

# The effects of diabetes on collagen within wound healing

Vanessa Goulding

**Diabetes is associated with recalcitrant wound healing as a result of vascular, neuropathic, immune and biomechanical abnormalities. The skin of people with diabetes has greater stiffness and less flexibility, which is thought to render it more prone to injury. It has been suggested that these skin changes are the result of imbalances in collagen synthesis and degradation. Wounds in these patients demonstrate a prolonged inflammatory phase, causing a delay in the formation of granulation tissue and, subsequently, reducing the wound tensile strength.**

Wound healing is a complex process that aims to restore the integrity of damaged skin (Stojadinovic et al, 2012). This involves many cellular processes and responses to inflammatory mediators, growth factors, cytokines and hormones (Schultz, 2007). These processes can be defined as a series of four overlapping phases of haemostasis, inflammation, proliferation and remodelling (Enoch and Leaper, 2005).

While acute wounds progress through these phases in a linear manner (Falanga, 2005), many wounds do not progress in a timely fashion due to intrinsic or extrinsic factors and are subsequently defined as chronic, non-healing wounds (Martin, 2013). Diabetes mellitus is an intrinsic factor associated with recalcitrant healing (Sibbald and Woo, 2008) as a result of vascular, neuropathic, immune and biomechanical abnormalities. Each of these factors can contribute to altered tissue repair in people with diabetes (Stojadinovic et al, 2012).

The skin of people with diabetes has inferior biomechanical properties, such as greater stiffness and less flexibility, which is thought to render it more prone to injury (Bermudez et al, 2011). It has been suggested that these properties are the result of imbalances in collagen synthesis and degradation (Bermudez et al, 2011).

Furthermore, dermal accumulation of advanced glycosylation end products (AGEs) has been increasingly implicated as a potential underlying

cause (Paul and Bailey, 1996). It is highly likely that this may affect the healing of diabetic wounds (Liao et al, 2009). These wounds demonstrate a prolonged inflammatory phase, causing a delay in the formation of granulation tissue and subsequently reducing the wound tensile strength (Acosta et al, 2008). Collagen synthesis and deposition are also reduced, affecting the final wound strength (Benbow, 2005).

## Structure and function of collagen

Collagen is the most abundant protein found in connective tissue, blood vessels and the skin (Haycocks et al, 2013). Within the dermal layer of the skin, it provides physical strength, accounting for up to 80% of the dry weight of the dermis (Hopkinson, 1992). Collagen is mainly produced by fibroblasts (Rangaraj et al, 2011), with at least 16 distinct types identified (Schultz et al, 2005). Within the dermal layer, collagen type I accounts for 80–85% of the collagen content with 10% of type III and trace amounts of IV, V, VI and VII (Rangaraj et al, 2011). Each collagen molecule consists of three polypeptide protein chains coiled into a triple helix (Bentley, 2001). Cross-linking of the molecules to adjacent molecules creates the additional strength and stability characteristic of collagen fibres in normal wound healing (Schultz et al, 2005).

Collagen is either directly or indirectly involved in each of the four phases of wound healing (Haycocks et al, 2013). Initially, within haemostasis, collagen

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## Article points

1. Diabetes is associated with recalcitrant wound healing.
2. The skin has greater stiffness and less flexibility, which is thought to render it more prone to injury.
3. These skin changes are thought to be the result of imbalances in collagen synthesis and degradation.

## Key words

- Collagen
- Skin
- Wound healing

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***“Defects in the tissue repair of people with diabetes may be the result of altered collagen metabolism.”***

exposed during wound formation activates both the intrinsic and extrinsic pathways of the clotting cascade (Gabriel, 2011). During the inflammatory phase, collagen influences cellular mitogenesis, differentiation and migration (Schultz et al, 2005). Collagen synthesis and deposition occurs within the proliferative phase. Type III collagen is laid down first within the granulation tissue of early healing wounds (Rangaraj et al, 2011). This is then replaced by type I collagen as degradation occurs and the wound bed is remodelled and healing occurs (Gabriel, 2011).

Early studies suggested that defects in the tissue repair of people with diabetes may be the result of altered collagen metabolism (Goodson and Hung, 1977; Yue et al, 1986; Spanheimer et al, 1988). As collagen is involved in each of the phases of wound healing, it is likely that any effects of diabetes within each of these phases will directly affect collagen within the extracellular matrix (ECM). This aspect will be explored in more detail, firstly, considering the effect of diabetes during the inflammatory phase.

**Inflammatory phase**

The inflammatory phase of wound healing is characterised by the sequential infiltration of neutrophils (polymorphonuclear leucocytes, PMNs), macrophages and lymphocytes (Guo and DiPietro, 2010). The main function of PMNs and macrophages is to ensure that any invading microbes and cellular debris are removed by phagocytosis (Guo and DiPietro, 2010). This, in conjunction with the production of various cytokines, orchestrates the resolution of the inflammatory phase and transition to the proliferative phase (Khan, 2005).

Hyperglycaemia has been shown to affect the function of PMNs (Lobmann et al, 2005). In an study of normal mice and mice with diabetes induced by streptozotocin, Fahey et al (1991) found that wound healing was impaired in diabetic mice. Over a 7-day period, the numbers of leucocytes infiltrating silicone wound chambers was significantly reduced in the diabetic mice. The connective tissue present within the wound chambers also appeared altered with less granulation tissue, decreased cellularity and organisation. While wound fluid was aspirated from the wound chambers for the analysis of the leucocytes, the precise methods used to analyse the connective tissue

were not described, only that the tissues were grossly and histologically examined. A lack of precise, repeatable methods may have reduced the reliability of the study. In addition, the use of rodents could also have influenced the results because wound healing in rodents is by contraction (Greenhalgh, 2005), rather than epithelisation and the formation of granulation tissue, characteristic of human wound healing (Galiano et al, 2004).

Similar results were reported by Chbinou and Frenette (2004), who found a reduced accumulation of PMNs and macrophages in the wounds of diabetic rats. The authors concluded that diabetes caused a decrease in proliferative activity. However, the methods employed to measure these effects may not be fully reliable and valid because the cells were viewed by a microscope and counted manually, a method which may be open to subjectivity.

While both studies may provide valuable insights into wound healing, the results may not be directly comparable to humans. Streptozotocin, the agent used to induce diabetes in the rodents, may have been partly responsible for the results because it has been found to alter T-cell function and reduce macrophage phagocytosis, both of which alter wound healing during the late inflammatory phase (Greenhalgh, 2005). Overall these studies do indicate that diabetes reduces the numbers of PMNs within wound healing, but further research is required using human subjects to establish if similar effects are observed.

In patients with diabetic foot ulcers (DFUs) of over 6 months duration, the total numbers of PMN and macrophages within the wound margins were decreased (Galkowska et al, 2005). This may be due to thickening of the basement membrane of blood vessels which acts as a barrier, reducing permeability and impeding the migration of activated PMNs into the ECM (Silhi 1998; Greenhalgh, 2003). Galkowska et al (2005) concluded that the healing process of DFUs may be hampered by mechanisms decreasing the accumulation of leukocytes.

In contrast, an earlier study by Loots et al (1998) found that chronic DFUs contained significantly higher numbers of granulocytes, B-cells, monocytes and macrophages (particularly 28 days post-wounding) compared to acute wounds in controls, when the inflammatory response is generally decreasing in healing wounds (Schultz et al, 2005).

***“Diabetes does alter wound healing during the inflammatory phase.”***

This was supported by Wetzler et al (2000) who found increased PMNs and macrophages in the wounds of genetically diabetic mice. They proposed the presence of neutrophil chemoattractants macrophage inflammatory protein-2 (MIP-2) and monocyte chemoattractant protein-1 (MCP-1) contributed to the prolonged presence of neutrophils and macrophages at the wound site, thus sustaining the inflammatory response. The increase in PMNs could be a result of a compensatory mechanism to attract more PMNs to the wound bed (Kidman, 2008) due to their increased adherence to the thickened vessel walls (Silhi, 1998) or to compensate for their reduced function.

From these studies, it seems that diabetes does alter wound healing during the inflammatory phase. Any defects in leucocyte chemotaxis and phagocytosis will prolong inflammation that will subsequently delay proliferation and the formation of granulation tissue which consequently affects collagen deposition (Acosta et al, 2008; Haycocks et al, 2013). An additional effect of altered leucocytes levels is the increased susceptibility to infection (Terranova, 1991), which is known to overstimulate the inflammatory response (Martin, 2013). The presence of infection and defective leucocytes is likely to continue a cycle of inflammation, delaying the progression to proliferation. It has also been suggested that presence of infection can directly increase collagen breakdown (Gabriel, 2011).

**Proliferative phase**

The proliferative phase of wound healing is characterised by fibroblast migration, deposition of the ECM, angiogenesis and the formation of granulation tissue (Enoch and Leaper, 2005). This phase is dependent on the interactions between different cell types and the ECM, which is consecutively remodelled and reconstructed during this process (Loots et al, 1999). Diabetes impairs cell migration during this phase by its direct effects on cells, enzymes and cytokines (Lioupis, 2005).

**Fibroblasts**

It has been suggested that the fibroblast is the most important cell within the proliferative phase (Gabriel, 2011). Fibroblasts are activated to the wound site by cytokines, produced by or released from the provisional matrix by platelets and

macrophages (Bainbridge, 2013). Fibroblasts produce and organise the ECM by laying down a collagen-rich matrix, communicating with each other and other cell types (Sorrell and Caplan, 2004). They are also implicated in wound contraction because myofibroblasts provide contractile properties to aid wound closure (Stojadinovic et al, 2012). These functions are tightly regulated by cytokines and growth factors through signalling during the course of wound healing (Stojadinovic et al, 2012), so any disturbance or dysfunction of fibroblasts may affect the synthesis and degradation of collagen.

Loots et al (1999) demonstrated that diabetes decreased the proliferation rate of fibroblasts. In a study of skin biopsies from DFUs, diabetic non-lesional skin and skin of healthy age-matched participants, the DFU fibroblasts proliferated much slower than the other two groups. The study also found morphological differences in the fibroblasts that the authors suggested indicated a high turnover and breakdown of intracellular structures which affected their function. The sample size in this study was small with only four subjects in the DFU group, six in the diabetic non-lesional group and five controls. The use of a small sample can affect the internal validity of the study, increasing the sampling error and reducing the probability of obtaining statistically significant results (Bowling, 2009), so the results in this study may not be truly representative. However, the findings suggest that slower fibroblast proliferation in addition to the altered structure of the fibroblasts within DFU may well contribute to a decreased production of ECM proteins such as collagen, which could contribute to impaired wound healing.

Black et al (2003) also identified decreased fibroblast proliferation and decreased deposition of collagen but only in type 1 diabetes. In their study, 34 people with type 1 diabetes, 25 with type 2 and 5 non-diabetic controls had two tubes inserted subcutaneously into the lateral part of the upper arm. After 10 days, these were removed and analysed. In those with type 1 diabetes, there was an altered fibroblast function that affected the capacity to synthesise collagen. This could be related to decreased insulin secretion, although the results indicate that collagen deposition was not correlated to glycaemia expressed by the HbA<sub>1c</sub> level in the type 1 or type 2 subjects. However, the number of

matched controls within this study was small, which may have influenced the results. In addition, the experimental situation could have had an influence on the results by way of a “reactive Hawthorne effect” (Bowling, 2009), with participants increasing their adherence to the metabolic regimen. This could conceal the true relationship between HbA<sub>1c</sub> and collagen formation.

Further support for fibroblast dysfunction and phenotypic change was acknowledged by Lerman et al (2003), who compared the *in vitro* behaviour of fibroblasts cultured from diabetic mice (leptin receptor-deficient) with non-diabetic mice. The authors demonstrated a reduced migration of fibroblasts from the diabetic mice even when grown in an *ex vivo* environment optimised for nutrient, growth factor and glucose concentrations. The study also revealed an increase in pro-MMP-9 and diminished production of VEGF in fibroblasts from the diabetic mice, indicating that diabetes may also have an effect on proteases and growth factors that in turn may alter the processes within proliferation, ultimately affecting collagen formation within wound healing.

### Matrix metalloproteinases (MMPs)

MMPs are proteases or enzymes produced by different cells in the skin such as neutrophils, macrophages, epithelial cells and fibroblasts (Gibson et al, 2009). In normal wound healing, MMPs function to remove denatured elements of the ECM and bacteria, revealing exposed areas of intact functional matrix (Fitzgerald and Steinberg, 2009). This degradation enables migration of epidermal cells and stimulation of angiogenesis required for the formation of granulation tissue (Gibson et al, 2009).

This process is highly regulated and controlled by tissue inhibitors of metalloproteinases (TIMPs) (Fitzgerald and Steinberg, 2009). Failure to regulate this activity results in further degradation of the ECM, inhibition of angiogenesis, breakdown of the granulation tissue and deposited collagen (Haycocks et al, 2013). It has been suggested that the presence of diabetes can affect the balance of proteases, delaying wound healing (Lobmann et al, 2005).

Wall et al (2002) observed a reduction in pro-MMP-9 and pro-MMP-2 in diabetic mouse wounds and reduced MMP-9 in human wound fluid from diabetic chronic wounds, although levels of MMP-2

were elevated in the human wound fluid. This is in contrast to the research by Lerman et al (2003), who identified an increase in pro-MMP-9. MMP-2 and MMP-9 are required to remove denatured fibrillar collagen and development of granulation tissue in wound healing (Lobmann et al, 2005). Therefore unbalanced levels are likely to cause excessive matrix degradation including breakdown of collagen.

While the findings of Wall et al (2002) suggest that diabetes may alter the fine balance of MMPs required in wound healing, this data must be interpreted with caution. The use of wound fluid to sample protease activity has been previously questioned (Ashcroft et al, 1997) because it may not adequately reflect the levels of proteinases involved in wound healing. In addition, other confounding variables such as glucose regulation, wound position and infection in the mouse model were not controlled, which could affect the reliability of the study. However, Lobmann et al (2002) demonstrated elevated levels of MMPs and reduced levels of TIMPs in DFUs compared to acute wounds in non-diabetics. This supports the theory that diabetes alters the balance of MMPs in wound healing by suggesting that an increased proteolytic environment contributes to delayed wound healing.

### Advanced glycation end products (AGEs)

Non-enzymatic glycosylation of proteins is a well-recognised metabolic consequence of diabetes (Di Girolamo et al, 1993). This involves the condensation reaction of the carbonyl group of sugar aldehydes with free-amino groups of proteins resulting in the initial formation of a Schiff base (Avery and Bailey, 2006). This is then rearranged to a more stable Amadori product that can undergo a further oxidative reaction, producing an irreversible AGE (Huijberts et al, 2008). The resulting AGE has been shown to affect the physical properties of collagen through intermolecular cross-linking between collagen molecules within the fibres, increasing stiffness, reducing solubility, flexibility and susceptibility to degradative enzymes which results in less elasticity and thickening of the basement membrane (Paul and Bailey, 1996).

It has been suggested that the increased matrix rigidity and increased resistance to protease degradation is responsible for delayed cell migration and formation of granulation tissue (Darby et al,

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1997). Glycation also modifies the amino acid side chains of collagen, consequently affecting the charge profile of the collagen molecule; this is likely to affect interactions with cell and other matrix compounds (Paul and Bailey, 1996).

Liao et al (2009) devised an *in vitro* model to mimic glycated tissues to establish the effect of AGES within wound healing. They found that both the mechanical properties of the collagen matrices and their interactions with fibroblasts were altered. ECM remodelling was impaired and contraction was inhibited. Loughlin and Artlett (2009) concur that AGES reduce fibroblast expression of collagen I and collagen III and fibroblast proliferation.

Acosta et al (2008) proposed a further disruption to wound healing occurs due to the action of AGEs with their cell surface receptor (RAGE), triggering the generation of pro-inflammatory cytokines, adhesion molecules and chemokines which stimulate the release of inflammatory cells into the tissue, thus sustaining the inflammatory response.

**Conclusion**

It is clear from the research reviewed for this paper that diabetes influences wound healing, specifically affecting collagen synthesis and degradation by prolonging the inflammatory phase and delaying proliferation and subsequent formation of granulation tissue.

Diabetes affects PMN by altering their chemotaxis and phagocytosis, resulting in a protracted inflammatory process. People with diabetes also have an increased susceptibility to infection, so the presence of infection and defective leucocytes is likely to continue the cycle of inflammation preventing the transition to proliferation phase and the formation of collagen. If the proliferative phase does proceed, diabetes has a direct effect on fibroblasts causing their dysfunction thus preventing collagen deposition and remodelling. The balance of MMPs and TIMPs within this phase also appears to be altered by diabetes causing an increased proteolytic environment, reducing the formation of the ECM (Stojadinovic et al, 2012).

The reasons for the changes in cells, enzymes, cytokines and growth factors are still to be elucidated, but hyperglycaemia and its involvement in the production of AGEs appears to be implicated. Further research is required to identify the precise

mechanisms by which diabetes affects collagen synthesis and degradation. This would provide opportunities to explore and develop interventions to restore the ‘normal’ functioning of collagen, aiding wound resolution. ■

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