Tissue repair and regeneration: The effects of diabetes on wound healing

Katherine Kidman

Diabetes can affect healing, resulting in delayed wound closure and increased risk of infection. It may also be associated with recurrence at the same site. This article will look at the processes involved in wound healing and how they are affected by diabetes.

A literature search was conducted on wound healing and diabetes using CINAHL, allied and complementary medicine database (AMED), national research register (NRR) and Medline, articles were searched in English. The search terms used are listed in *Box 1*. Boolean operators were used to focus the search and there were no limits to the years searched. An initial search identified 14 articles, while a search of their reference lists increased the total number of relevant research studies.

Normal wound healing can be split into 4 stages (Falanga, 2005).

- Coagulation.
- Inflammation.
- Proliferation.
- Remodelling.

Each stage overlaps allowing a cascade of events to occur, resulting in re-epithelialisation and scar formation (McLennnen et al, 2006).

This review will take a broad view of the molecular and cellular processes involved in wound healing relating to the negative effect of diabetes.

Substance P

It is well established that diabetes and hyperglycaemia can impair the function of motor, sensory and autonomic nerves. If these nerves are damaged, the release of neuropeptides can be affected and this can in turn influence the processes of wound healing. Substance P is a nerve-derived mediator or neuropeptide with a role in initiating the production of growth-factor promoting cells and movement of cells through the endothelium. Substance P is secreted by sensory nerves after trauma and its release may be impaired in people with diabetic neuropathy (Spenny et al, 2002). Gibran et al (2002) examined tissue from eight chronic wounds in people with diabetic neuropathy and controls with diabetes but no neuropathy, as well as from diabetic and non diabetic mice; while Spenny et al (2002) studied wounds in genetically diabetic mice (db/db) and healthy mice. Both studies showed that neuropathy increased the time taken for wound closure and that this may have been the consequence of defective release of Substance P. There were

Article points

1. A literature search was conducted on wound healing and diabetes using CINAHL, allied and complementary medicine database (AMED), national research register (NRR) and Medline, articles were searched in English.
2. A variety of growth factors are involved in the process of wound healing and these are mediated through an effect on cell movement, as well as other aspects of cell function.
3. The research reviewed suggests that the addition of growth factors or amelioration of substances lacking in the diabetic wound may improve wound healing.

Key words

- Substance P
- Growth factors
- Fibroblasts
- MMPs

Katherine Kidman is a Diabetes Specialist Podiatrist at Medway Maritime Hospital, Kent.

Page points

1. When Jude et al (2002) compared skin taken from people with diabetes and non-diabetic controls, with diabetic foot ulcers and eight venous ulcers, they found that the expression of TGF-b1 was lower in diabetes than in other groups.
2. Reduction in levels of IGF-1 was observed in a study of cutaneous wounds in diabetic and non diabetic mice, as well as in rats with streptozotocin-induced diabetes.
3. Apoptosis is the term used for controlled cell death and is a necessary part of the healing process and may be disordered in the chronic wound..

Box 1: Search terms used in the literature search.

- Diabetes.
- Pathophysiology.
- Physiology.
- Cell.
- Molecular.
- Ulceration.
- Chronic wound.
- Acute wound.
- Wound healing.
- Repair.
- Regeneration.

associated changes in the number of nerves in the epidermis and dermis. Defective release of Substance P may therefore be one mechanism by which diabetic neuropathy may impede healing.

Growth factors: TGF-β, IGF-1 and VEGF

A variety of growth factors are involved in the process of wound healing and these are mediated through an effect on cell movement, as well as other aspects of cell function (Jude et al, 2002; Blakytny and Jude, 2006). Doxey et al (1995) found that the expression of platelet derived growth factor (PDGF) in rats with streptozotocin-induced diabetes was lower than in rats without diabetes. The effect was lost by day 20, possibly as a result of recovery from the effects of streptozotocin (Doxey et al, 1995). Bitar and Labbad (1996) wounded streptozotocin-induced diabetic rats and examined tissue samples after 7 days. The results showed a reduction in the levels of transforming growth factor-beta (TGF-β) and insulin-like growth factor-1 (IGF-1) in serum and wound fluid compared with controls.

When Jude et al (2002) compared skin taken from diabetic foot ulcers from people with diabetes and eight venous ulcers from controls without diabetes, they found that the expression of TGF-β1 was lower in diabetes than in other groups.

Galkowska et al (2006) biopsied 12 diabetic foot ulcers from people with type 2 diabetes. The mean age was 61.8 years and duration of diabetes was between 6 and 16 years. The control group consisted of 5 people without diabetes undergoing elective surgery. Skin samples were also taken from the border of the diabetic foot ulcers and the plantar surface of the foot in the control group. Tests were conducted in vitro. Raised levels of TGF-β1 were found in keratinocytes and epidermis of the skin of the people with diabetes. However, they were reduced in the endothelial cells in the dermis of the diabetic foot ulcers. These results are to some extent in conflict and this may result from the high levels of TGF-β3 in the study by Jude et al (2002). TGF-β3 can influence the expression of TGF-β1 (Jude et al, 2002), and it is not clear how this may have affected the results of the study by Galkowska et al (2006).

Reduction in levels of IGF-1 was observed in a study of cutaneous wounds in diabetic and non diabetic mice (Brown et al, 1997a), as well as by Bitar (2000) in rats with streptozotocin-induced diabetes. Similar observations were made in humans by Blakytny et al (2000), who examined skin biopsies from the lower limbs of eight people with diabetes without ulceration, ten people with chronic diabetic foot ulcers (neuropathic or ischaemic, or both) and ten controls without diabetes.

Using in vitro analyses Lerman et al (2003) studied fibroblasts and their ability to migrate and to release growth factors in 20-week-old db/db diabetic mice. They found that the migration of fibroblasts and the production of growth factors in the diabetic mouse was significantly reduced in vitro when compared with controls. Under hypoxic conditions fibroblasts from non-diabetic mice responded with increased migration and production of vascular endothelial growth factor (VEGF), whereas fibroblasts from diabetic mice did not respond. The neonate cell cultures were taken before the onset of diabetes and showed no reduction of VEGF production. This could show that it is not only complications of diabetes but diabetes itself that affects the function of fibroblasts in wound healing.

Apoptosis and growth factors

Apoptosis is the term used for controlled cell death and is a necessary part of the healing process (Brown et al, 1997b), it may be disordered in the chronic wound (Rai et al, 2005). Brown et al (1997b) studied experimental wounds in 8–12-week-old db/db mice, and found that apoptosis was delayed when compared with non-diabetic littermates. Brown et al (1997b) also

Page points

1. Nitric oxide (NO) facilitates infection control and apoptosis in the healing of wounds, although its effect can be inhibitory if levels are raised, and can initiate tissue destruction.
2. Fibroblasts are essential for the production of extracellular matrix, fibrous tissue and, subsequently, wound contraction. Lerman et al (2003) found abnormal fibroblast proliferation with an impaired response to hypoxia.

showed that this delay in apoptosis could be reversed by the topical administration of IGF-2, PDGF, or a combination of the two, over a five day period.

Rai et al (2005) looked at the effect of hyperglycaemia on apoptosis in humans. Twenty people with diabetes presenting with chronic wounds of more than four weeks' duration were split into two groups, with half receiving insulin and the other half receiving oral hypoglycaemic agents. The control group consisted of ten individuals without diabetes with chronic wounds of differing aetiology. A tissue biopsy was taken from all wounds for histology and DNA fragmentation. They found evidence of an increase in apoptosis which was associated with hyperglycaemia, indicating the effect that some of the abnormalities observed may be corrected by improved glycaemic control.

Leucocytes

As part of the process of inflammation, leucocytes such as neutrophils and macrophages move towards injured tissue due to the effect of chemokines (King, 2001). Chronic wounds, in both mice and humans, have been found to be in a state of prolonged inflammation (Goren et al, 2003), with resultant impaired healing and tissue breakdown (McLennan et al, 2006). Wetzler et al (2000) reported an early increase in chemokines in wound tissue from genetically diabetic mice which contrasted with controls in which levels reduced from day 5. Galkowska et al (2005) found that the total numbers of macrophages and other leucocytes were decreased in the margins of diabetic foot ulcers which had persisted for over 6 months. Such a decrease in leucocyte migration could conceivably result from the thickening of the basement membrane of blood vessels, which would affect the movement of cells into the extravascular space, and it is possible that the high levels of chemokines observed by Wetzler et al (2000) is a compensatory attempt to attract more leukocytes to the wound bed.

Nitric oxide

Nitric oxide (NO) facilitates infection control and apoptosis in the healing of wounds (Blakytny and Jude, 2006), although its effect can be inhibitory if levels are raised, and can initiate tissue destruction (Jude et al, 1999). Jude et al (1999) investigated samples of diabetic foot ulcers (n=22), skin of people with diabetes (n=22) and normal skin (n=14) and determined by immunocytochemistry that people with diabetes had a reduced plasma nitrite concentration compared with the controls, while the diabetic foot ulcer group showed an increase.

Jude et al (2001) examined 98 individuals with type 1 or 2 diabetes aged between 25 and 85 years with a duration of diabetes of longer than 3 years, in order to assess NO levels and its effect on wound healing.Forty-eight patients had current or a history of ulceration while the remaining 50 had no ulcer. Blood samples were taken and plasma separated to determine levels of NO; as measured by nitrite and nitrate quantities. It appears that levels of NO are present in greater quantities in those people with ulceration or recurrent ulceration and it is possible that the high plasma NO levels may delay healing.

Fibroblasts

Fibroblasts are essential for the production of extracellular matrix, fibrous tissue and, subsequently, wound contraction (Close-Tweedie, 2002). Lerman et al (2003) found abnormal fibroblast proliferation with an impaired response to hypoxia in db/db mice. Hehenberger et al (1998a) looked at the proliferation of fibroblasts in chronic wounds and skin in people with diabetes on insulin therapy. The comparison was with non diabetic chronic wounds and skin from a person without diabetes. Biopsies were taken and it was determined that a significant decrease in proliferation was found in the chronic wound fibroblasts compared to skin from a person without diabetes which was reversed by the addition of heparin (Hehenberger et al 1998a).

Page points

1. A decrease in fibroblast proliferation and collagen formation was found in those with type 1 diabetes, although not in those with type 2 disease.
2. Matrix metalloproteinases (MMPs) are produced by fibroblasts and are responsible for breaking down the extracellular matrix, which is an essential part of the process of wound healing. An abnormal increase in MMP expression could, however, delay wound healing.
3. The expression of MMPs was elevated in chronic diabetic foot ulcers, when compared with acute wounds, while levels of TIMP were reduced.
4. It was found that the application of TGF-β led to an increase in wound tensile strength in rats with streptozotocin-induced diabetes, measured by stretching tissue strips until breaking point.

A later study by Loots et al (1999) took biopsies for cell cultures from four type 2 diabetic foot wounds which had been present for longer than 8 weeks. Biopsies from the skin of age-matched people without diabetes were used on controls. The fibroblasts showed a slower proliferation in vitro, and there were associated changes in morphology when compared with the control group. The results mirror the Hehenberger et al (1998a, 1998b) studies and suggest that proliferation of fibroblasts is slower in wounds in people with diabetes.

These results are supported by an in vivo study by Black et al (2003) on 34 people with type 1 diabetes and 25 people with type 2 diabetes, but without chronic wounds, neuropathy or ischaemia. HbA_{1c} levels were raised in the type 2 diabetes group. Five controls were used. A technique was used to document fibroblast proliferation and collagen formation over 10 days. They found a decrease in fibroblast proliferation and collagen formation in those with type 1 diabetes, although not in those with type 2 diabetes. However, this study does not represent the patients typically seen within a high risk diabetes clinic who tend to present with chronic wounds, neuropathy, ischaemia and a long history of hyperglycaemia. Acute wound healing may demonstrate these differences between type 1 and type 2 diabetes however it would be interesting to see the fibroblast proliferation over a longer period than 10 days.

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are produced by fibroblasts and are responsible for breaking down the extracellular matrix (Khan, 2005) – this is an essential part of the process of wound healing. An abnormal increase in MMP expression could, however, delay wound healing. Wall et al (2002) examined concentrations of MMPs in studies undertaken both in mice and in humans with diabetic foot ulcers. Wound closure was found to be faster in non diabetic mice, and levels of MMP were slightly lower in diabetic mice than in controls. Wound fluid from diabetic foot ulcers in humans showed increased concentrations of MMP-3 when compared with acute wounds. In 2002, Lobmann et al found that the expression of MMPs was elevated in chronic diabetic foot ulcers, when compared with acute wounds, while levels of tissue inhibitor metalloproteinase (TIMP) were reduced. In terms of wound healing, the imbalance between the MMP inhibitors (TIMPs) could cause an increase in levels of MMPs. This could in turn slow wound healing by increasing the destruction of the extracellular matrix and creating a chronic wound.

Remodelling

Goodson and Hunt (1977) studied the effect of streptozotocin-induced diabetes on tensile strength and collagen formation in wounds. After 21 days the wounds were excised and the tensile strength was found to be reduced in the diabetic wounds compared with the non diabetic wounds. A second study was conducted on streptozotocin-induced diabetic rats and also found a reduction in tensile strength when compared with control. In the study by Bitar and Labbad (1996) referred to above, it was found that the application of TGF-β led to an increase in wound tensile strength in rats with streptozotocin-induced diabetes, measured by stretching tissue strips until breaking point.

Discussion

Limitations of research

The above studies indicate that diabetes may impose a wide range of negative effects on key components of wound healing and cellular function. It should be noted that in all the animal studies, the wounds investigated were acute and any changes observed may not necessarily apply to the chronic wounds that are commonly seen in people with diabetes. Acute wounds move through the phases of wound healing while chronic wounds may be arrested in one or

more phases (Falanga, 2005). Moreover, experimental wounds in rats and mice heal by a process of contraction, as opposed to granulation and epithelialisation as seen in the diabetic foot wound (McLennan et al, 2006). It is also important to recognise that the diabetes of experimental animals is also acute, and factors involved in the development of ulcers in humans are often dependent on the duration of the condition and the associated development of chronic complications.

Future studies

The research reviewed suggests that the addition of growth factors and other substances lacking in the diabetic wound may improve wound healing. This information may help with the development of new dressings which would be specific to each stage of wound healing, and could adjust the environment of the wound bed to ensure the cells are working in the correct way. Further research will help us understand the effects of diabetes on wound healing and may help the dressing choice and treatment. Future research, preferably in humans, needs to use larger samples and concentrate on how to reverse the effects of diabetes, bearing in mind the need for cost-effective and acceptable treatment in order to improve the quality of life of poeple with diabetes. ■

Bitar MS and Labbad ZN (1996) Transforming growth factor-β and insulin-like growth factor-I in relation to diabetes-induced impairment of wound healing. *Journal of Surgical Research* **61**: 113–9

Bitar MS (2000) Insulin and glucocorticoid-dependent suppression of the IGF-I system in diabetic wounds. *Surgery* **127**: 687–95

Page points

1. Further research will help us understand the effects of diabetes on wound healing and may help the dressing choice and treatment.
2. Future research, preferably in humans, needs to use larger samples and concentrate on how to reverse the effects of diabetes, bearing in mind the need for cost-effective and acceptable treatment in order to improve the quality of life of poeple with diabetes.

The above studies indicate that diabetes may impose a wide range of negative effects on key components of wound healing and cellular function.

Black E, Vibe-Petersen J, Jorgensen LN et al (2003) Decrease of collagen deposition in wound repair in type 1 diabetes independent of glycaemic control. *Archives of Surgery* **138:** 34–40

Blakytny R, Jude EB, Gibson JM et al (2000) Lack of insulin-like growth factor I (IGF I) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. *Journal of Pathology* **190**: 589–94

Blakytny R and Jude E (2006) The molecular biology of chronic wounds and delayed healing in diabetes. *Diabetic Medicine* **23**: 594–608

Brown DL, Kane CD, Chernausek SD Greenhalgh DG (1997a) Differential expression and localization of insulin-like growth factors I and II in cutaneous wounds of diabetic and nondiabetic mice. *American Journal of Pathology* **151**: 715–24

Brown DL, Kao WW-Y, Greenhalgh D (1997b) Apoptosis down-regulates inflammation under the advancing epithelial wound edge: Delayed patterns in diabetes and improvement with topical growth factors. *Surgery* **121**: 372–80

Close-Tweedie J (2002) Diabetic foot wounds and wound healing: A review. *The Diabetic Foot Journal* **5**: 68–80

Doxey DL, Ng MC, Dill RE, Iacopino AM (1995) Platelet-derived growth factor levels in wounds of diabetic rats. *Life Sciences* **57**: 1111–23

Falanga V (2005) Wound healing and its impairment in the diabetic foot. *Lancet* **366:** 1736–43

Galkowska H, Wojewodzka U, Olszewski WL (2005) Low recruitment of immune cells with increased expression of endothelial adhesion molecules in margins of the chronic diabetic foot ulcers. *Wound Repair and Regeneration* **13**: 248–54

Galkowska H, Wojewodxka U, Olszewski WL (2006) Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. *Wound Repair and Regeneration* **14**: 558–65

Gibran NS, Jang YC, Isik FF et al (2002) Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *Journal of Surgical Research* **108**: 122–8

Goodson WH 3rd, Hung TK (1977) Studies of wound healing in experimental diabetes mellitus. *Journal of Surgical Research* **22**: 221–7

Goren I, Kämpfer H, Podda M et al (2003) Leptin and wound inflammation in diabetic ob/ob mice. Differential regulation of neutrophil and macrophage influx and a potential role for the scab as a sink for inflammatory cells and mediators. *Diabetes* **52**: 2821–32

Hehenberger K, Kratz G, Hansson A, Brismar K (1998a) Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. *Journal of Dermatological Science* **16**: 144–51

Hehenberger K, Heilborn JD, Brismar K, Hansson A (1998b) Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of L-lactate. *Wound Repair and Regeneration* **6**: 135–141

Jude EB, Boulton AJM, Ferguson MWJ and Appleton I (1999) The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: Possible modulatory effects by transforming growth factor beta 1. *Diabetologia* **42**: 748–57

Jude EB, Tentolouris N, Appleton I (2001) Role of neuropathy and plasma nitric oxide in recurrent neuropathic and neuroischemic diabetic foot ulcers. *Wound Repair and Regeneration* **9**: 353–9

Jude EB, Blakytny R, Bulmer J et al (2002) Transforming growth factor-beta 1,2,3 and receptor type I and II in diabetic foot ulcers. *Diabetic Medicine* **19**: 440–7

Khan MN (2005) The influence of diabetes on wound healing. *The Diabetic Foot Journal* **8**: 144–53

King L (2001) Impaired wound healing in patients with diabetes. *Nursing Standard* **15**: 39–45

Lerman OZ, Galiano RD, Armour JP and Gurtner C (2003) Cellular dysfunction in the diabetic fibroblast. Impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *American Journal of Pathology* **162**: 303–12

Lobmann R, Ambrosch A, Schultz G et al (2002) Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non diabetic patients. *Diabetologia* **45**: 1011–6

Loots MAM, Lamme EN, Mekkes JR et al (1999) Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Archives of Dermatological Research* **291**: 93–9

McLennen S, Yue DK Twigg SM (2006) Molecular aspects of wound healing in diabetes. *Primary Intention* **14**: 8–13

Rai NK, Ansari M, Kumar M et al (2005) Effect of glycaemic control on apoptosis in diabetic wounds. *Journal of Wound Care* **14**: 277–81

Spenny ML, Muangman P, Sullivan SR et al (2002) Neutral endopeptidase inhibition in diabetic wound repair. *Wound Repair and Regeneration* **10**: 295–301

Wall SJ, Bevan D, Thomas DW et al (2002) Differential expression of matrix metalloproteinases during impaired wound healing of the diabetes mouse. *The Journal of Investigative Dermatology* **119**: 91–8

Wetzler, C, Kämpfer, H, Stallmeyer, B et al (2000) Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: Prolonged persistence of neutrophils and macrophages during the late stage of repair. *The Journal of Investigative Dermatology* **115**: 245–53