The influence of diabetes on wound healing

Muhammad N Khan

ARTICLE POINTS

1 Diabetes is a major cause of impaired wound healing and is responsible for significant morbidity and mortality.

2 Hyperglycaemia can disrupt cellular functions and lead to non-enzymatic glycation, which can have an adverse physiological effect.

3 Insulin is involved in the metabolism of proteins, carbohydrates and fats, which are all linked to wound healing.

4 Fluid and electrolyte imbalances resulting from hyperglycaemia also have adverse effects.

5 Other related problems include defects in immune function, fibroblast function, angiogenesis and collagen production.

KEY WORDS
- **Wound healing**
- **Hyperglycaemia**
- **Metabolism**
- **Non-enzymatic glycation**

Muhammad N Khan is a Specialist Registrar in Surgery at North Hampshire Hospital, Basingstoke.

Introduction

Diabetes is a chronic systemic disorder of glucose metabolism. The World Health Organization (1999) described the condition as 'a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both' (World Health Organization, 1999). Among the associated morbidities is impaired wound healing. This article reviews the possible mechanisms by which diabetes can affect wound healing.

Diabetes is a systemic disorder that affects almost all body systems, either directly or indirectly through its complications. Among the acute complications, acute metabolic derangements, urinary tract infections, skin and other infections and side effects of drugs are important. The major chronic complications are retinopathy, nephropathy, neuropathy, ischaemic heart disease, cerebrovascular disease, peripheral arterial disease and skin lesions. Among these, peripheral arterial disease is one of the major morbidities. In the US, 35–45% of all limb amputations are performed on people with diabetes (Stonebridge, 1996). Type 2 diabetes has a stronger association with these morbidities than type 1 diabetes does.

The effects of diabetes on healing are diverse, multifactorial, complex and inter-related (Greenhalgh, 2003). It is one of the well-known intrinsic factors which affect wound healing. In fact, diabetes affects almost all stages of wound healing to some extent. The underlying mechanisms have been extensively investigated in the past few decades.

Effects of non-enzymatic glycation

The hyperglycaemia associated with diabetes can cause tissue damage in two ways. The first pathway is the intracellular hyperglycaemia caused by increased flux through different metabolic pathways, which can adversely affect cellular functions. This is the underlying mechanism of early diabetic cataracts and peripheral neuropathy. The second, and more important, pathway for long-term complications in diabetes is the non-enzymatic glycation of proteins. In this process, glucose chemically attaches to the amino group of proteins without the involvement of enzymes. These stable products then accumulate over the surface of cell membranes, structural proteins and circulating proteins. They are called 'Amadori products'.

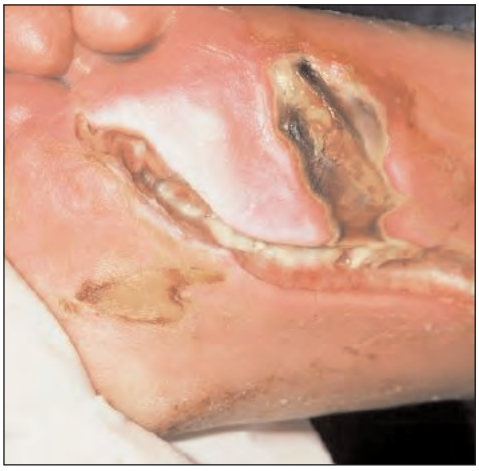
Proteins with a longer half-life, such as collagen, fibrin, albumin and haemoglobin, accumulate advanced glycation end products, which form slowly from Amadori products through series of further reactions. The extent of these reactions depends on the concentration of glucose, the duration of hyperglycaemia and the half-life of these proteins. This non-enzymatic glycation can affect a number of physiological processes in the body, ranging from enzymatic activity and binding of regulatory molecules to cross-linking of proteins and susceptibility to proteolysis (Reiser, 1998).

The microtubular protein tubulin forms non-reducible aggregates during

PAGE POINTS

1 Non-enzymatic glycation (which is linked to hyperglycaemia) can affect a number of physiological processes in the body.

2 Insulin is involved in the metabolism of proteins, carbohydrates and fats, which are all linked to wound healing.



A diabetic foot wound.

non-enzymatic glycation and contributes to the defective axoplasmic transport seen in diabetic neuropathy. Non-enzymatic glycation is also responsible for the thickening of the glomerular basement membrane seen in diabetic nephropathy (Makita et al, 1991). This results from accumulation of albumin and trapping of immunoglobulin G.

Non-enzymatically glycated collagen binds soluble proteins to form *in situ* immune complexes characteristic of diabetic nephropathy (Brownlee et al, 1984). Similarly, thickening of basement membrane in the microcirculation can lead to ischaemia and decreased tissue perfusion, which in turn results in impaired wound healing (Cavallo et al, 1984). The important proteins from a wound healing perspective that are affected by non-enzymatic glycation are collagen, fibrin and keratin.

Fibronectin is the major glycoprotein secreted by fibroblasts during initial synthesis of extracellular matrix proteins. It serves important functions, being a chemo-attractant for macrophages, fibroblasts and endothelial cells. It promotes re-epithelialisation and acts as a transduction agent in wound contraction. Non-enzymatic glycation of fibronectin decreases its ability to bind to collagen, gelatin and heparin. Di Girolamo et al (1993) were unable to show significant differences in functional activities of fibronectin between people with diabetes and controls. They postulated, however, that defects in wound healing are caused by the hyper-glycosylation of the locally synthesised cellular fibronectin and are not due to the effect on plasma fibronectin.

Altered metabolism of proteins, carbohydrates and fats

Insulin is an anabolic hormone which promotes protein synthesis and utilisation of glucose. Diabetes affects the metabolism of carbohydrates, proteins and fats, which play an important role in cellular activities, proliferation, and migration and wound healing (Cooper, 1990).

Proteins are the structural units of healing wounds. Collagen and proteoglycans are the important proteins in the context of wound healing (Deodhar and Rana, 1997). A lack of insulin in diabetic wounds results in more protein degradation uncoupled from protein synthesis (Marchesini et al, 1982). Collagen formation is reduced and the existing collagen lacks tensile strength (Gottrup and Andreassen, 1981). This also adversely affects fibroblast and polymorphonuclear (PMN) cell functions (Sawant, 1993).

Insulin is integral to the metabolism of carbohydrates. The majority of cells – with notable exceptions being red blood cells and lens tissue cells – are dependent on insulin for the intracellular shift of glucose, which is the major source of energy for cellular functions. Fibroblasts and PMN cells require glucose to carry out their vital functions in wound healing.

In the absence of insulin, there is more proteolysis, glycogenolysis and lipolysis. Fatty acids and triglycerides are mobilised to provide energy. Fatty acids are important in the synthesis of cell membranes and if the fat stores are depleted, this synthesis is slowed down (Young, 1988). The liver handles the excess of free fatty acids by converting them into ketone bodies. The synthesis of ketone bodies is enhanced by the counter-regulatory hormones. In a healing wound, the presence of ketone bodies is a sign of inadequate nutrient supply or lack of insulin (Gavin, 1989).

PAGE POINTS

1 Hyperglycaemia can lead to decreased intravascular volume, which, in turn, can cause decreased perfusion pressure and tissue oxygenation and hence delayed wound healing.

2 Diabetes is associated with a number of defects in the cellular and humoral immune system.

Fluid and electrolyte imbalances

Hyperglycaemia leads to osmotic diuresis and loss of water and electrolytes. This occurs when the plasma glucose concentration exceeds the renal threshold value. The resulting decreased intravascular volume can lead to decreased perfusion pressure and decreased tissue oxygenation and hence delayed wound healing. Insulin is essential for the entry of potassium into the cell. Regulation of electrolyte balance can be impaired because of diabetes itself or becaues of an increase in counter-regulatory hormones such as glucagon and corticoids.

Effects on white blood cells, macrophages and immune function

Neutrophils and macrophages play a crucial role in the inflammatory and proliferative stages of wound healing. They perform important functions, including phagocytosis, migration to the wound bed, clearing the debris and producing a wide range of cytokines that orchestrate the healing cascade. Experimental studies have shown that impaired PMN cell function leads to delayed wound healing (McMurry, 1984).

Hyperglycaemia affects the whole range of neutrophil functions, including migration, chemotaxis, adherence, and phagocytic and bactericidal activity (Wall et al, 2003). PMN neutrophils also play a role in angiogenesis by releasing angiogenic factors.

Diabetes is associated with a number of defects in the cellular and humoral immune system. The severity of these defects is directly related to the duration and severity of diabetes. In the proliferation and maturation phases of wound healing, the lymphocyte function and production of lymphokines is impaired. This places people with diabetes at an increased risk of infections and delayed wound healing. Repine et al (1980) found that PMN neutrophils from people with diabetes were unable to increase their bactericidal activity in response to infection. Goodson and Hung (1977) postulated that defective wound healing in diabetes is related to the abnormal tissue responses during the inflammatory phase.

Cytokines are released from different cells in response to injury and play a vital role in wound healing. Fahey et al (1991) carried out a study to determine the relationship between diabetic healing and altered PMN neutrophil influx by measuring tumour necrosis factor (TNF) and interleukin (IL)-6 in wound chambers implanted subcutaneously in diabetic and control mice. The initial inflammatory responses were similar in the two groups but the number of white blood cells and IL-6 levels were decreased towards the end of the inflammatory phase in diabetic mice.

They used 8- to 12-week-old male BALB mice that were rendered diabetic by intraperitoneal injection of streptozotocin. Wound chambers were implanted in flanks and wound fluid was aspirated at days 1, 3 and 7. There was a clear difference in the number of white blood cells at day 7 in diabetic and control groups, with diabetic mice having a significantly lower count ($P<0.005$). Histology of the wound chambers also revealed decreased cellularity in the diabetic group. The levels of IL-6 were comparable in the two groups on days 1 and 3, but on day 7 they were significantly higher in the control group ($P<0.05$). These data provide *in vitro* evidence for the altered function and migration of white blood cells in the group with diabetes.

Similar results were reported by Chbinou and Frenette (2004), when they found that in diabetic rats there was decreased accumulation of neutrophils and macrophages at days 3 and 7 post-injury as compared with the non-diabetic mice. There was also decreased proliferative activity. They suggested that these alterations in the inflammatory, proliferative and angiogenic processes secondary to hyperglycaemia will eventually have effects on healing and remodelling.

PAGE POINTS

1 Defects in fibroblast migration could be the main underlying cause of delayed wound healing seen in people with diabetes.

2 It has been shown that the fibroblasts from people with diabetes display decreased proliferation in cell cultures.

Doxey et al (1988) demonstrated that platelet-derived growth factor (PDGF) levels are deficient at the wound site in diabetic rats. There was an overall reduction of cytokine release. Non-diabetic hyperlipidaemic rats showed a similar decrease in cytokine production. This supports the hypothesis that elevated serum lipid levels are the primary determinants of diabetes-induced reductions in macrophage cytokine release.

Defects of fibroblast function

Fibroblasts play a crucial role in the proliferative stage of wound repair. They function as synthetic cells, depositing a collagen-rich matrix, and as signalling cells, producing important cytokines. Defects in fibroblast migration could be the main underlying cause of delayed wound healing seen in people with diabetes. Lerman et al (2003) carried out experiments on mice and studied the behaviour of fibroblasts from normal and diabetic (leptin receptor deficient) mice. They showed that the fibroblasts from diabetic mice exhibited a 75% reduction in migration compared with the normal fibroblasts ($P<0.001$) and, furthermore, this migration was not stimulated even after exposing these fibroblasts to hypoxia. Diabetic fibroblasts produced twice the amount of pro-matrix metalloproteinase (MMP)-9 as controls and also showed seven-fold reductions in the production of vascular endothelial growth factor.

It has been shown that the fibroblasts from people with diabetes display decreased proliferation in cell cultures (Hehenberger et al, 1998), a0nd this proliferation inhibition is not a direct effect of non-enzymatic glycation, as it can be reversed by protein kinase C inhibitors and antioxidants. Diabetic fibroblasts produce higher lactate levels, which could further inhibit their proliferation. Hehenberger et al (1999) carried out experiments on GK rats – used as an animal model of type 2 diabetes – and found similar results.

Loot et al (2002) compared the mitogenic response of fibroblasts cultured from diabetic ulcers, non-diabetic ulcers, people with diabetes but no ulcers and age-matched controls to PDGF, epidermal growth factor, basic fibroblast growth factor (bFGF) and insulin-like growth factor-1. They found that simultaneous administration of all growth factors always resulted in a higher response than the sequential administration ($P<0.05$).

Fibroblasts also express MMPs, which are proteolytic enzymes responsible for extracellular matrix and basement membrane protein degradation. It is believed that an abnormal expression of MMPs could be an underlying factor in the impaired healing response in people with diabetes. Wall et al (2003) have demonstrated that dermal fibroblasts from people with diabetes show significantly elevated levels of MMP-2 and pro-MMP-3. They studied four healthy volunteers and four people with diabetes and found that fibroblasts from unwounded skin of the people with diabetes exhibited high production of MMPs.

Defects of angiogenesis

Neovascularisation is an important step in the proliferative phase of healing. It results in the formation of new capillaries of the granulation tissue. PMN neutrophils secrete angiogenic factors that results in budding of new blood vessels. Hamuro et al (2002) showed that higher glucose levels inhibit endothelial cell migration via activation of nuclear factor kappa B (NF-κB). They used human aortic endothelial cells to study this. The described inhibitory effect was nullified by the use of NF-κB inhibitors.

bFGF plays an important role in angiogenesis. It is secreted by fibroblasts, macrophages and endothelial cells. bFGF has angiogenic properties and has its action through specific receptors resulting in cellular proliferation, migration, morphogenesis and angiogenesis (Gospodarowicz et al, 1987). The effects of fibroblast growth factor (FGF) have been evaluated in a clinical trial on patients with diabetic

PAGE POINTS

1 Studies of skin fibroblasts from people with diabetes have shown decreased collagen production *in vitro*.

2 An insight into possible mechanisms by which diabetes can affect wound healing provides us with the opportunity to explore possible interventions and to avoid the complications of delayed wound healing.

ulcers in 1995, but the results were not convincing (Richard et al, 1995). *In vitro* experiments have shown that glycation of FGF significantly reduced its activity. It resulted in a decrease in the ability of FGF to bind to the tyrosine kinase receptor and activate signal transduction pathways responsible for mitogenesis and angiogenesis (Duraisamy et al, 2001).

Defects of collagen production

It has already been noted that diabetes is associated with a generalised defect of tissue metabolism. Collagen is the most abundant protein and a major component of the connective tissue. It provides tensile strength, organisation and integrity for the connective tissue. It also plays a role in haemostasis through interaction with the platelets and participates in differentiation and morphogenesis during embryonic development (Ruszczak, 2003).

Studies of skin fibroblasts from people with diabetes have shown decreased collagen production *in vitro* (Seibold et al, 1985). In addition to defects in collagen production, post-translational modifications of collagen peptide and increased cross-linking of collagen is enhanced in diabetes. This leads to decreased solubility and increased accumulation of collagen in tissues coupled with decreased synthesis of new collagen. Spanheimer et al (1988) have shown that diabetic rats produce less collagen. In their experiments, within 2 weeks after onset of diabetes, collagen production was reduced to less than half that seen in control animals. But, at the same time, no significant difference was found in the production of non-collagen proteins.

Conclusion

Diabetes mellitus is a chronic metabolic disorder having diverse effects on all body systems. It is one of the leading causes of morbidity in developed countries. It adversely affects almost all stages of wound healing. An insight into possible mechanisms by which diabetes can affect wound healing provides us with the opportunity to explore possible interventions and to avoid the complications of delayed wound healing in people with diabetes. ■

Brownlee M, Vlassara H, Cerami A (1984) Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Annals of Internal Medicine* **101**(4): 527–37

Cavallo T, Pinto JA, Rajaraman S (1984) Immune complex disease complicating diabetic glomerulosclerosis. *American Journal of Nephrology* **4**(6): 347–54

Chbinou N, Frenette J (2004) Insulin-dependent diabetes impairs the inflammatory response and delays angiogenesis following Achilles tendon injury. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **286**(5): R952–7

Cooper DM (1990) Optimizing wound healing. A practice within nursing's domain. *The Nursing Clinics of North America* **25**(1): 165–80

Deodhar AK, Rana RE (1997) Surgical physiology of wound healing: a review. *Journal of Postgraduate Medicine* **43**(2): 52–6

Di Girolamo N, Underwood A, McCluskey PJ, Wakefield D (1993) Functional activity of plasma fibronectin in patients with diabetes mellitus. *Diabetes* **42**(11): 1606–13

Doxey DL, Nares S, Park B, Trieu C, Cutler CW, Iacopino AM (1998) Diabetes-induced impairment of macrophage cytokine release in a rat model: potential role of serum lipids. *Life Sciences* **63**(13): 1127–36

Duraisamy Y, Slevin M, Smith N, Bailey J, Zweit J, Smith C et al (2001) Effect of glycation on basic fibroblast growth factor induced angiogenesis and activation of associated signal transduction pathways in vascular endothelial cells: possible relevance to wound healing in diabetes. *Angiogenesis* **4**(4): 277–88

Fahey TJ 3rd, Sadaty A, Jones WG 2nd, Barber A, Smoller B, Shires GT (1991) Diabetes impairs the late inflammatory response to wound healing. *The Journal of Surgical Research* **50**(4): 308–13

Gavin LA (1989) Management of diabetes mellitus during surgery. *The Western Journal of Medicine* **151**(5): 525–9

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

Gospodarowicz D, Neufeld G, Schweigerer L (1987) Fibroblast growth factor: structural and biological properties. *Journal of Cellular physiology. Supplement* (Suppl 5): 15–26

Gottrup F, Andreassen TT (1981) Healing of incisional wounds in stomach and duodenum: the influence of experimental diabetes. *The Journal of Surgical Research* **31**(1): 61–8

Greenhalgh DG (2003) Wound healing and diabetes mellitus. *Clinics in Plastic Surgery* **30**(1): 37–45

Hamuro M, Polan J, Natarajan M, Mohan S (2002) High glucose induced nuclear factor kappa B mediated inhibition of endothelial cell migration. *Atherosclerosis* **162**(2): 277–87

Hehenberger K, Hansson A, Heilborn JD, Abdel-Halim SM, Ostensson CG, Brismar K (1999) Impaired proliferation and increased L-lactate production of dermal fibroblasts in the GK-rat, a spontaneous model of non-insulin dependent diabetes mellitus. *Wound Repair and Regeneration* **7**(1): 65–71

Hehenberger K, Kratz G, Hansson A, Brismar K (1998) 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**(2): 144–51

Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC (2003) Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *The American Journal of Pathology* **162**(1): 303–12

Loot MA, Kenter SB, Au FL, van Galen WJ, Middelkoop E, Bos JD, Mekkes JR (2002) Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls. *European Journal of Cell Biology* **81**(3): 153–60

Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V (1991) Advanced glycosylation end products in patients with diabetic nephropathy. *New England Journal of Medicine* **325**(12): 836–42

Marchesini G, Forlani G, Zoli M, Vannini P, Pisi E (1982) Muscle protein breakdown in uncontrolled diabetes as assessed by urinary 3-methylhistidine excretion. *Diabetologia* **23**(5): 456–8

McMurry JF Jr (1984) Wound healing with diabetes mellitus. Better glucose control for better wound healing in diabetes. *The Surgical Clinics of North America* **64**(4): 769–78

Reiser KM (1998) Nonenzymatic glycation of collagen in aging and diabetes. *Proceedings for the Society of Experimental Biology and Medicine* **218**(1): 23–37

Repine JE, Clawson CC, Goetz FC (1980) Bactericidal function of neutrophils from patients with acute bacterial infections and from diabetics. *The Journal of Infectious Diseases* **142**(6): 869–75

Richard JL, Parer-Richard C, Daures JP, Clouet S, Vannereau D, Bringer J et al (1995) Effect of topical basic fibroblast growth factor on the healing of chronic diabetic neuropathic ulcer of the foot. A pilot, randomized, double-blind, placebo-controlled study. *Diabetes Care* **18**(1): 64–9

Ruszczak Z (2003) Effect of collagen matrices on dermal wound healing. *Advanced Drug Delivery Reviews* **55**(12): 1595–611

Sawant JM (1993) Biochemical changes in polymorphonuclear leucocytes in diabetic patients. *Journal of Postgraduate Medicine* **39**(4): 183–6

Seibold JR, Uitto J, Dorwart BB, Prockop DJ (1985) Collagen synthesis and collagenase activity in dermal fibroblasts from patients with diabetes and digital sclerosis. *The Journal of Laboratory and Clinical Medicine* **105**(6): 664–7

Spanheimer RG, Umpierrez GE, Stumpf V (1988) Decreased collagen production in diabetic rats. *Diabetes* **37**(4): 371–6

Stonebridge PA (1996) Surgical management of peripheral vascular disease in diabetes. *Diabetes Reviews International* **5**(4): 9–12

Wall SJ, Sampson MJ, Levell N, Murphy G (2003) Elevated matrix metalloproteinase-2 and -3 production from human diabetic dermal fibroblasts. *The British Journal of Dermatology* **149**(1): 13–6

World Health Organization (WHO; 1999) *Definition, diagnosis and classification of diabetes mellitus and its complications*. WHO, Geneva. Available at http://www.who.int/diabetes/currentpublications/en/ (accessed 16.08.2005)

Young ME (1988) Malnutrition and wound healing. *Heart & Lung* **17**(1): 60–7

'An insight into possible mechanisms by which diabetes can affect wound healing provides us with the opportunity to explore possible interventions and to avoid the complications of delayed wound healing in people with diabetes.'