogenesis.

3. Increases breakdown of dead tissue and fibrin contributing to autolytic debridement.

4. Potentiates interaction of growth factors with their target cells.

5. Reduces the incidence of infection.

6. Is associated with less pain.

In the contrast, exposure of the wound to air causes wound dessication and scabbing, which delay epithelialisation. The healing time of superficial and deep cutaneous wounds has been shown to be faster and less painful when treated with a moisture- retaining dressing as against a simple gauze dressing (8-10).

Wound bed preparation

Wound bed preparation is a concept which attempts to systematize the management of the chronic wound with a view to accelerate endogenous healing and to facilitate the effectiveness of other therapeutic measures. The 3 pillars of wound bed preparation are management of necrotic burden by debridement, managing bacterial burden and maintaining moisture balance. Additional and related goals include correcting cellular dysfunction and achieving biochemical balance.

Debridement could be achieved by surgical, mechanical or enzymatic means.

Mechanical debridement refers to a range of techniques like wet-to-dry dressings, which cause separation of eschar and its removal with the dressing; wound irrigation, which ideally is irrigation of the wound

by saline using a 20 gauge catheter at 15 pounds per square inch of pressure; and whirlpool or foot soaks in medicated saline to remove debris. Enzymatic debridement refers to the topical use of bacterial collagenase, DNAse/fibrinolysin, papain/urea combination or trypsin to achieve clearance of debris.

Bacterial burden may be reduced by a combination of debridement; correcting underlying causes like ischaemia and immunosuppression, and topical antiseptics delivered through dressings like cadexomer iodine and silver based dressings.

Maintaining moisture balance involves the control of exudate while maintaining a moist wound environment that is conducive to healing. Exudate may be controlled using specially designed absorbent dressings or mechanical devices such as topical negative pressure therapy. Moisture-retaining dressings like hydrogels, hydrocolloids and foams aid in maintaining a balance and preventing dessication.

Though the individual components mentioned above are not entirely new, the concept of wound bed preparation has unified the different approaches, giving clinicians a global perspective of the problem facing them at the wound bed while at the same time stressing the importance of each of the components.

Advances in the assessment and investigation of wounds

Wound assessment has several dimensions– clinical, physical, physiological, biochemical, histological and genetic. While advanced techniques employed in wound healing research like cytological, immunohistochemical and molecular methods have made significant contributions to our understanding, only those of immediate relevance to the practicing clinician are considered here.

Scoring and Grading: Wounds are heterogeneous not only in terms of aetiology but also in quantitative (size, shape) and qualitative terms (wound bed, slough, discharge, effects produced etc). Over the years scoring and grading systems have been designed to objectively assess the wound to minimise inter-observer variation. Scoring or grading wounds also helps in classifying wounds and to an extent provides prognostic information. Uniformity in reporting allows for comparisons amongst and between various wounds as well as following the progress of a wound over time.

Some systems like those used for assessing pressure ulcers are useful in day to day practice. Others, like the ASEPSIS score (11) and the Southampton grading system (12) pertaining to postoperative wounds, are useful tools that are mainly employed in the research setting. Because of the heterogeneity of the wounds, to date there is no single acceptable system that could be adopted universally to assess all wounds.

Infection: Wound infection has been and continues to be an elusive term to define and reach a consensus on. One systematic review (13) addressing the quality of measurement of surgical wound infection identified 41 different definitions of wound infection in the 90 prospective studies included in the analysis. This aside, attempts to correlate clinical signs and symptoms with wound infection have met with some success. Cutting and Harding (14) identified friable bright red granulation, exuberant granulation, increased discharge and new areas of slough at the wound base as possible signs of infection. Gardner et al. (15) validated pain, increasing wound size, new areas of breakdown and odour as signs with a high correlation with $> 10^5$ colony forming organisms per gram of tissue. Grayson et al. (16) validated the exposure of or probing to bone in diabetic foot ulcers as a useful bedside test for deep infection with a specificity of 85% and positive predictive value of 89%.

Quantitation of bacterial load in a wound by tissue biopsy is expensive and time consuming. A semiquantitaive swab technique has been proposed as an alternative. The wound is first swabbed after the bed is cleaned with saline. The swab is then used to streak 4 quadrants of a solid medium sequentially. A growth in the fourth quadrant of > 30 colony forming units has been correlated with a bacterial burden of $> 10^5$ organisms per gram of tissue (17).

Biochemical assessment: Advances in technology have made measurement of cytokines, growth factors and proteases possible. Wound fluid and biopsy specimens of wounds have been the tools of investigation with respect to these factors.

The pro-inflammatory cytokine content and protease content of chronic wounds has been observed to be higher than in acute healing wounds. The levels of TNF α and IL-1 remain elevated in chronic wounds (18,19). Chronic wounds also tend to have a lower growth factor content.

The protease-antiprotease balance seems to be critical and a close correlation between the high ratios of TIMP/MMP-9 and healing of pressure ulcers has been noted (20). Wounds that are healing normally show a peak MMP-9 level at 24 h which declines significantly by 48 h. Persistent elevation of MMP-9 in wound fluid has been shown to be a useful marker of delayed healing. Absence of a decline in MMP-9 between 24 and 48 h in postoperative wounds has been correlated with infection and chronicity of wounds (21).

Though currently wound fluid analysis remains largely a research tool, in future it may evolve further as a source of vital prognostic wound information or may even be useful in directing therapy.

Developments in therapy

Physical methods

Topical negative pressure therapy (TNP)

Pioneered by Fleischmann et al. (22) and Morykwas and Argenta (23) in the mid 90s, TNP involves the application of an external sub-atmospheric pressure to exert a suction force across the wound. Vacuum assisted closure (VAC), sub-atmospheric pressure dressing (SPD), vacuum sealing technique (VST), sealed surface wound suction (SSS), and negative pressure therapy (NPT) are alternative terminologies.

The wound is compartmentalised by an airtight seal around it and through a dressing interface (which is usually a polyurethane or polyvinyl alcohol foam) the compartment is connected to an external suction apparatus. The application of pressure may be continuous or intermittent. Pressures used range between -125 and -175 mmHg. Dressings are usually changed every 48-72 h.

TNP has been shown to increase dermal perfusion, stimulate granulation tissue, decrease interstitial fluid, control wound exudate and decrease bacterial load.

These effects have clinically translated to better healing rates of wounds in a variety of specialities including plastic, cardiothoracic and orthopaedic surgery. The clinical indications of TNP are ever expanding – from ulcers and burns to wound dehiscences and fistulae. Other useful roles are as an adjunct in tissue salvage in reconstructive surgery, burns and trauma where the application of TNP has had the effect of preserving the vitality of tissues and flaps of borderline viability. In reconstructive surgery, the ability of TNP in reducing the size of a tissue defect, which has been termed 'the reverse expansion effect', has been exploited to enable downgrading of the wound on the reconstructive ladder so that a simpler procedure (for example, secondary suture) may be used instead of a more complex one (such as a local flap).

Problems with TNP include pain, fluid loss especially in large wounds and risk of bleeding. Large controlled trials which are underway will hopefully define its precise role in the therapeutic armamentarium of the wound care provider.

Warming (therapeutic heat)

Although the application of warmth to wounds in the form of compresses is a well known practice since time immemorial, the primary objective had been pain relief. The science behind the beneficial effects of heat on tissues has only been explored relatively recently. Although per-operative warming is now standard anaesthetic practice, the application of warming as a modality to reduce wound infection and to augment wound healing is a novel approach which holds promise.

The following facts are now established

- Warming improves blood flow and oxygen tension in tissues (24,25)
- Warming decreases the rate of wound infection in clean elective surgery (26)
- Warming reduces the risk of developing pressure ulcers (27)
- Warming may eradicate established MRSA infection in pressure sores (28)

Warming may be applied systemically or locally. Systemic warming is usually done using specially designed warming blankets (Bair-Hugger, Arizant Health Care) or mattresses (Pegasus-Inditherm mattresses). The mechanisms used include circulating warm air/water or electrically powered coils. Local warming is applied using specially designed pads which may use an external source of electricity (Warm up dressing, Augustine Medical Inc.) or an exothermic reaction triggered by exposure to oxygen.

The ideal duration and intensity of heat needed for optimising the wound milieu is yet to be determined. Warming is attractive as an option for many reasons:

1. It can be applied prophylactically as well as therapeutically.
2. Is simple to apply (systemically or locally) and well tolerated.
3. Is cheap and cost effective.
4. May help to reduce the use of antibiotics.
5. May be useful in circumstances where antibiotics fail.

Electrical stimulation

Research into the effects of electricity on living tissues has unravelled the pivotal role of electrical charge in interactions in biological systems both at cellular and molecular level. Electricity-induced tissue hyperaemia and galvanotaxis, the purposeful predictable movement of cells under the influence of electricity, are well documented. However, only over the past couple of decades has electrical stimulation made the slow transition from the realms of research to the clinical arena.

The devices used to deliver the current are heterogeneous in terms of the type of current, voltage, intensity and waveforms used. Gardner et al. in a meta-analysis reviewed the effect of electrical stimulation in healing chronic wounds (29). Of the 14 studies included, 9 were randomised controlled trials. The primary outcome measure was the mean percentage area of the wound healed per week. There were 591 ulcers in the electrical stimulation group and 212 in the control group. The mean percentage area of the wound healed per week in the ES group was 22.5% compared to 9% in the control group. Pressure ulcers had the greatest response.

Further research is needed to define the optimum dose-response and the relative effectiveness of the different modalities of electrical stimulation.

4. Miscellaneous physical methods

Magnetism, laser phototherapy, cycloidal vibration therapy and ultrasound are some of the other physical modalities which have been observed to have beneficial effects on various steps of the wound healing cascade, both in the laboratory and in the clinic. However, most of the evidence to date has been in the form of observational studies with very few controlled trials employing small numbers of patients. Magnetism in particular was more effective than control in healing plantar ulcers in leprosy (30) and resulted in better wound outcomes in patients after liposuction (31). Large well powered, blinded, randomised controlled trials are needed before the clinical role of these modalities can be judged.

B. Biological Therapy

1. Larva therapy

Although conceptually many centuries old, larva therapy in its current form was 'rediscovered' in the late 1980s and was given a fresh breath of life by the work of Sherman (32) in the US and popularised in the UK by the biological research unit at Bridgend in Wales.

Also termed maggot therapy and biosurgery, it involves the application onto the wound of necrophagous larvae of the green bottle fly *Lucilia Sericata*, reared in a controlled and sterile environment.

Maggots augment wound healing by a number of mechanisms:

- Antimicrobial effect: Larvae ingest and kill bacteria and may secrete chemicals with antimicrobial action. The larval secretions increase the pH of the wound to 8-8.5, which has an inhibitory effect on certain bacteria.
- Selective debridement: Several proteases capable of degrading many matrix components have been characterised in their secretions, which cause rapid and selective debridement of dead tissue (33,34).
- Promotion of tissue interactions: Larval secretions favourably influence the fibroblast to extracellular matrix interactions, which is critical in many processes in wound healing. Secretions by removing the fibrin cuff might kick- start the healing process in a static chronic wound (32-34).

The larvae are 1-2 mm in size and do not multiply in the wound. The recommended dose of larvae is 10 larvae per square centimetre of wound. Usually 1-2 applications achieve adequate debridement. The dressing, which is left in place for 2-3 days, needs to be oxygen-permeable and provide a moist environment. Larva therapy is best suited for wounds with slough and infection and has been shown to be useful in diabetic ulcers, pressure sores and venous ulcers. It can be a useful tool against drug resistant strains of bacteria, for example Methicillin resistant Staphylococcus aureus (MRSA). Larva therapy is cost effective and tolerance is excellent. Apart from the presence of fistulas, and the proximity of the wound to major blood vessels or vital organs, there appear to be no

contraindications. Limitations are the lack of aesthetic appeal and the short shelf-life of maggots.

2. Skin Substitutes

That "skin is the best dressing" is a well known surgical aphorism. The normal structural and cellular components of skin not only have a barrier function on the wound but also exert a complex, benign and active influence on the wound environment which has been termed the biological effect of a skin dressing.

Tissue engineering has provided us with a number of clinically viable alternatives to autograft skin that may be used in a broad spectrum of clinical scenarios – from covering raw areas as in burns to stimulating the healing processes in a static chronic wound. The products may be classified into single layered products (containing the equivalent of either the epidermis or the dermis) or bilayered products (containing layers which mimic both the dermis and epidermis). A brief discussion on a few of the currently available products follows.

Single layered products

A. Cultured keratinocytes *(Epicel®, Genzyme biosurgery)*

Keratinocytes harvested from the host can be grown to produce stratified sheets of keratinocytes in vitro. The major advantage of this technique is that only a 2-8 cm^2 area of the host skin is needed to generate sheets of autologous keratinocytes which can cover 60-90% of the patients' surface area. Expansion of the order of 100-1000 -fold is possible in 2-4 weeks. The main use of keratinocyte sheets has been as a method of achieving early wound closure in extensive burns. It has also been applied over allogenic cryopreserved graft skin in this situation. Long term survival of keratinocytes has not been proven, but at least it functions as a moist occlusive dressing and stimulates endogenous healing by generating cytokines and growth factors (35).

B. Human dermal replacement *(Dermagraft®, Smith and Nephew Inc.)*

New- born foreskin derived fibroblasts when cultured on a 3-dimensional polymer scaffold remain metabolically active, producing growth factors that support wound healing. Dermagraft is single layered and cryopreserved. The polymer scaffold is absorbable. It has been used in burns and diabetic foot ulcers.

C. Cadaver derived processed dermis *(Alloderm®, Life Cell Corporation)*

Cadaveric skin is processed to remove all dermal and epidermal cells, resulting in an acellular dermal collagen matrix. Following application the graft revascularises from the wound bed and is repopulated by recipient cells.

Bilayered Products

D. Bilayered skin equivalent *(Apligraf®, Organogenesis Inc.)*

Bioengineered bilayered skin substitutes contain a dermal and epidermal layer closely resembling the architecture of skin. They contain matrix proteins and expresses cytokines. They do not, however, contain melanocytes, Langerhans cells, macrophages, lymphocytes, blood vessels or appendages. *Apligraf®* is one such substitute which contains neonatal foreskin fibroblasts and keratinocytes on a dermal matrix made of bovine type I collagen. It has been proven to be effective in achieving faster healing in diabetic and venous and pressure ulcers when compared to conventional treatment (36-39). An infected wound and allergy to bovine products are contraindications.

E. *TransCyte® (Smith and Nephew Inc.)*

This biological dressing contains human neonatal fibroblasts cultured on the silicone membrane bonded nylon mesh. This has been successfully used in treating partial thickness burn wounds (40).

F. Integra® (Integra Life Sciences)

The dermal equivalent of this bilayered substitute is made of cross linked bovine collagen and chondroitin sulphate. The epidermal equivalent is made of silicone. As healing progresses, the dermal layer is replaced by an endogenous matrix. The silicone layer may then be removed and an epidermal autograft applied.

3. Stem cell therapy

Bone marrow derived stem cells are pleuripotent, being capable of differentiating into a variety of cells. This property is being exploited in the wound healing environment. Early reports from case series on the application of bone marrow derived cells on the chronic wound are promising (41). However, large controlled trials are needed to clarify their role in therapeutics.

4. Growth factors

Normal wound healing is heavily dependent on a plethora of growth factors and cytokines which variably interact with cells and the matrix at different stages. Use of growth factors topically to augment wound healing, although a very intuitive prospect, is fraught with difficulties for the following reasons:

- The multitude of factors involved
- Problems in matching the spatial and temporal profile found in wounds
- Rapid degradation in the wound by proteases
- Lack of a vehicle for sustained release
- Cost

Although animal studies have evaluated a number of growth factors, only the following have shown promise in clinical studies.

• Platelet derived growth factor (PDGF)

PDGF is currently the only growth factor licensed for topical use. The efficacy of recombinant PDGF-BB (becaplermin gel) in diabetic foot ulcers has been proven in a number of randomised trials (42,43). In a meta-analysis of 4 randomised controlled trials, patients with a median ulcer area of 1.5 cm^2 treated with becaplermin gel at a concentration of 100 µg/g, achieved a 39% higher healing rate when compared to placebo gel (44).

Efficacy of PDGF in healing pressure ulcers has been demonstrated in phase I and II human studies (45,46).

• Keratinocyte growth factor 2 (KGF 2)

A multicentric double blind controlled study using recombinant KGF-2 (Repifermin) on venous ulcers showed that it healed twice the number of ulcers and at twice the rate of controls in 12 weeks (47).

• EGF and FGF

EGF and basic FGF have been used topically to augment epithelialisation of skin donor sites. Improvements in healing have, however, been negligible to modest (48-50).

While some studies found EGF to be effective in non-healing chronic wounds (51), diabetic foot ulcers (52) and in corneal wounds (53), Falanga did not detect any significant improvement in venous ulcers (54).

FGF has proved useful when applied to pressure sores (55).

5. Gene therapy

Gene therapy, put simply, aims to help the cell to help itself by providing it with specific genes. Genes once incorporated in the cell affect the cell and its milieu through their products of expression. These in the context of wound healing are growth factors, their receptors, adhesion molecules and inhibitors of proteases. Gene therapy has the potential to circumvent most of the shortcomings of topical growth factor therapy mentioned above.

Classical gene therapy involves incorporating the gene to directly influence the wound by its product of expression. Epigenetic therapy is a variation wherein a nucleotide sequence is used to modulate the expression of the endogenous genes.

Genes can be delivered by biological (viral vectors), physical (e.g., microinjection, microseeding), and chemical (e.g., cationic liposomes) methods. A new method of sustained delivery of genes to the wound milieu called matrix–enabled gene transfer or gene activated matrix (GAM) therapy is evolving. This involves embedding genes onto a scaffold matrix which remains in the wound increasing the length of exposure of target cells to the genes (56).

Transfer could be established in vivo (the gene being delivered to the cells in the wound directly) or ex vivo (gene transfection is achieved outside the wound environment in a selected population of cells which are then transplanted into the wound).

Once in the wound environment, the genes may also be controlled by externally applied substances by specific mechanisms termed genetic switches.

The scope of gene therapy is expanding:

Genes may be employed to augment an effect – (e.g., promote healing)

- Genes for growth factors and their receptors
- Genes for tissue inhibitors of metalloproteinases

Alternatively they may be used to inhibit an effect (e.g., suppress overhealing or excessive scarring)

- Genes for antibodies against specific growth factors
- Genes for soluble receptors of growth factors

Gene therapy as applied to wound healing is understandably in its infancy. Currently trials are

underway exploring the use of PDGF, VEGF and FGF genes in diabetic foot and venous ulcers.

Future directions include using multiple genes concurrently (e.g., genes for growth factors and their receptors), and fine tuning the gene expression as required using genetic switches.

6. Other biological therapies

Fibrin sealant, primarily used for its adhesive and haemostatic properties, has been claimed to accelerate revascularisation, decrease wound contraction and produce less inflammatory response when compared to sutures. It has also been used as a vehicle for delivery of growth factors. However, its use in chronic non-healing wounds is limited.

Platelet gel prepared from autologous blood contains supraphysiological doses of platelets which provide growth factors and proteins like fibronectin. In the field of maxillofacial surgery they have been found to reduce oedema and ecchymosis in the early postoperative phase and to accelerate the ingrowth of autogenous bone grafts. However, a randomised trial of its effect on venous ulcers did not find any significant effect on healing (57).

C. Miscellaneous Therapies

1. Hyperbaric oxygen

Oxygen is more than a nutrient. Most vital cellular and molecular mechanisms are influenced either directly or indirectly by the available tissue levels of oxygen. Well known impediments to healing like ischaemia act so because of the tissue hypoxia they impose, whereas the beneficial effects of modalities like warming are at least in part attributable to enhancement of tissue oxygenation. Hyperbaric oxygen therapy is one method of increasing oxygen delivery to tissues and involves breathing 100% oxygen in pressurised chambers (2-2.5 atmospheres). The P_{O_2} may reach 1500 mmHg.

A recent systematic review (58) on the use of hyperbaric oxygen in wounds identified 6 controlled trials (2 of which were randomised) involving diabetic ulcers and 1 non-randomised trial involving chronic non-healing

wounds. All of these studies reported statistically significant positive clinical outcomes in favour of hyperbaric oxygen therapy.

Hyperbaric oxygen therapy needs special equipment and expertise and is not without complications. This restricts its use as an adjunct in recalcitrant ulcers.

2. Dressings

The sheer variety of dressings available today is probably matched only by the diversity in the wounds they are employed to treat. Practices steeped in tradition like the simple gauze soaked in antiseptics applied directly to the open wound have given way to more evidence-based approaches aimed at maintaining a moist environment that supports the healing wound. Another conceptual shift is targeted therapy. The appreciation that wounds have different barriers to healing at different points in time has guided the evolution of the various groups of dressings addressing specific problems. The choice of dressing is dictated by the needs of the wound and this is reflected in the classification system shown in Table 3.

However, this classification is not a rigid one as, in practice, there are many hybrid or composite dressings which combine aspects of members of different groups to achieve an optimum effect. A brief discussion on some of the types of dressings follows.

- **Films** are usually polyurethane based, transparent allowing inspection, retain moisture, provide occlusion, prevent contamination but provide no absorption. Typical use includes the postoperative wound.
- **Soft silicone mesh** (Mepitel*) is a non-adherent, porous, semi-transparent dressing with a wound contact layer consisting of a flexible polyamide net coated with soft silicone. Although it is non-absorbant, the porous nature allows fluid to pass through to the secondary dressing. Primarily used in skin donor areas.
- **Hydrocolloids** are composed of a mixture of adhesive, absorptive (carboxymethyl cellulose) and elastomeric ingredients. They are waterproof, impermeable to bacteria, promote autolytic debridement and can be changed once every 3-7 days. However, absorption is limited. Useful in granulating and epithelialising wounds with minimal exudates.
- **Hydrogels** are composed of a polymer (carboxymethyl cellulose or modified starch), propylene glycol and up to 80% water. Their main use is as hydrating agents in drying wounds. They promote autolytic debridement but absorption is limited.

Table 3. Types of Dressings.

Broad category	Primary goal	Group	Examples /Comments
General purpose dressings	1. Cover with varying occlusion	Augmented Fabrics Films Aerosols/Sprays	Mepitel*, Vaseline gauze, tulle Opsite*, Tegaderm*, Opsite*
	2. Occlusion, Absorption, Hydration	Foams	Allevyn*, Lyofoam*
		Hydrogels	Intrasite*, Tegagel*
		Hydrocolloids	Duoderm*,Comfeel*, Tegasorb*
		Alginates	Sorbsan*, Kaltostat*
Specialised dressings	3. Delivery	Medicated dressings	Iodine, silver, zinc and antibiotics can be delivered by a variety of vehicles-ointments/creams/ impregnated beads
		Nitric oxide releasing dressings	Experimental
		Cell platforms	Experimental. Being developed to enable cell based therapy
		Gene activated matrix	Experimental. Being developed to deliver genes effectively
	4. Tissue substitution	Skin substitutes	Epicel*, Demagraft*
	5. Deodorisation	Adsorbant and antibiotic impregnated types	Charcoal based dressings, Inadine* , Polysaccharide beads
	6. Protease sequestration		Promogran* Modified dialdehyde cotton gauze
	7. Compression	Three and four layered bandages	Tensopress*, Coban*

- **Alginates** are derived from seaweed and are available as ropes, sheets or wafers. Are highly absorbent and convert to a gel on absorption. However, if the wound is not producing enough fluid, they may cause drying of the wound. Useful in packing cavities, but usually require a secondary dressing.
- **Foams** are polyurethane based, non-adherent, heavily absorbent dressings which conform to wound contours and can be left in place for up to 4 days. However, they do not protect from external contamination and usually need a secondary dressing. Useful in wounds with large volume exudates.
- **Promogran*** is a protease modulating matrix dressing made of collagen and oxidised regenerated cellulose. Promogran binds proteases which, when present in excess, are a major barrier to healing. This also results in the increased availability of growth factors in the wound. Promogran has been found to be useful in venous and diabetic ulcers.
- **Modified dialdehyde cotton gauze** is ordinary cotton gauze modified by oxidation, phosphorylation and sulphonation; it has been shown in in vitro studies to have a 4-fold affinity to sequestrate neutrophil elastase (59). The technology although promising needs to be tested in the clinical setting.

- **Cell platform** is an evolving method of delivering cell based therapy to the wound. Keratinocytes and fibroblasts grown on bioreactive microporous beads enclosed in a polyethylene bag have shown potential when applied to wounds in mice (60).

Conclusion

The healing wound has been recognised for millennia as a minefield of activity– an activity which we now know is orchestrated by native and infiltrating cells interacting with the matrix. The language of this complex machinery is only beginning to be uncovered by the discovery of many superfamilies of molecules. Defining the interactions precisely in space and time are the challenges for the future. Developments in therapy would be expected to follow naturally.

From a clinical standpoint it is tempting to speculate that wound management, which is increasingly becoming multidisciplinary, may evolve into a specialty in its own right. However, wounds, as ubiquitous as they are, will continue to be seen and treated by different strata of health care providers. This means that while technologies like tissue engineering and gene therapy hold great potential it is the appreciation of the pathophysiology of wounds along with the awareness of the different modalities of treatment from which to choose which will have the greatest impact on the average patient. This article seeks to address that need for dissemination.

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References

1. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 14: 2123-33, 2000.
2. Gomez DE, Alonso DF, Yoshiji H et al. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Eur J Cell Biol. 74: 111-22, 1997.
3. Ravanti L, Kahari VM. Matrix metalloproteinases in wound repair. Int J Mol Med 6: 391-407, 2000.
4. Koch AE, Halloran MM, Haskell CJ et al. Angiogenesis mediated by soluble forms of E-selectin and Vascular cell adhesion molecule-1. Nature 376: 517-9, 1995.
5. Winter GD. Formation of the scab and the rate of epithelialisation of superficial wounds in the skin of the young domestic pig. Nature 193: 293-294, 1962.
6. Winter GD. Effect of air exposure and occlusion on experimental human skin wounds. Nature 200: 378-379, 1963.
7. Winter GD, Scales JT. Effect of air drying and dressings on the surface of a wound. Nature 197: 91-92, 1963.
8. Nemeth AJ, Eaglstein WH, Taylor JR, et al. Faster healing and less pain in skin biopsy sites treated with an occlusive dressing. Arch Dermatol 127: 1679-83, 1991.
9. Leipziger LS, Glushko V, DiBernardo B et al. Dermal wound repair: role of collagen matrix implants and synthetic polymer dressings. J Am Acad Dermatol 12: 409-19, 1985.
10. Pirone LA, Monte KA, Shannon R et al. Wound healing under occlusion and non-occlusion in partial-thickness and full-thickness wounds in swine. Wounds 2: 74-81, 1990.
11. Wilson APR, Sturridge MF, Treasure T et al. A scoring method (ASEPSIS) for postoperative wound infections for use in clinical trials of antibiotic prophylaxis. Lancet 1(8476): 311-12, 1986.
12. Bailey SI, Karran SE, Toyn K et al. Community surveillance of complications after hernia surgery. Brit Med J. 304: 469-471, 1992.
13. Bruce J, Russell EM, Mollison J, et al. The quality of measurement of surgical wound infection as the basis for monitoring: a systematic review. Journal of Hospital Infection. 49: 99-108, 2001.
14. Cutting KF, Harding KGH. Criteria for identifying wound infection. J Wound Care 3: 198-201, 1994.
15. Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Rep Reg 9: 178-86, 2001.
16. Grayson ML,Gibbons GW, Balogh K et al . Probing to bone in infected pedal ulcers: A clinical sign of underlying osteomyelitis in diabetic patients. JAMA 273: 721-3, 1995.
17. Thompson P, Taddonio T, Tait M. Correlation between swab and biopsy for the quantitation of burn wound microflora. Proct Int Cong Burn Inj 8: 381-3, 1990.
18. Trengove NJ, Bielefeldt-Ohmann H, Stacey MC. Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers. Wound Rep Reg 8: 13-25, 2000.

19. Tarnuzzer RW, Schultz GS. Biochemical analysis of acute and chronic wound environments. Wound Rep Reg 4: 321-5, 1996.

20. Ladwig GP, Robson MC, Liu R et al. Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. Wound Rep Reg 10: 26-37, 2002

21. Tarlton JF, Vickery CJ, Leaper DJ et al. Postsurgical wound progression monitored by temporal changes in the expression of matrix metalloproteinase-9. Br J Dermatol. 137: 506-16, 1997.

22. Fleischmann W, Becker U, Bischoff M et al. Vacuum sealing indication, technique and results. Eur J Orthop Surg & Trauma 5: 37-40, 1995.

23. Argenta LC, Morykwas MJ. Vacuum assisted closure: a new method for wound control and treatment: Clinical experience. Ann Plast Surg. 38: 563-576, 1997.

24. Rabkin JM, Hunt TK. Local heat increases blood flow and oxygen tension in wounds. Arch Surg 122: 221-25, 1987.

25. Ikeda T, Tayefeh F, Sessler DI et al. Local radiant heating increases subcutaneous oxygen tension. Am J Surg 175: 33-37, 1998.

26. Melling AC, Ali B, Scott EM et al. Effects of preoperative warming on the incidence of wound infection after clean surgery: a randomised controlled trial. Lancet. 358: 876-80, 2001.

27. Scott EM, Leaper DJ, Clark M et al. Effects of warming therapy on pressure ulcers – a randomised trial. AORN Journal 73: 921-38, 2001.

28. Ellis SL, Finn P, Noone M et al. Eradication of methicillin–resistant staphylococcus aureus from pressure sores using warming therapy. Surgical infections. 4: 53-55, 2003.

29. Gardner SE, Frantz RA, Schmidt FK. Effect of electrical stimulation on chronic wound healing: a meta-analysis. Wound Rep Reg 7: 495-503, 1997.

30. Sarma GR, Subrahmanyam S, Deenabandhu A et al. Exposure to pulsed magnetic fields in the treatment of plantar ulcers in leprosy patients – a pilot, randomised double blind controlled trial. Indian Journal of Leprosy 69: 241-50, 1997.

31. Man D, Man B, Plosker H. The influence of permanent magnetic field therapy on wound healing in suction lipectomy patients: a double blind study. Plastic and Reconstructive Surgery. 104: 2261-6, 1999.

32. Prete PE. Growth effects of Phaenicia sericata larval extracts on fibroblasts: mechanism for wound healing by maggot therapy. Life Sci 60: 505–10, 1997.

33. Chambers L, Woodrow S, Brown AP et al. Degradation of extracellular matrix components by defined proteases from the greenbottle fly larva Lucilia sericata used for chemical debridement of non-healing wounds. Br J Dermatol 148: 14–23, 2003.

34. Horobin, AJ, Shakesheff, KM, Woodrow S et al. Maggots and wound healing: an investigation of the effects of secretions from Lucilia sericata larvae upon interactions between human dermal fibroblasts and extracellular matrix components. Br J Dermatol 148: 923-933, 2003.

35. Phillips TJ. Keratinocyte grafts for wound healing. Clin Dermatol 12: 171-181, 1994.

36. FalangaV, Margolis D, Alvarez O et al. Rapid healing of venous ulcers and lack of clinical rejection with an allogenic cultured skin equivalent. Arch Dermatol 134: 293-300, 1998.

37. Falanga V, Sabolinski M. A bilayered skin construct (APLIGRAF) accelerates complete closure of hard–to-heal venous ulcers. Wound Rep Reg 7: 201-7, 1997.

38. Veves A, Falanga V, Armstrong DG et al. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomised multicentre clinical trial. Diabetes Care 24: 290-5, 2001.

39. Brem H, Balledux J, Bloom T et al. Healing of diabetic foot ulcers and pressure ulcers with human skin equivalent. Arch Surg 135: 627-634, 2000.

40. Noordenbos J, Dore C, Hansbrough JF. Safety and efficacy of TransCyte for the treatment of partial-thickness burns. J Burn Care Rehabil 20: 275-81, 1999.

41. Badavias EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. Arch Dermatol 139: 510-16, 2003.

42. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers: a phase III, randomised, placebo-controlled, double-blind study. Diabetes care 21: 822-7, 1998.

43. d'Hemecourt PA, Smiell JM, Karim MR. Sodium carboxymethyl cellulose aqueous based gel versus beclapermin in patients with nonhealing, lower extremity diabetic ulcers. Wounds 10: 69-73, 1998.

44. Smiell JM, Wieman TJ, Steed DL et al. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomised studies. Wound Rep Reg 7: 335-46, 1999.

45. Robson MC, Phillips LG, Thomason A et al. Platelet derived growth factor BB for the treatment of chronic pressure ulcers. Lancet 339: 23-5, 1992

46. Mustoe TA, Cutler NR, Allman RM et al. A phase II study to evaluate recombinant platelet-derived growth factor – BB in the treatment of stage 3 and 4 pressure ulcers. Arch Surg 129: 213-9, 1994.

47. Robson MC, Phillips TJ, Falanga V et al. Randomised trial of topically applied Repifermin (rh-KGF-2) to accelerate wound healing in venous ulcers. Wound Rep Reg 9: 347-52, 2000.

48. Brown GL, Nanney LB, Griffin J et al. Enhancement of wound healing by topical treatment with epidermal growth factor. N Engl J Med 321: 76-9, 1989.

49. Cohen IK, Crossland MC, Garrett A et al. Topical application of epidermal growth factor onto partial-thickness wounds in human volunteers does not enhance reepithelialization. Plast Reconstr Surg 96: 251-4, 1995.

50. Greenhalgh DG, Rieman M. Effects of basic fibroblast growth factor on the healing of partial-thickness donor sites: A prospective, randomized, double-blinded trial. Wound Rep Reg 2:113-6, 1994.

51. Brown GL, Curtsinger L, Jurkiewicz MJ et al. Stimulation of healing of chronic wounds by epidermal growth factor. Plast Reconstr Surg 88: 189-94, 1991.

52. Tsang MW, Wong WK, Hung CS et al. Human epidermal growth factor enhances healing of diabetic foot ulcers. Diabetes Care 26: 1856-61, 2003.

53. Daniele S, Frati L, Fiore C et al. The effect of the epidermal growth factor (EGF) on the corneal epithelium in humans. Graefes Arch Clin Exp Ophthalmol 210: 159-65, 1979.

54. Falanga V, Eaglstein WH, Bucalo B et al. Topical use of human recombinant epidermal growth factor (h-EGF) in venous ulcers. J Dermatol Surg Oncol 18: 604-606, 1992.

55. Robson MC, Phillips LG, Laurence WT et al. The safety and effects of topically applied recombinant basic fibroblast growth factor on healing of chronic pressure sores. Ann Surg 216: 401-8, 1992.

56. Chandler LA, Gu DL, Ma C et al. Matrix enabled gene transfer for cutaneous wound repair. Wound Rep Reg 8: 473, 2000.

57. Senet P, Bon FX, Benbunan M et al. Randomized trial and local biological effect of autologous platelets used as adjuvant therapy for chronic venous leg ulcers. Journal of Vascular Surgery. 38:1342-8, 2003.

58. Wang C, Schwaitzberg S, Berliner E et al. Hyperbaric oxygen for treating wounds. Arch Surg 138: 272-9, 2003.

59. Edwards VJ, Yager DR, Cohen IK et al. Modified cotton gauze dressings that selectively absorb neutrophil elastase activity in solution. Wound Rep Reg 9: 50-58, 2001.

60. Rees RS, Adamson BF, Lindblad WJ. Use of a cell-based interactive wound dressing to enhance healing of excisional wounds in nude mice. Wound Rep Reg 9: 297-304, 2001.