Current status of genetic modulation of growth factors in wound repair (Review)

KATRIN RIEDEL[1], FRANK RIEDEL[2], ULRICH R. GOESSLER[2], GISBERT HOLLE[3], GÜNTER GERMANN[1] and MICHAEL SAUERBIER[1]

[1]Department of Hand, Plastic and Reconstructive Surgery, Burn Centre, BG-Trauma Centre, Plastic and Hand Surgery of the University of Heidelberg, Ludwigshafen; [2]Department of Otolaryngology, Head and Neck Surgery, University Hospital Mannheim, University of Heidelberg, Mannheim; [3]Department of Plastic, Reconstructive and Hand Surgery, Markus Hospital, Frankfurt, Germany

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Abstract. Growth factors are members of a large functional group of polypeptide regulatory molecules secreted by different cells. They are important players in orchestrating all stages of wound healing exerting their influence through autocrine and paracrine fashions within sites of injury and repair. They are mitogen, chemotactic, they regulate cell-cell interactions and influence synthesis and composition of extracellular matrix components. The use of growth factors to stimulate wound healing is a promising therapeutic approach to repair chronic tissue defects. The delivery of genetic material offers an attractive treatment modality to produce an appropriate amount of growth factor proteins within the wound site. Gene therapy might become a significant treatment modality for those wound healing pathologies refractory to other wound management approaches. This review discusses several methods of growth factor gene transfer into wound tissue.

Contents

1. The process of wound healing
2. Impaired wound healing
3. Growth factors
4. Growth factors in preclinical studies
5. Growth factors in clinical trials
6. Limitations of topical or systemic growth factor therapy
7. Basic principle of genetic modulation
8. Methods of gene delivery
9. Non-viral techniques (transfection)
10. Viral techniques (transduction)
11. Current gene therapy results in wound healing
12. Conclusions

Correspondence to: Dr Katrin Riedel, Department of Hand, Plastic and Reconstructive Surgery, Burn Centre, BG Trauma Centre, Plastic and Hand Surgery at the University of Heidelberg, Ludwig-Guttmann-Strasse 13, D-67071 Ludwigshafen, Germany
E-mail: k_riedel1@gmx.de

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1. The process of wound healing

Wound healing involves a complex series of consecutive, but overlapping, stages, characterised by the sequential movement of different cell populations into the wound site. This complex process, relying on the collaboration of many different extracellular matrix components, cell types and soluble mediators, ultimately leads to the restoration of injured tissue. Simplified, the process of wound healing is often subdivided into three phases: i) inflammation, ii) granulation formation, and iii) matrix formation and remodelling (1). Wound repair is initiated with the aggregation of platelets, formation of a fibrin-based provisional matrix, and release of growth factors from the activated coagulation pathways, injured cells, platelets, and extracellular matrix (ECM), followed by migration of inflammatory cells to the wound site. Thereafter, keratinocytes migrate into the wound and the growth of new blood vessels from pre-existing ones (angiogenesis) is initiated. During the process of angiogenesis, fibroblasts deposit and remodel the granulation tissue. Cell migration, angiogenesis, degradation of provisional matrix, and remodelling of newly formed granulation tissue, all require co-ordinated breakdown, synthesis and remodelling of the ECM (2,3).

2. Impaired wound healing

Acute and chronic wounds represent major clinical problems. It is estimated that at any given time, approximately 1.5% of the population have chronic wounds that require treatment (4). Chronic wounds that fail to close and re-epithelialize (5) include pressure sores, lower-extremity diabetic and venous stasis ulcers, and wounds in immunocompromised subjects. The latter group comprises patients with uncontrolled diabetes mellitus, chronic steroid use, sepsis, and those undergoing systemic chemotherapy and/or radiation therapy. Although

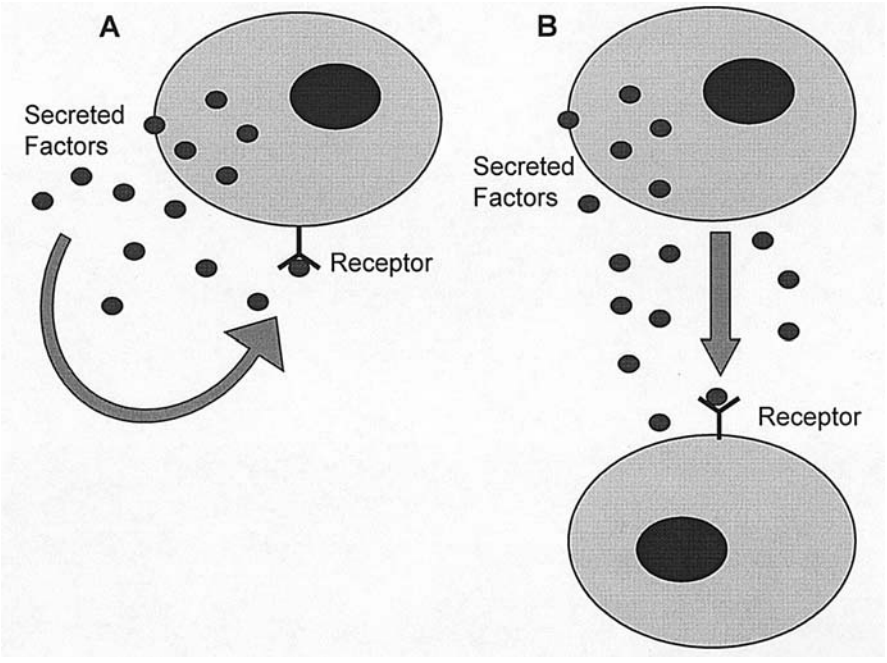

Figure 1. Autocine and paracrine secretory pathways of growth factors [modified from Shenaq and Rabinovsky (9)]: growth factors can act on the same cell type from which they were secreted (A: autocrine pathway) or act on adjacent cells (B: paracrine pathway).

many reasons for why these wounds fail to heal have been proposed, no unifying theory exists, and the cause is most likely multifactorial. Pressure sores are best treated with prevention and alteration in the local forces associated with the wound breakdown and local debridement, when indicated. Chronic diabetic ulcers secondary to large-vessel occlusive disease often necessitate surgery. On the other hand, lower-extremity ulcers secondary to diabetic microvascular, neuropathic disease and/or venous stasis are often not amenable to revascularization. Once again, altering local factors, including excess pressure, regular surveillance, and meticulous foot care, are conterminous in preventing wound progression. Patients with overwhelming infection or who are in a state of profound sepsis also demonstrate impaired wound healing. As elucidated earlier, septic states are associated with a robust pro-inflammatory cytokine response that delays wound healing. In these septic patients, the neutralisation of the pro-inflammatory cytokines and growth factors that may be responsible for delayed and impaired wound healing offers an attractive means for therapy. Patients who are being treated with chronic steroid therapy or who are undergoing systemic chemotherapy and/or radiation therapy are known to have incompetent wound-healing mechanisms. In these patients, the endogenous administration of cytokines and growth factors may be of particular use (6).

3. Growth factors

Growth factors are members of a large group of polypeptide regulatory molecules released by many cell lines in the body. Growth factors are cytokines whose primary role is directing the maturation of cells during normal turnover and in the postinjury tissue repair response (7,8). They are a major part of the process of wound healing, being involved in cell infiltration, cell proliferation, matrix deposition and scar formation (9). They can be involved in the triggering, control, and determination of these events by acting through autocrine or paracrine pathways, or combinations of these (9). Several growth factors that are released at the wound site are presumed to be necessary for wound healing (10). Over the past two decades, much research has been conducted in characterising the role and potential treatment applications of individual growth factors in impaired wound healing states. A growth factor can affect other cell types as well as the cell where it was produced (Fig. 1). These interactions result in the recruitment of macrophages, the proliferation of fibroblasts and keratinocytes, the expression of other growth factors, the formation of neovascularised granulation tissue and the expression of extracellular matrix molecules (9). The role of different growth factors in the process of wound healing is discussed below.

Platelet-derived growth factor. Platelet-derived growth factor (PDGF) was initially described in the early 1970s as a platelet-derived mitogen, specific for target fibroblasts and smooth muscle cells (11). It is a dimer consisting of two polypeptide chains, A and B. PDGF naturally occurs as three isomers (AA, AB and BB) (12,13), named according to the arrangement of the two polypeptide chains, which share 60% homology (7,8). These isomers exert their influence by binding to either of two receptors (14). PDGF is produced by platelets, macrophages, vascular endothelium, fibroblasts and keratinocytes (15,16), which are all cells involved in early wound healing. Platelets entering the wound site soon after injury are the earliest and largest source. Thus, PDGF is essential in initiating and sustaining the wound-healing response. Its immediate effects include the recruitment and activation of immune cells and fibroblasts (14,17). There-

after, PDGF is also secreted by macrophages and stimulates collagen and proteoglycan synthesis (13,18). PDGF is, therefore, involved in cell recruitment at all stages of wound healing: the stimulation of epithelial and endothelial cells, matrix formation and modulation of other growth factor activities. Pierce *et al* reported that all three PDGF isomers are found in extremely low levels in both normal skin and chronic nonhealing ulcers (19). Such observations have led to widespread clinical applications of PDGF.

Transforming growth factor-ß. Transforming growth factor-ß (TGF-ß) was first described in 1983 based upon its ability to stimulate the anchorage-independent growth of mouse embryo fibroblasts and normal rat kidney fibroblasts (20). Since then, it has been shown to represent a large family of structurally related factors with various activities. TGF-ß exists as three isomers (TGF-ß1, -ß2, and -ß3), whose most important effects include fibroblast-migration, maturation, and extracellular matrix synthesis (21,22). Although all three isomers are similar in function in *in vitro* assays, emerging evidence suggests that their biological *in vivo* activities may be quite different (23). TGF-ß isomers exert their influence through three receptors, which are referred to as types I, II, and III. Numerous cell types have receptors to TGF-ß. The type I receptor seems to modulate extracellular matrix synthesis, whereas the type II receptor plays an important role in cell growth. The type III receptor presents biologically active TGF-ß to the other two receptor complexes (23). Released by degranulating platelets, activated macrophages, keratinocytes and fibroblasts at the site of injury (21,22), TGF-ß influences the inflammatory response, angiogenesis (24,25), re-epithelialisation, extracellular matrix deposition and remodelling by promoting collagen and proteoglycan synthesis while inhibiting protease activity (21-24,26). These effects make TGF-ß important in wound repair and have prompted considerable attention in the context (27). Of the three isomers, TGF-ß1 has been the most widely studied. TGF-ß1 is released early in the wound healing process, and there is a second peak in concentration at 5-7 days (28,29). Specifically, TGF-ß1 seems to play an important role in the collagen metabolism (21). The absence of TGF-ß1 delays wound healing, as shown in immunodeficient TGF-ß1 knockout mice (30). In addition, TGF-ß1 accelerated wound healing in normal, steroid-impaired, and irradiated animals in experimental wound models (22,23,31,32).

Epidermal growth factor. EGF was discovered in mouse salivary glands in 1962 (33) and was the first growth factor to be described. It interacts with the EGF receptor on epidermal cells and fibroblasts (34). Epidermal growth factor (EGF) is mainly secreted by keratinocytes and directs epithelialisation in an autocrine fashion (35). In addition, EGF stimulates fibroblast collagenase secretion and thus may be important in wound remodelling (36). Recent evidence suggests that aged dermal fibroblasts have a decreased EGF receptor expression and may contribute to the impaired healing seen during ageing (37). In contrast, EGF inhibits fetal wound contraction and thus may contribute to the scarless repair seen in utero (38).

Vascular endothelial factor. Vascular endothelial growth factor (VEGF) was first isolated as a mediator of vascular permeability in the late 1970's (39). There are now five known isoforms, detectable in different human tissues (40). VEGF acts via two tyrosine kinase receptors located predominantly on endothelial cells (40). VEGF is released primarily by keratinocytes but also by macrophages and fibroblasts (41). VEGF levels rise steadily after wounding and serve as a potent angiogenic factor (42). VEGF production is influenced greatly by local tissue conditions, including hypoxia, and also by nitric oxide production (43). VEGF causes increased vascular permeability and deposition of a proangiogenic matrix, as well as formation of blood vessels (40). VEGF administration improved granulation-tissue formation in both normal and hypoxic tissues during experimental wounding (44).

Fibroblast growth factor. Fibroblast growth factor (FGF) is actually a family of heparin-binding growth factors. Originally, acidic FGF and basic FGF were the two forms identified, but more recently the family has been expanded to include seven additional forms. These are now named FGF1 to FGF9 (45). FGFs are produced by fibroblasts, endothelial cells, smooth muscle cells, chondrocytes and mast cells (46). The fibroblast growth factor (FGF) family of proteins is an important mediator of wound angiogenesis and epithelialisation. Both FGF isomers are released by macrophages and endothelial cells within wounds and stimulate fibroblast and keratinocyte proliferation and migration (47,48). In addition, bFGF promotes the endothelial-cell growth and migration that are essential for angiogenesis (49). Finally, bFGF plays an important role in preventing wound contraction and in collagen remodelling (50,51).

Keratinocyte growth factor. Keratinocyte growth factor (KGF) was discovered in 1989 and takes its name from its action on keratinocytes (52). It is a member of the FGF family of polypeptides (53) and as such is now also called FGF7 (54). Two forms, KGF-1 and KGF-2, have been identified and interact with the same receptor (55-57). Interestingly, both isomers are secreted by fibroblasts and endothelial cells (both of mesenchymal origin), whereas KGF receptors are found exclusively on epithelial cells of ectodermal origin (55). There is increased expression of KGF during re-epithelialisation of normal skin, and it induces the proliferation and migration of keratinocytes (52). It may constitute the dermal/epidermal signal stimulating re-epithelialisation of a wound.

Insulin-like growth factor. Insulin-like growth factor (IGF), also known as somatomedian, exists as two isomers, IGF-I and IGF-II, which help regulate cell metabolism and growth. IGF is produced primarily in the liver and skeletal muscle but is also secreted by fibroblasts, neutrophils, and macrophages within wounds (58,59). IGF is largely bound to a carrier protein, which seems to play a major role in regulating its effects (60). These primary effects of IGF include stimulating fibroblast and keratinocyte proliferation, as well as collagen synthesis. In addition, IGF influences endothelial-cell turnover and may promote neovascularisation (58,61). IGF-I seems to play a greater role than IGF-II in the response to postnatal injury because IGF-II concentrations, although high during fetal life, rapidly decline after birth in most tissues (58). IGF-I

Table I. Growth factors involved in the process of wound healing.

Growth factor	Main stage of involvement	Major source	Target cells and major effects
PDGF	Inflammation, tissue formation, remodelling	Platelets, macrophages, endothelial cells	Chemoattractant for neutrophils and fibroblasts; mitogenic for endothelial cells and fibroblasts
FGF	Tissue formation, remodelling	Fibroblasts, endothelial cells	Mitogeneic for endothelial cells, fibroblasts and keratinocytes, stimulates angiogenesis
EGF	Tissue formation	Platelets, macrophages, keratinocytes	Mitogeneic for endothelial cells, fibroblasts and keratinocytes
TGF-ß	Inflammation, tissue formation, remodelling	Platelets, macrophages, fibroblasts, keratinocytes, neutrophils	Mitogeneic for fibroblasts and smooth muscle cells
VEGF	Tissue formation	Neutrophils, platelets	Stimulating angiogenesis
KGF	Tissue formation	Fibroblasts	Mitogeneic and chemotactic for keratinocytes
IGF	Inflammation, tissue formation, remodelling	Fibroblasts, neutrophils, and macrophages	Stimulating fibroblast and keratinocyte proliferation, collagen synthesis

concentrations are normally low in unwounded skin but steadily rise within 24 h after experimental wounding. Thereafter, elevated levels of IGF may persist locally for several weeks (59), (Table I).

4. Growth factors in preclinical studies

The administration of several recombinant growth factors has shown clinical improvement in wound-healing rates in preclinical animal models (62-65). In incisional wound healing, PDGF induces fibroblast proliferation and differentiation, collagen deposition and angiogenesis (66,67). Topical application of PDGF has been shown to improve the breaking strength of incisional wounds and, in particular, it partially reverses the reduction in breaking strength in wounds in aged ischaemic animals (66). Wound healing was accelerated (47,68), and healed wounds appeared microscopically normal after treatment. The PDGF-BB isomer is the most widely preclinically studied. The administration of PDGF-BB has improved wound closure in chronic and diabetic nonhealing ulcers in both rodents and humans (19). By contrast, PDGF-BB did not improve wound healing or contraction in steroid-impaired animals (69).

Topical TGF-ß1 stimulates wound repair in young ischaemic rabbit skin (66). It exerts its effect in young rabbits by increasing the rate of granulation tissue formation, but without altering scar prominence (66). TGF-ß1 also accelerated wound healing in irradiated animals (32,70,71). In addition, the administration of exogenous TGF-ß1 and partially reversed wound-healing deficits in steroid-impaired animals (31,72).

Given its profound mitogenic effect on keratinocytes and fibroblasts, recombinant bFGF has been used in animal studies during both normal and impaired wound-healing conditions. A single application of recombinant bFGF accelerated the rate of epithelialisation by 20% in porcine wounds (48). In addition, the administration of recombinant bFGF reverses the impairment in the strength of healing wounds seen in diabetic rats (73-75). In addition, FGF-2 restores the reduction in angiogenesis in diabetic mice to levels found in non-diabetic mice (76). Furthermore, animal studies have shown that topical application of recombinant FGF-2 improves the strength of ischaemic wounds (77) and accelerates the rate of their closure (75), suggesting that it may play an important role in the management of locally compromised wounds (75,78).

Application of EGF significantly accelerates epidermal regeneration of partial- and full-thickness skin wounds in pigs (79), and continuous or prolonged EGF exposure increases tensile strength in rat skin wounds (80). These effects make the use of EGF attractive in experimental wound-healing models. In fact, several experiments have shown that EGF accelerates wound-healing time and epithelialisation in animal burn and wound models (81,82). Topically applied VEGF improves granulation tissue formation in ischaemic rabbit wounds, although it has no effect on epithelium formation (44). Topical VEGF has recently been found to improve wound closure by 25% in a diabetic mouse model, perhaps because of reduced VEGF production by macrophages, neutrophils and keratinocytes in diabetic mice (83). Similar findings of decreased VEGF in diabetic wounds have been observed in other cutaneous wound healing models (84) and tissue samples from chronic wounds (85,86).

The mitogenic effect of KGF on epithelialisation makes it particularly attractive in modulating wound healing. The administration of recombinant KGF-1 or KGF-2 improved

re-epithelialisation, collagen content, and wound-breaking strength in experimental murine wound models (87,88). Accordingly, topical application of KGF results in an increased rate of re-epithelialisation in pig skin (87). KGF also promotes epithelialisation in ischaemia-impaired rabbit ear wounds, but does not affect the final scar (55). Induction of KGF expression in diabetic mice is reduced and delayed (57).

IGF-I concentration has been shown to be decreased in the experimental wounds of both diabetic and steroid-impaired animals (59,60,72). More importantly, the exogenous administration of IGF-I improved wound healing in both diabetic and steroid-impaired subjects. These results suggest an important role for IGF in the wound healing of both normal and impaired subjects.

5. Growth factors in clinical trials

Based upon promising preclinical results using administration of growth factors in the treatment of acute and chronic wounds, various clinical trials have been conducted. In humans, PDGF is found in surgically created acute wounds, but not in chronic non-healing wounds (19). Based on this, the result of local application of PDGF to wounds has been examined with the expectation that vascular granulation tissue formation would be enhanced, resulting in the promotion of epithelial growth (89). The PDGF-BB isomer is the most widely clinically studied, because the receptor for PDGF is predominant in fibroblasts, vascular smooth muscle cells and microvascular endothelium. In fact, becaplermin (Regranex, Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ), a recombinant human PDGF-BB, is the first FDA-approved growth factor available for the treatment of diabetic neuropathic ulcers. A multicenter, double-blinded, placebo-controlled trial showed a 48% complete closure rate in a PDGF-BB-treated group, versus 25% in the control group of locally debrided and adequately oxygenated diabetic neuropathic ulcers (90,91). Recent clinical trials with recombinant PDGF-BB have also shown promising results in accelerating wound-healing rates of advanced pressure sores (92,93). It is worth noting, however, that there is wide variation in the response rates. Study design, patient selection and quantification methods may account for the different results.

Regarding the use of FGF, the results from the few trials that have been performed are less encouraging. A human clinical trial of topical FGF-2 in the treatment of patients with pressure ulcers showed a non-significant reduction in wound volume of 69% in those treated with FGF-2 compared with 59% in those treated with placebo (89), suggesting that topical FGF-2 has no advantage over placebo. In contrast, a recently completed large phase III trial in China demonstrated that recombinant bFGF administration accelerated wound-healing times in burns, operative wounds, and chronic dermal ulcers by an average of 3-4 days, with a successful closure rate of >90% for all groups (94).

EGF stimulates epithelialisation in early human wound repair (34). In a trial of skin graft donor site healing in 10 patients, the addition of topical EGF increased epithelialisation and shortened healing time by 1.5 days compared with standard treatment alone (80). Treatment of venous ulcers with EGF resulted in complete healing in 35% of those treated with EGF compared with 11% receiving placebo, with a reduction in ulcer size of 73 versus 33% by 10 weeks (95). A cross-over trial of topical EGF in patients with chronic wounds, with no evidence of healing over an initial period of 3 weeks to 6 months, resulted in healed wounds in 8 of 9 patients in a mean of 34 days after initiation of topical EGF twice daily (96).

In conclusion, the early optimism for growth factor-directed therapy due to positive results in animal models was followed by controversial clinical results being not that promising as expected (97). Further basic science research as well as large clinical trials will be necessary.

6. Limitations of topical or systemic growth factor therapy

With ongoing characterisation of growth factors and their function, our understanding of their effects grows. Due to synergistic effects of these mediators, therapy directed at any one growth factor may not attenuate pathological wound-healing responses (4,102,104). The timing of these strategies is essential to effective growth factor therapy. Any adverse cellular events triggered by growth factors may continue long after they become undetectable. In such situations, the simple neutralisation of these factors alone is not enough. Instead, opposing and antagonising growth factors added to the local milieu may be necessary to initiate a compensatory response. Finally, the further development of delivery systems to administer these factors into wounds may be required. From local topical application to systemic therapy, these growth factors must be sufficiently bioavailable to exert their effects in a timely and appropriate fashion. The limited success of most other purified growth factors may be because of properties such as short half-life, low bioavailability, enzymatic inactivation, and the need for carrier molecules. Topical preparations in particular may have unreliable absorption and utilisation by their targets. On the other hand, systemic administration of these factors may have profound unwanted global responses. Such shortcomings have led to experimentation with targeted gene therapy. Many of the limitations exhibited by purified growth factor use may be circumvented by gene therapy. The limited time of cytokine effect and the expense of production and application of cytokines has led to the development of gene delivery systems that produce a more prolonged effect. Gene vectors produce a higher transduction efficiency and are more cell-selective (98). For this reason, gene therapy of growth factors is under intense investigation (99-104). Following, we review the current techniques used for gene transfer and the potential application of these techniques in the treatment of chronic wounds.

7. Basic principle of genetic modulation

Gene therapy was originally developted as a way to correct heritable gene defects in targeted populations of mutant cells (105,106). Now, however, the basic concept of gene therapy is more generally defined as a treatment approach for several types of human disease based on the introduction of genetic material (DNA) into an individual which confers a therapeutic effect either directly by the transgene product or via an additional agent, e.g. by activation of an inactive pro-drug

(107). The transfer of a therapeutic gene can lead to a cure of a disease or offer a transient advantage for tissue growth and regeneration. The initial effort toward developing somatic gene therapy relied on an indirect means of introducing genes into tissues, called *ex vivo* gene therapy or autologous cell transfer. By this approach, target cells were removed from the body, transduced with viral vectors carrying recombinant genes, and then re-implanted (105). More recently, somatic gene therapy has relied on the direct introduction of DNA into tissues (105). To achieve this goal, a therapeutic gene first has to be efficiently delivered to the specific target cell. Second, its expression has to reach and sustain a certain level to achieve its therapeutic purpose. Third, the levels and timing of the gene's expression within the target cells should be reversibly controlled by a pharmacological agent. In this way, the therapeutic dose can be finely tuned as the disease or tissue regeneration evolves, and stopped when it is no longer needed.

8. Methods of gene delivery

The choice of gene delivery system (vector) is crucial for gene therapy (107). Methods of gene transfer may be virus-independent (transfection) of virus-dependent (transduction). The following review covers these two major strategies of gene delivery systems. The method chosen is dictated largely by the condition for which gene therapy is being utilised. Transfection may be required, for example, when the introduction of large pieces of DNA is required, as current viral vectors are limited in the size of the DNA sequence they can incorporate successfully. Both strategies may be carried out either *in vivo*, whereby genes are delivered directly to target cells; or *ex vivo*, in which cells are removed from the patient, genetically modified, and implanted back into the tissue. In addition, potential side-effects on the host, such as toxicity and immunisation, must be prevented. Trials in humans have to date largely proved safe, although the death in 1999 of a patient emphasises the need for continued vigilance in the planning and performance of such trials (104).

9. Non-viral techniques (transfection)

Transfer of genetic material into mammalian cells can be achieved using various chemical or physical methods and is dependent on cellular transport mechanisms for uptake and expression in the host cell. Such techniques are attractive for their ease of manipulation. Its efficiency *in vivo*, however, is low, and gene expression is transient.

Direct injection of DNA. Perhaps the simplest method of gene delivery is the direct injection of naked DNA into tissues and cells. Direct injection of genetic material into muscle was associated with a low level of spontaneous DNA uptake/incorporation and resultant gene expression (108). This discovery propelled further research on DNA injection and has led to the development of various injection techniques and their use in many clinical settings. Advantages of direct DNA injection include simplicity in technique and preparation, local delivery to the target organ, and the ability to act on non-dividing cells. Their drawbacks are that they can only be applied to tissues accessible by injection, are unable to target specific cells, and result in low transfection efficiencies, thus requiring repeated treatments (109).

Liposomes. Liposomes are self-assembling particles formed from phospholipids. The phospholipids are able to encapsulate substances within their layers. This feature was first used as drug carriers, but later found application in genetic engineering for delivery of viruses and plasmid DNA (110). Although the earliest liposomes were relatively inefficient modes of gene transfer, the discovery of 'cationic liposomes' greatly improved transfection efficiency (111,112). Cationic liposomes are distinguished from more primitive liposomes by their positive charge, which facilitates their interaction with both cell membranes and negatively charged DNA. Transfection with liposomes, or 'lipofection', is achieved by either direct application or by injection into a defined arterial or venous system. Once liposomes are delivered, they are able to gain access into the cytoplasm by cellular phagocytosis. The efficiency of cationic liposome-mediated gene transfer *in vitro* can vary greatly depending on the type of cells targeted and the route of administration (113). Liposomes offer a number of advantages as a method of gene transfer including technical simplicity, the ability to package relatively large amounts of genetic material, and low immunogenicity. The major disadvantages of cationic liposomes are their non-specific targeting and relatively low transfection efficiency (4). The future success of cationic liposomes as a method of gene therapy will rely on further improvements in targeting strategies and transfection efficiency.

Electroporation. Electroporation uses the application of electric pulses to cells to create pores in the plasma membrane allowing DNA diffusion into the cell. Electroporation has been primarily used as an *ex vivo* method of gene therapy. However, recent reports have demonstrated that electroporation, used in conjunction with traditional techniques such as intra-arterial DNA and direct muscle injection, can increase transfection efficiency (114,115). Like most non-viral methods of gene delivery, electroporation has the advantage of being non-toxic. Other benefits of electroporation include the potential to deliver relatively large pieces of DNA, and the ability to transfect non-replicating cells. The main limitations of electroporation are its low transfer rate, its non-specific nature, and the need to deliver electrical pulses to the target tissue (116).

Particle bombardment. Particle bombardment ('gene gun technology') involves coating microscopic gold particles with DNA and blasting them into tissue. It was originally developed for gene transfer to plant cells (117), however, particle bombardment has also been shown to be an efficient means of gene delivery in mammalian cells (118) including cells from skin dermis and epidermis (119). The advantages of this technique include easy use, ability to deliver large amounts of DNA, and a wide spectrum of action on various tissues. The disadvantages of this transfer system are its inability to reach internal organs, low frequency of stable gene integration, non-specific cell delivery, and potential damage to the bombarded tissues (120).

Antisense oligonucleotides. Although gene therapy is commonly thought of as the delivery of new genes for expression, many consider therapy intended to inhibit gene expression to also fall under the rubric of 'gene therapy'. One such application is antisense oligonucleotide therapy, which involves binding of oligonucleotide sequences (single-stranded DNA) to complementary mRNA to block translation into proteins (123,124). Antisense oligonucleotide delivery is an appealing technique because it can diminish the expression of genes implicated in disease by simply blocking protein translation. Currently, numerous therapeutic options of antisense oligonucleotides are being studied for a variety of human diseases. Although it is generally accepted that oligonucleotides exert their effects by antisense activity, other mechanisms that are less well established may also be involved (125). The future of antisense oligonucleotides will depend on better elucidating these mechanisms and on improving binding affinity and uptake of oligonucleotides. Efforts to improve the effectiveness of oligonucleotides by combining them with other gene therapy techniques appear promising (126).

10. Viral techniques (transduction)

Viral vectors. Viral vectors are derived from the introduction of new genes within genetically modified viruses and are appealing because they are generally associated with higher transfection efficiency. Once in the cell, they utilise the cellular metabolism of the host to complete their replicative cycle. Regions of the virus genome that are dispensable are deleted and replaced with the foreign gene(s) to be introduced into the cell. This manipulation renders the virus incapable of replication (replication-defective) in the host. There are, however, some potential hazards. Firstly, the integration of the virus in the genome of the targeted host cell may affect cell function. Mutagenesis of a cell toward a more tumorigenic state is clearly of concern. Other potential hazards include the rescue of an infectious virus from the replication-defective virus by recombination with host retroviral sequences, causing clinical infection. Encouragingly, none of these potential adverse effects has been observed in gene therapy clinical trials to date (4,127). Although numerous viruses are under investigation as potential vectors, only the viruses most often used in gene therapy are discussed below.

Recombinant adenoviruses. The potential role of adenoviruses as viral vectors was uncovered by the discovery that a replication-defective adenovirus lacking a portion of its genome could grow in the presence of cells expressing this missing region (128). This concept of inducible gene expression was later applied to recombinant adenoviruses carrying foreign DNA, leading to first application of adenoviruses (129). The genome of adenoviruses contains a linear, double-stranded DNA molecule. The virus exhibits two cycles of replication: an early phase and a late phase. Genes expressed during the early phase are found in the regions termed early transcriptional regions (E1, E2, E3, and E4) (130). The earliest adenoviral vectors are commonly constructed by deletion of the essential E1 gene to prevent viral replication. Furthermore, deletion of this region creates space for inserting genes of interest (131). The E3 gene is also commonly used for insertion of exogenous DNA sequences (132). The viral genome does not integrate into host chromosomes and remains episomal. This extrachromosomal location makes them a safer alternative than other viral vectors which may exhibit random chromosomal integration and possible insertional mutagenesis. Other advantages are their ability to express high levels of gene, act on nondividing cells, and accommodate large DNA inserts (133). On the other hand, the mode of action of adenoviruses remaining outside the host chromosome is associated with relatively short-term expression (134). A potential limiting factor for this virus system is the fact that they trigger a strong host immune response against transfected cells due to expression of viral antigens (4).

Recombinant adeno-associated viruses. Adeno-associated viruses are non-pathogenic viruses infecting about 80% of the human population. This fact makes them appealing vectors for gene delivery (4,135). In the absence of co-infection with a helper virus, adeno-associated viruses are unable to grow in human cells (136). Adeno-associated viruses are single-stranded DNA viruses with a genome size <5 kb. The viral genome consists of two genes that are flanked by two inverted terminal repeats. In recombinant adeno-associated virus vectors, viral genes are replaced by foreign DNA and flanked by the inverted terminal repeat regions (137). Adeno-associated viruses are integrated into the host genome, which makes this vector system a promising tool for gene therapy. It can achieve long-term transgene expression. Another advantage of adeno-associated viruses is their ability to infect nondividing cells. Disadvantages of adeno-associated virus vectors are an exogenous DNA insert capacity of ≤4.5 kb, and the potential for insertional mutagenesis.

Retroviruses. Retroviruses are enveloped single-stranded RNA viruses containing ~8 kb of nucleotides. They are the only available vectors that selectively infect proliferating cells. Upon entering a host cell, the RNA is reverse transcribed to double-stranded DNA molecules, which are then integrated into the host cell chromosome. Retroviruses are perhaps the best studied of gene therapy vectors. Currently, the most frequently used recombinant retroviral vectors for clinical gene therapy trials are derived exclusively from murine leukaemia virus, in which the genes encoding for essential viral proteins such as *gag* (encodes viral core proteins), *pol* (encodes reverse transcriptase and integrase), and *env* (encodes viral envelope glycoprotein) are replaced by a therapeutic gene. The major advantage to retroviral vectors is stable and permanent integration enabling sustained gene expression. The two major disadvantages of this virus system are that they only infect dividing cells and that they can potentially induce cell transformation due to their ability to integrate into chromosomes in a random manner. Other disadvantages include small size of DNA insert, and a lower yield of infectious viral titers compared with adeno-associated virus and adenovirus (138).

Recombinant herpes simplex virus. Herpes simplex virus is a linear double-stranded DNA virus with a genome size of about 150 kb. The genome is encapsulated by a capsid surrounded by a viral envelope. The herpes simplex virus

replicates in epithelia cells and establishes a life-long latent infection in neural bodies within the sensory ganglia of infected individuals. The predilection of herpes simplex virus for the central nervous system renders it a unique candidate for gene therapy. Its large genomic size allows a loading capacity of up to 30 kb of exogenous DNA. The advantages of herpes simplex virus are its large size, wide spectrum of action, and continuous expression of genes from long-lived infection. In addition, there is little risk of insertional mutagenesis with herpes simplex virus because it remains outside the nucleus (episomal). Unfortunately, herpes simplex virus also has its limitations, which include low infection efficiency, wild-type breakthrough, and a large genome size that makes it more difficult to manipulate (139). Similar to other viral vectors, replication-defective herpes simplex virus vectors are propagated in helper cells engineered to express gene product. However, this method of preparation creates a risk for wild-type breakthrough (140).

11. Current gene therapy results in wound healing

Gene therapy with PDGF-B has been successful at improving wound repair. For example, single dose adenoviral transfer with PDGF-BB to excisional wounds in an ischemic rabbit ear improved wound healing and corrected the deficits resulting from ischemia (141). Thus, 7 days after treatment the PDGF-BB adenoviral treated wounds were characterised by increased granulation tissue formation and re-epithelialization. Accordingly, other groups have tried promoting repair by enhancing levels of TGF-ß. Benn *et al* showed that particle-mediated *in vivo* transfer of TGF-ß gene can accelerate healing of murine incisional wounds and can significantly increase wound strength after surgery (142). Increase in wound tensile strength was also shown when wounds were transfected with PDGF-expressing plasmid by gene gun (143). Andree *et al* studied gene delivery of EGF by means of particle bombardment to porcine partial-thickness wounds and demonstrated that wounds treated with EGF plasmid exhibited an increase in EGF protein concentration and healed 20% earlier when compared with controls (81). Other groups have altered EGF biologic activity in wounds by introducing the gene for the EGF receptor. Nanney *et al* had favorable wound healing outcomes in pigs after delivering the gene encoding human EGF receptor through particle bombardment (144). The group first showed that the EGF receptor gene was successfully delivered to both grafted and excisional wounds, and then attempted to boost EGF biologic activity by topically applying EGF ligand (144). Human keratinocytes transfected with the IGF-1 gene through retroviral-mediated gene transfer secreted significant levels of IGF-1 *in vitro*. These modified cells were grafted into athymic mice, and when compared with unmodified grafts, they produced an epidermis of similar appearance and showed higher levels of certain markers of proliferation (145). A recent study evaluated the effects of injecting cationic liposomes containing the IGF-1 complementary DNA (cDNA) into rat skin that had received full-thickness scald burns. In this study, subcutaneous delivery of IGF cDNA-containing liposomes resulted in only localized transfection of skin cells and spared blood cells and distant organs. These injections were associated with significant increases in IGF-1 levels promoting epithelial cell proliferation and accelerating re-epithelialisation (146,147).

Gene therapy targeting the vascular blood supply is most applicable to the field of plastic surgery when it focuses on one of two major processes: i) promoting blood vessel ingrowth, or ii) preventing graft failure (104). Increasing angiogenesis due to genetic modulation of genes encoding angiogenic factors may significantly enhance wound healing (104). For instance, liposome-mediated administration of VEGF to anterior abdominal skin flaps was shown to increase the survival of ischemic experimental skin flaps (148). Accordingly, Roth *et al* demonstrated that direct treatment of transverse rectus abdominis muscle flaps in rats with an adenovirus expressing the VEGF gene resulted in a significant increase in flap survival and was associated with markedly increased new vessel ingrowth (Roth *et al*, Proceedings of the 44th Annual Meeting of the Plastic Surgery Council, Pittsburgh, PA, 1999). The role of recombinant FGF to promote skin flap angiogenesis has also been analysed. However, the conflicting results, in terms of the best delivery method, time, and dose (104) have led researchers to look for other methods of enhancing FGF biologic activity, such as combining exogenous FGF with other modalities such as hyperbaric oxygen or pharmacologic agents (149,150). However, gene therapy of FGF may soon share the same success in promoting blood vessel growth in ischemic flaps as it has in other clinical settings.

12. Conclusions

Gene therapy provides the opportunity to manipulate both local and systemic growth factors. With continuing advances in gene technology, gene therapy is becoming a reality, and it is a particularly attractive approach for the treatment of both acute and chronic wounds, because the wound site is often exposed, the treatment and condition should be transient, and gene products such as growth factors and cytokines suffer from problems with bioavailability and stability. The regulated delivery of growth factors in the wound microenvironment would maximize their biological effect and decrease the potential toxicity resulting from overexpression (120). Ultimately, multiple growth factor gene therapy and/or combination of delivering genes encoding for growth factor and its specific receptor might be more effective than single growth factor therapy alone (4). In the future, chronic wounds due to poor vascularity, previous radiation treatment or chronic steroid usage will likely be treated by growth factor gene insertion, obviating the need for repeated applications of topical growth factors. The efficacy of *in vivo* gene transfer of growth factor receptor genes to wounds, however, will be limited due to low *in vivo* transfection efficiency associated with the present gene delivery systems (4).

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