Diabetic foot, growth factors and tissue engineering

Vanessa Jones

Introduction

The normal physiological processes of wound healing are often disrupted by diabetes. Diabetes can interfere with growth factor and cytokine production, causing an alteration in the role of these proteins in controlling wound healing. Elimination of the factors that delay healing by the application of appropriate growth factor to the wound is receiving much attention and has become an exciting area of research. This article presents a critical review of some of the recent studies on growth factors and tissue-engineered skin and discusses the potential role of these products in healing diabetic foot ulcers.

The wound environment contains many growth factors, which are involved in all phases of wound healing. Growth factor is a broad term used to describe various proteins that cause panacrine, autocrine and some endocrine stimulation in a large number of cells.

The name growth factor was assigned to the first compounds found to display this activity, and reflects the initial activity, cell of origin or target cell. However, this name does not reflect the multiple functions that growth factors are now known to have, and a more correct term may be multifunctional peptides (Falanga, 1992). The term growth factor can refer to cytokines, peptides, inflammatory mediators, interleukins, tumour necrosis factor and interferons (Robson, 1997).

The action of these proteins may be inhibitory or stimulatory, depending on the local environment in the wound and how these interact with each other at any specific time (Cox, 1993). Given the complexity of these multiple interrelated processes, it is difficult to imagine how the application of a single growth factor would have any major effect on the healing process.

Studies of growth factors

It is now possible to study the role of growth factors in wound healing, since they can be produced in their pure form using recombinant DNA technology.

The majority of experimental work has been performed on the less expensive rat rather than pigs, even though pig skin is physiologically closer to human skin.

In 1985, a single application of platelet-derived growth factor (PDGF) was shown to accelerate the growth of granulation tissue in normal and diabetic rats (Grotendorst, 1985). Twelve years later, a combination of transforming growth factor β1 (TGFβ1) and basic fibroblast growth factor (bFGF) was shown to be more effective than either factor alone in restoring granulation tissue formation in diabetic rats (Davidson et al, 1997).

PDGF was one of the first growth factors to be identified (Declair, 1999) and is one of the first growth factors to reach the wound site following release from alpha granules in the platelets. It is therefore not surprising that much of the work on human subjects has been performed using this pivotal growth factor.

Steed (1995) investigated the efficacy and safety of recombinant human platelet-derived growth factor BB (rhPDGF-BB) in a double-blind placebo-controlled trial involving 118 patients with neuropathic ulcers. The results showed a statistically significant improvement in the 61 patients randomised to rhPDGF-BB, with 48% of this group progressing to complete healing compared with only 24% of the control group.

This work provided a benchmark for further development of a commercially available rhPDGF-BB gel 0.01%, which was marketed in the USA as becaplermin and is now licensed for use in the UK as

ARTICLE POINTS

1 Diabetes can interfere with growth factor and cytokine production, causing an alteration in the role of these proteins in controlling wound healing.

2 Elimination of the factors that delay healing by the application of appropriate growth factor to the wound has become an exciting area of research.

3 Growth factor and skin substitute will provide an important therapeutic option for some, but not all, diabetic foot ulcers.

4 An understanding of the basic and applied knowledge of growth factors will be essential for all practitioners involved in providing this new technology for their patients.

KEY WORDS

- **Diabetic foot**
- **Growth factors**
- **Tissue engineering**
- **Wound healing**

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Publisher's note: This image is not available in the online version.

Regranex 0.01% gel.

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Dermagraft applied to a diabetic foot ulcer.

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Hyalofill.

Regranex®. Trials of rhPDGF-BB gel 100µg/g (0.01%) have shown promising results on various types of chronic wounds.

The concentration of growth factor in the gel was investigated in four trials involving 922 patients with full-thickness diabetic foot ulcers (Smiell, 1998). Patients were divided into four treatment arms: two groups received different concentrations of rhPDGF-BB, one group placebo gel, and one group no gel (the control group). All groups received good wound care. Although numbers vary in each study, the results of two of the trials demonstrate significant improvement in closure rate with the application of rhPDGF-BB gel 100µg/g (0.01%) (Weiman, 1998).

Another study of the healing of diabetic ulcers (Richard et al, 1995) involved the application of topical human recombinant bFGF. This trial did not demonstrate any improvement in healing in the treatment group over the placebo group. However, the numbers in this trial were small (17) and it was concluded that single growth factors were not sufficient to accelerate wound healing in the diabetic patient.

Knighton et al (1989) applied a platelet-derived wound healing formula (Procuren solution) to 20 diabetic patients, and achieved 90% healing during a period ranging from 12 to 30 months. Procuren is made from the patients' own platelets, which are treated with thrombin to obtain the growth factors. The growth factors released from treated platelets' alpha granules include PDGF, TGFα, TGFβ, epidermal growth factor (EGF), bFGF and platelet factor-4.

The purpose of this study, however, was to review the outcome of an 'aggressive, comprehensive amputation intervention program in a well-defined group of high risk patients for whom limb loss was imminent'. Procuren provided only a small part of the intensive and aggressive care given to these patients, and the results need to be interpreted with this in mind.

The exact role that growth factors play in chronic wounds is still unclear (Falanga et al, 1995). It is unknown at which stage of the healing process any one combination of, or at what concentration, these growth factors would be most effective.

Tissue engineering (skin substitutes)

An alternative method of introducing growth factors into the wound is through tissue engineering. Skin substitutes can be classified into one of three categories depending on their composition:

- Epidermal components
- Dermal components
- Composite grafts containing both epidermal and dermal components. (Phillips, 1998).

Cultured skin grafts have been available for many years, but it is only recently that scientists have attempted to mimic the structure and function of the skin with biological engineering.

Grafts and substitutes can be autologous, with a decreased risk of rejection, or allogenic, which are easier to obtain for immediate use (Leah, 1998). Depending on its composition, tissue-engineered skin can facilitate cell proliferation and production of extracellular matrix components and increase the local concentrations of growth factors in the wound.

Dermal replacement

The first cultured human dermal replacement, consisting of neonatal fibroblasts, has now been produced and is marketed as Dermagraft (Gentzkow et al, 1996). For this product, fibroblasts are cultured in vitro onto a bioabsorbable mesh to produce a metabolically active tissue. This secretes matrix proteins and cytokines which remain active after implantation onto the patient's wound bed (Landeen et al, 1992).

Trials of Dermagraft on diabetic foot ulcers have yielded promising results. In a controlled prospective randomised pilot study, Gentzkow et al (1996) evaluated healing in 50 patients with diabetic foot ulcers over a 12-week period. They found that complete healing in those receiving Dermagraft was 50% compared with 7.7% in the control group.

Further work replicated these results, with a median time to complete healing of 13 weeks for the Dermagraft group compared with 28 weeks for the control group (Pollak et al, 1997).

A smaller trial conducted in the UK concluded that this product would appear

PAGE POINTS

1 The application of growth factor or tissue-engineered skin is not an alternative to good wound care.

2 Growth factor and skin substitute will provide an important therapeutic option for some, but not all, diabetic foot ulcers.

3 Preparation and application of the growth factor or skin substitute may take between half an hour and one hour daily.

4 All practitioners using this new technology will require an understanding of the basic and applied knowledge of growth factors.

to have a place in the treatment of diabetic foot ulcers, but only with appropriate preparation of the wound bed and in a controlled environment (Grey et al, 1998).

Epidermal and composite grafts, although not yet trialled on patients with diabetic foot ulcers, have demonstrated some promising results in healing other chronic wounds such as leg ulcers.

Epidermal replacement

Cultured epidermal grafts are generally more successful when placed on a dermal bed as it is well known that the dermis influences epithelial migration, differentiation, attachment and growth (Choucair and Phillips, 1997).

One of the most recent developments in this area is the production of an autologous keratinocyte delivery system called Vivoderm. A biopsy specimen of the patient's skin is taken and cultured on a membrane of HYAFF, an ester of hyaluronic acid that acts as a support surface for keratinocyte cell growth and delivery (Hollander et al, 1999).

One of the advantages of this system is that the keratinocytes do not need to grow to confluence before grafting takes place. The presence of this 'preconfluent' state allows grafting to take place in as little as several days. Hyaluronic acid is known to influence the inflammatory response, enhance angiogenesis and aid the deposition of collagen (Chen and Abatangelo, 1999).

Vivoderm can be used on a wound bed pre-treated with Hyalofill, or other artificial dermal components, to enable skin regeneration (Leigh and Navsaria, 1997).

Composite grafts

Apligraf is a bilayered skin equivalent consisting of type I bovine collagen and live allogenic human skin fibroblasts and keratinocytes. Apligraf looks like human skin, makes its own matrix proteins and growth factors, and can heal by itself if wounded (Falanga et al, 1998).

Although considered a human skin equivalent (HSE), Apligraf lacks many cellular components of skin, including endothelial cells and Langerhans' cells (Falanga, 1999). Rejection of Apligraf is considered unlikely, owing to the absence of Langerhans' and other immune cells, which are thought to be the main cause of rejection (Thivolet et al, 1986; Falanga et al, 1998).

Although skin substitutes are an exciting development, it is not possible to compare their clinical performance as comparative studies have yet to be performed (Phillips, 1998). Given the cost, both in time and resources, it is doubtful whether such trials will be conducted in the UK within the foreseeable future.

Conclusion

It is important to remember that, although growth factors have shown promising results in clinical studies, the application of growth factors or tissue-engineered skin is not an alternative to good wound care. It should also be borne in mind that the conduct of these trials involved thorough assessment of vascular status, wound debridement, and treatment of any existing infection. Interpretation of the results should therefore take into account the comprehensive and thorough care afforded to these patients.

This field of wound care is undoubtedly exciting and promising, and will provide an important therapeutic option for some, but not all, patients with diabetic foot ulcers.

These trials can be extremely labour intensive, with preparation and application of some of the growth factors or skin substitutes taking between half an hour and one hour daily. It is for this reason, along with what may appear in some cases to be prohibitive costs, that only time will tell which of these products may be used on a routine clinical basis.

Perhaps more importantly, an understanding of the basic and applied knowledge of growth factors will be essential for all practitioners involved in providing this new technology for their patients (Declair, 1999). Until all the processes in wound healing are fully understood, finding ways to stimulate healing in chronic wounds such as diabetic foot ulcers will continue to pose a major challenge. ■

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