

ABC of wound healing

Recent advances and emerging treatments

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This is the last in a series of 12 articles

The healing of acute wounds involves a complex and dynamic series of events leading to the repair of injured tissues. These events, triggered by tissue injury, involve four overlapping but well defined phases: haemostasis, inflammation, proliferation, and remodelling.

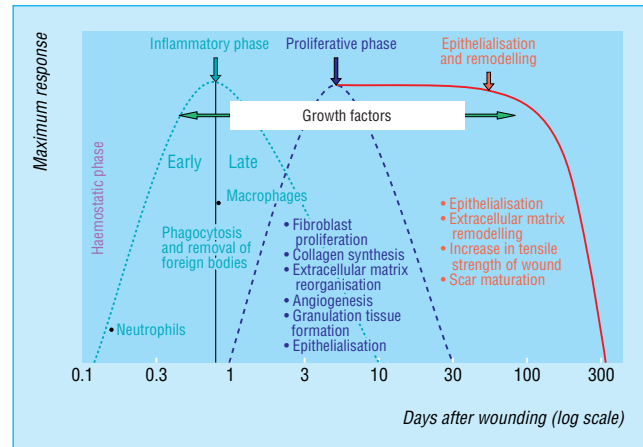
Haemostasis is secured by platelet aggregation and clot formation. The inflammatory phase begins with the arrival of phagocytic neutrophils and, later, macrophages at the wound site; they are important sources of and substrates for growth factors. The proliferative phase is characterised by the formation of new blood vessels (angiogenesis), synthesis of extracellular matrix components such as collagen, granulation tissue formation, and re-epithelialisation. The extracellular matrix is continually remodelled during the final phase; an avascular scar is the end result of the healing process.

Chronic wounds may be arrested in any of the four phases; commonly, however, disruption occurs in the inflammatory or the proliferative phases. Many mediators—including inflammatory cells, growth factors, proteases such as matrix metalloproteinases (MMPs), and cellular and extracellular elements—play important roles in different stages of the healing process. Alterations in one or more of these components may account for the impaired healing observed in chronic wounds.

Biological based treatments

Cryopreserved human cadaver skin (used in the UK), and human amniotic membrane and frog skin (used in other parts of the world) have long been used to treat wounds, particularly burns. More recently, artificial “skin substitutes” and growth factors have been developed to help achieve healing in chronic, non-healing wounds of varying aetiologies. These treatments target different stages of the healing process and, in the case of skin substitutes, replace lost tissue.

Artificial skin substitutes, products of tissue engineering, consist of a microengineered, biocompatible, polymer matrix in combination with cellular and/or extracellular elements such as collagen. Several growth factors (proteins involved in coordinating and regulating various interrelated processes during wound healing) produced by recombinant DNA technology have also been developed to aid healing of such wounds.



The four phases of acute wound healing

Composition of some tissue engineered skin substitutes

Product by type Content and description

Epidermal

Epicel, Laserskin	Cultured epidermal autograft (sheet)
CellSpray	Cultured epidermis in suspension form
BioSeed-S	Keratinocyte-fibrin-glue suspension
LyphoDerm	Lysate of cultured human keratinocytes, comprising cytokines and growth factors

Dermal (acellular)

Integra	Two layered skin substitute comprising biodegradable matrix and bovine collagen, and outer silicone layer
AlloDerm	Processed human cadaver skin with acellular dermal matrix and intact basement membrane
Biobrane	Porcine dermal collagen bonded to semipermeable silicone membrane

Dermal (cellular)

TransCyte	Allogenic human fibroblasts cultured on nylon mesh coated with porcine collagen
Dermagraft	Allogenic human fibroblasts cultured on bioabsorbable scaffold

Composite

Apligraf, OrCel	Allogenic cultured skin containing keratinocytes, fibroblasts, and bovine collagen
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Tissue engineered skin substitutes in wound healing

Product	Uses/advantages	Disadvantages
Epicel, Laserskin*	Permanent coverage for superficial and partial thickness burns	2-3 week lag period between biopsy and obtaining epidermis; lacks dermal component
Integra†	Immediate permanent coverage for surgically excised full thickness burns; reconstructive surgery	Requires healthy and non-infected wound base; in burns, autograft is needed after 3-4 weeks for epithelial cover
AlloDerm†	Intended to permanently cover full thickness burns and deep ulcers; reconstructive surgery	In burns, may necessitate removal after 2-3 weeks; autograft is needed for epithelial cover; not suitable for infected wounds
Biobrane†	To cover extensive partial thickness burns and donor sites	Temporary; not suitable for infected burn wounds
TransCyte‡	To cover surgically excised full thickness burns and non-excised partial thickness burns	Temporary (may need skin grafting after 2-3 weeks); not suitable for infected wounds and patients allergic to porcine collagen
Dermagraft‡	Non-healing diabetic foot ulcer and venous leg ulcer	Not for infected wounds or ulcers with sinus tracts
Apligraf§	Non-healing diabetic foot ulcer and venous leg ulcer	Not for infected wounds or patients allergic to bovine collagen
OrCel§	Acute and chronic deep dermal ulcers, partial thickness burns and donor site wounds	Not for infected wounds or patients allergic to bovine collagen

*Epidermal; †Dermal, acellular; ‡Dermal, cellular; §Composite.

Selected growth factors in wound healing*

Growth

factor	Wound healing related function	Indication
VEGF	Stimulates angiogenesis and collateral blood vessel development; accelerates granulation tissue formation	Has potential for use in diabetic ulcers; gene transfer encoding VEGF shown to be effective in ischaemic legs and ulcers by formation of collateral blood vessels
FGF	Promotes fibroblast proliferation, matrix deposition, wound contraction, and angiogenesis	Trials show that topical recombinant bovine FGF is effective in burns, donor site wounds, and pressure ulcers
KGF	Promotes proliferation and migration of keratinocytes	Trials show that topical recombinant human KGF-2 (repifermin) is effective in venous leg ulcers
EGF	Stimulates keratinocyte differentiation, proliferation, migration, and adhesion	Trials show that topical recombinant human EGF is effective in partial thickness burns
PDGF	Chemoattractant for neutrophils and fibroblasts; stimulates fibroblast proliferation	Topical recombinant human PDGF-BB (becaplermin) shown to be effective in diabetic foot ulcers
G-CSF	Stimulates production of neutrophils; enhances neutrophil and monocyte function; promotes keratinocyte proliferation	Recombinant human G-CSF injected subcutaneously shown to be effective in infected diabetic foot ulcers
GM-CSF	Mediates epidermal cell proliferation	Trials show that topical recombinant human GM-CSF enhances healing of venous leg ulcers
HGF	Recruits neutrophils, monocytes, and mast cells; has mitogenic and morphogenetic properties	Topical recombinant HGF may accelerate healing of chronic venous leg ulcers
TGF- β	Attracts macrophages and fibroblasts to wound site; stimulates angiogenesis and collagen metabolism	Trials show that recombinant human TGF- β 2 is effective in diabetic foot ulcers

VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; KGF = keratinocyte growth factor; EGF = epidermal growth factor; PDGF = platelet derived growth factor; HGF = hepatocyte growth factor; G-CSF = granulocyte colony stimulating factor; GM-CSF = granulocyte macrophage colony stimulating factor; TGF- β = transforming growth factor β . *Only PDGF is licensed for commercial use in the United Kingdom.

Products targeting inflammatory phase

The production and activity of several proteases—including metalloproteinases, serine proteases, and neutrophil elastases, which are tightly regulated in acute wound healing—may be altered in chronic wounds. Raised levels of such proteases can be detrimental to wound healing, and products aimed at counteracting their effect have been developed. One such product is Promogran, which is designed to inactivate proteases and also protect the host's naturally produced growth factors. It may be useful in the treatment of chronic wounds refractory to conventional treatments, but it is not effective in infected wounds or those with unhealthy wound beds.



Left: Chronic venous leg ulcer suitable for protease inhibitor dressing. Right: Infected diabetic foot ulcer, associated with Charcot's arthropathy, suitable for G-CSF treatment

Growth factors, including granulocyte colony stimulating factor (G-CSF) and transforming growth factor- β (TGF- β), have also been used to target this phase of healing. G-CSF, an endogenous haemopoietic growth factor, induces terminal differentiation and release of neutrophils from the bone marrow, enhances neutrophil and macrophage function, and promotes keratinocyte proliferation. Recombinant human G-CSF, injected subcutaneously, has been shown to enhance healing in infected diabetic foot ulcers.

TGF- β is chemotactic for macrophages, induces the production of collagen and fibronectin, and inhibits metalloproteinase activity. TGF- β 1 has been shown to accelerate wound healing in animal models, and topical application of TGF- β 2 has been shown to be effective in the healing of diabetic foot ulcers

Products targeting proliferative phase

Growth factors

Fibroblasts, the key type of cell in the healing process, are attracted to the wound site by several growth factors, including platelet derived growth factor (PDGF) and TGF- β . They proliferate and produce the matrix proteins fibronectin, hyaluronan, and later, collagen and proteoglycans, all of which help to construct the new extracellular matrix.

PDGF attracts keratinocytes and promotes the formation of granulation tissue. Recombinant PDGF was developed to expedite the proliferative phase. Becaplermin (as Regranex gel)



Topical application of recombinant platelet derived growth factor on a diabetic foot ulcer

is the only growth factor currently licensed for commercial use in the United Kingdom. A multicentre, double blind randomised controlled trial in patients with chronic diabetic foot ulcer showed topical PDGF to be superior to placebo in promoting healing. Its effectiveness was further enhanced when used in conjunction with debridement of the wound bed, emphasising the importance of good basic wound care.

Fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) are active in this phase of repair. FGF promotes fibroblast proliferation and collagen accumulation and accelerates the formation of granulation tissue. VEGF plays a crucial role in angiogenesis.

Cell and matrix based treatments

Autologous fibroblasts (that is, from the patient's own dermis) seeded onto a matrix derived from hyaluronic acid have been shown to be useful in treating diabetic foot ulcers and venous leg ulcers. Similarly, acellular collagen based matrices designed to mimic the extracellular matrix have been successfully used to treat chronic ulcers of varying aetiologies.

Loss of the dermal layer occurs frequently in deep ulcers and burns. Allogenic fibroblasts, obtained from neonatal human foreskin and cultured in vitro, have been used to provide the dermal replacement in such wounds. They are seeded either on a biologically absorbable scaffold (for example, Dermagraft) or on a nylon mesh (for example, TransCyte). The proliferating fibroblasts secrete collagen, matrix proteins, and growth factors and promote healing. They are designed to provide dermal replacement in a variety of wounds, though most evidence to date comes from the treatment of diabetic foot ulcers and burns.

Processed human cadaver skin, in which the cells are removed to leave a non-antigenic dermal scaffold (for example, AlloDerm), was one of the first dermal replacement treatments. Composite skin substitutes comprising allogenic keratinocytes (epidermal equivalent) and fibroblasts (dermal equivalent) are also effective in treating diabetic foot ulcers and venous leg ulcers.

Products targeting epithelialisation and remodelling

Growth factors

Epidermal growth factor (EGF) plays a vital role in keratinocyte differentiation, proliferation, migration, and adhesion. Topical application of recombinant EGF is effective in inducing epithelialisation of partial thickness burns and superficial granulating wounds. Keratinocyte growth factor (KGF) also induces proliferation and migration of keratinocytes. Recombinant human KGF-2 induces proliferation of epithelial cells and has been shown to enhance healing of venous leg ulcers. Granulocyte macrophage colony stimulating factor (GM-CSF), secreted by keratinocytes shortly after injury, mediates epidermal cell proliferation in an autocrine manner. Topical recombinant human GM-CSF is effective in the healing of venous leg ulcers.

TGF-β3 causes reduced deposition of collagen during the proliferative and remodelling phases, thus reducing scar formation. Trials into the efficacy of TGF-β3 in the treatment of hypertrophic scars are under way; the role of TGF-β3 in the treatment of keloid scars is unclear.

Cell based treatments

Autologous keratinocyte grafts or suspension (obtained after biopsy and culture of the individual's own keratinocytes) and allogenic cultured keratinocyte grafts are used to treat diabetic foot ulcers, venous leg ulcers, and partial thickness burns. A keratinocyte suspension in a fibrin sealant matrix has recently been developed to aid adherence of the keratinocytes to the

Recombinant bovine FGF accelerates the formation of granulation tissue and epidermal regeneration in patients with pressure ulcers and burns. Topical VEGF improves angiogenesis and granulation tissue formation in ischaemic wounds in animal models. Intramuscular gene transfer of VEGF has been shown to improve the collateral circulation in patients with peripheral vascular disease, leading to healing of ischaemic ulcers



Venous leg ulcer suitable for use of dermal or composite skin substitute



Partial thickness burn suitable for an epidermal skin substitute



Wounds suitable for therapies aimed at the remodelling and epithelialisation phases of wound healing



Left: Hypertrophic scar complicating healing of a sternotomy wound. Right: Keloid scarring of acne in an adolescent

wound bed (keratinocyte-fibrin-glue suspension). In addition, a total lysate of cultured human keratinocytes—comprising growth factors, cytokines, and matrix molecules, in a hydrophilic gel—has been developed to treat non-healing venous leg ulcers. The keratinocyte products are primarily used in specialist centres in the treatment of chronic wounds and burns.

Delivery of growth factors

Growth factors are currently delivered to the wound either topically (such as platelet derived growth factor) or by subcutaneous injections (such as granulocyte colony stimulating factor). However, their effectiveness is limited owing to their short half life and the presence of proteases in chronic wounds. To overcome this, gene therapy (the transfer of nucleic acid) has been investigated as a means of providing a longer lasting source

Concerns about biological products

Lack of level 1 evidence

Randomised controlled trials are lacking for many biological products, and the current evidence for many biological based treatments is based on non-randomised prospective trials, retrospective reviews, small case series (institutional or personal), and isolated case reports. Furthermore in the case of growth factors, there is little evidence on dose and duration of treatment.

No trials have evaluated the effectiveness of two comparable products (for example, dermal versus dermal skin substitute). Large, multicentre double blind randomised trials are ongoing for some of the products.

Transmission of diseases

Before human tissue is obtained for use in tissue engineered skin substitutes, the donors' medical histories are extensively reviewed and blood samples are screened for a wide variety of infectious diseases, including hepatitis, HIV, and syphilis. However, products obtained from human sources cannot be terminally sterilised owing to the presence of viable human cells, and existing tests cannot provide absolute assurance that such products will not transmit unknown diseases.

Similarly, tissue (for example, serum, collagen, cells) obtained from animal sources also carries the theoretical risk of transmitting infection, particularly prion diseases such as Creutzfeldt-Jakob disease.

Other concerns

Most biological based products contain bovine, porcine, or human constituents and thus have religious and ethical implications.

Tissue engineered skin substitutes and growth factors produced by recombinant DNA technology are expensive, which may limit their widespread use.

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Competing interests: For series editors' competing interests, see the first article in this series.

Products targeting different phases of wound healing process

Phase	Product	Growth factors
Inflammatory phase	Promogran	G-CSF, TGF- β 1 and β 2
Proliferative phase	Dermagraft, TransCyte, Alloderm, Apligraf, OrCel	PDGF, FGF, VEGF
Epithelialisation	Epicel, Laserskin, CellSpray, BioSeed-S, LyphoDerm, Trancell	EGF, KGF, GM-CSF
Remodelling and scarring	Silicone based products, such as silicone gel sheet (Cica-care)	TGF- β 3

For abbreviations, see footnote to previous table.

Current areas of research include regulation of target genes of cells involved in wound healing; new methods of delivery of specific cell products to the wound (including nanotechnology); use of adult pluripotent stem cells, which are capable of differentiating into essential cells involved in wound healing (such as fibroblasts, endothelial cells, keratinocytes). To date, there is only experimental (though promising) evidence for gene and stem cell therapy in the treatment of chronic wounds

Basics of advanced wound care

- Tissue engineered skin substitutes and other biological wound manipulations are seldom effective in sloughy and exudative wounds with unhealthy wound beds
- Good wound care (wound debridement and exudate management), adequate rest, compression, pressure relief, and skin care must be provided as clinically indicated
- The new approaches and biological based treatments should complement, not replace, the tenets of good, basic wound care

Cutaneous wound healing is a multistep process requiring the interaction and coordination of many different cell types and molecules—including growth factors and proteases. Given the multiple molecular mechanisms involved, no single mediator, growth factor, or gene is likely to be successful in accelerating healing. Similarly, there is heterogeneity within wound types; identification of the cellular and molecular dysfunction in individual wounds and targeting or supplementing them is one of the goals for the future

Key references and further reading

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