Wound healing and the role of fibroblasts

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extra cellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound. This article explores and summarises the research evidence on the role of fibroblasts, their origins and activation, and how they navigate the wound bed, as well as how their activity leads to wound contraction. This article also explores the local conditions at the wound site, which activate, regulate and ultimately reduce the fibroblast activity as the skin's integrity returns on healing.

fibroblast; migration; extracellular matrix; contraction

Healthy wound healing follows the processes of haemostasis, inflammation, proliferation, epithelialisation and remodelling, which are confined to the local wound area.[1] This article provides a brief overview, exploring the role of fibroblasts, specifically focusing on when and how they migrate to the wound site and the current understanding of how their activity is regulated.

Fibroblasts are present in the healthy healing wound, from the late inflammatory phase until full epithelialisation has occurred.[2] They are summoned to migrate to the wound area, proliferate and carry out a number of key activities under the tight regulation of injury mediated factors and the progressively changing environment of the healing wound, which is critical to the end state of the wound.[3] After the wound has been remodelled sufficiently, although not identical to the surrounding healthy tissue, the fibroblast levels return to pre-injury levels.[4]

Method

The aim of this article was to provide a focused commentary on the research evidence regarding the role of fibroblasts, their origins and activation, and how they navigate the wound bed, as well as how their activity leads to wound contraction. This article also explores the local conditions at the wound site, which activate, regulate and ultimately reduce the fibroblast activity as the skin's integrity returns on healing.

MEDLINE ScienceDirect, Online Research at Cardiff University (ORCA) and Google Scholar were searched for English language papers, using the search terms ['human dermal fibroblast'] OR ['would healing']. The search covered the years 1995–2013, and included contemporary studies on this subject; however, relevant citations prior to these dates, identified from the included articles' reference list, were also included, to give context to more recent discoveries. Articles to be included were hand selected on the basis of their methodological rigour and relevance to the topic. To preserve the quality of the article, only articles published in peer-reviewed journals were considered.

Activation of the fibroblasts

In the context of the healing process, at the end of the inflammatory phase and beginning of the proliferative phase (24–48 hours post injury), the first fibroblasts appear at the site of injury.[2] Fibroblasts infiltrate and degrade the fibrin clot by producing various matrix metalloproteinases (MMPs), replacing it with extracellular matrix (ECM) components, such as collagen I–IV, XVIII, glycoproteins, proteoglycans, laminin, thrombospondin, glycosaminoglycans (GAGs), hyaluronic acid (HA) and heparan sulphate.[5] This complex matrix supports and regulates the migration and activity of the fibroblasts, as well as acting as a support and signal for angiogenesis, granulation-tissue generation and epithelialisation to occur.[6]

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Declaration of interest: There were no external sources of funding for this study. The author is currently employed as a product development specialist for 3M HealthCare, who had no role in the current manuscript. The research for the article was performed as part of a Masters in Wound Healing and Tissue Repair at Cardiff University.

Fibroblasts are found in uninjured tissue where they maintain the ECM.[6] Fibroblasts are summoned to the wound site via chemoattractants, such as platelet-derived growth factor (PDGF), interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), which are produced by, or released from, the provisional matrix by platelets and macrophages as part of the inflammatory response.[7] Chemoattractants are critical in directing the fibroblasts to the site of injury by binding to specific receptors on the cell surface.[8] This binding allows the fibroblast to orientate themselves according to the concentration of the attractant and establish a leading edge in the direction of the highest concentration.

Fibroblast origin and differentiation

In the presence of transforming growth factor-β (TGF-β1, TGF-β2 and TGF-β3), which originates from macrophages at the wound site as part of the inflammatory response,[7] fibroblasts undergo a phenotypical differentiation, whereby the structure and function is altered.[9] Firstly, TGF-β1 and TGF-β2 stimulate fibroblasts to attach, via integrin containing adhesions, to fibrous proteins in the ECM. Then this binding causes them to begin to express stress fibres or actin filaments in the cytoplasm (Fig 1).[9] This phenotype is termed a promyofibroblast. In the presence of TGF-β1, TGF-β2 and a rigid substrate these further differentiate into myofibroblasts.

Tomasek et al.[10] suggested that fibroblasts proximal to the wounded area differentiate owing to changes in the microenvironment, as the fibroblasts in uninjured tissue are stress shielded by the cross-linked structure in the ECM. On wounding, this shielding is lost and may be important in the recruitment and differentiation of fibroblasts to myofibroblasts. Myofibroblasts are characterised by the expression of α-smooth muscle actin (α-SMA), which gives them increased contractile power, as well as cell–matrix and cell–cell adherins, which is in stark contrast to those fibroblasts found in uninjured ECM.[5] The stress fibres and α-SMA are critical to both the fibroblasts migration action and wound contracting forces.[11]

The exact origin of the myofibroblasts seen in the healing wound is not clear. The majority are recruited locally from the dermis and tissues around the wound site;[9] however, bone marrow-derived peripheral blood fibrocytes are observed to have some fibroblast features and may also be a source.[12] Tubular epithelial cells that alter their phenotype via a process known as epithelial-mesenchymal transition (EMT) are another potential source.[13] It is possible that fibroblasts associated with tissue repair are summoned from a number of sources to meet the high, but temporary, need of wound healing. They may be recruited in a phased approach, dependent on wound site and severity or longevity, beginning with the local fibroblast recruitment.

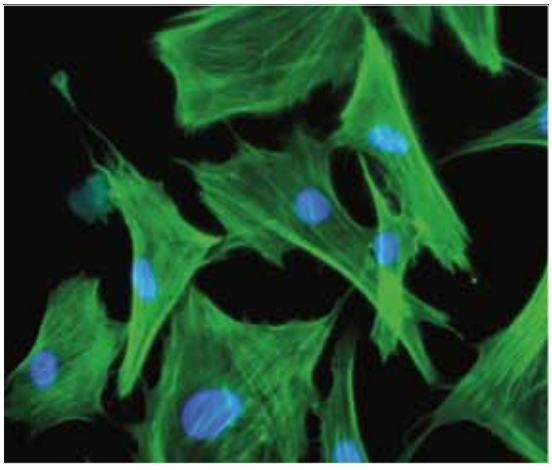

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Fig 1. Human dermal fibroblasts growing *in vitro* on synthetic ECM visualised for actin

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Fig 2. Hypertrophic scarring

Naturally, the question arises that if fibroblasts do have different origins, do they perform and interact differently once they arrive at the wound site, do they exhibit different residency times at the site of injury, or are their origins influenced by disease states, such as diabetes? If they do, this could be important to the clinical outcome of healing. The consequences of excessive or incomplete activity are detrimental to healing and will be discussed later.

Fibroblast migration

Wherever fibroblasts originate from, they must migrate to the wound bed and into the fibrin clot.[14] Guido and Tranquillo[15] observed that fibroblasts migrate into the wound bed via a mechanism called 'contact guidance'. They observed that the fibroblasts do not invade in a haphazard manner or via the fastest route, but instead travel in line with the collagen orientation that is already in place.

It is likely that the fibroblasts adhere to, and travel along, the fibronectin in the wound bed, rather

than collagen fibres (the predominant material in ECM).[16] Experimentation by Hsieh and Chin[17] demonstrated a specificity for fibronectin fibrils as a means of migration. According to Xu and Clark,[18] inhibition of fibronectin-binding integrins (namely, α3β1 and α5β1) also blocked migration, demonstrating the requirement of both specific substrate and binding integrins to facilitate migration.

Myofibroblasts have also been shown to express integrin (αvβ3), which allows them to adhere to and migrate on fibrin.[11] *In vitro* fibroblast experimentation showed no migration without fibronectin, but further stimulation in the presence of the ECM protein, tenascin-C.[14] This protein is rapidly produced by fibroblasts within 24 hours of tissue damage, but is cleared from the wound by the time the tissue is repaired.[19] Trebaul et al.[14] suggested that when tenascin-C was broken down by proteolysis, the fragments signalled to the fibroblasts to cease migrating; however, this was not supported in their study. Clark et al.[20] also demonstrated the requirement of a further proteoglycan (dermatan sulphate-CD44) in order to encourage the migration of fibroblasts into fibronectin/fibrin gels.

These studies suggest that a complex and specific set of circumstances are required in order to initiate fibroblast migration. The studies are carried out *in vivo* and cannot replicate the complex and dynamic nature of the wound; however, they do illustrate the complex regulation required to maintain normal wound healing. Too little activity and reduced granulation tissue can occur,[21] and the wound does not heal, whereas excessive fibroblast activity can be detrimental to wound healing and lead to complications, such as hypertrophic scarring (Fig 2), keloids and contracture.[22]

The locomotion of the myofibroblasts occurs through orchestrated ECM adhesion (via the integrins mentioned previously, in the presence of TGF-β1), cytoskeletal rearrangement and subsequent contraction in the direction of the cells leading edge, with the myofibroblasts moving in a dragging motion.[8]

The first stage of the movement is protrusion, where the leading edge of the cell (initiated by chemoattractants) expresses a thin extended layer called a lamellipodia, containing the actin-myosin and/or α-SMA filaments.[10] These protrude outwards from the centre of the cell cortex. Further actin molecules are polymerised to these structures, extending them outward, without penetrating the plasma membrane.[23] The lamellipodia reach out from the cell, and once they find a suitable substrate in the ECM, they adhere via integrins at focal adhesions (FAs), sometimes referred to as the fibronexus.[24] This is referred to as the adherence phase.[23] The transmembrane integrins themselves are attached to stress fibres that extend into the cell and can transfer information about the microenvironment to the cell. Finally, once adhesion at the leading edge is established, a contraction of the cytoskeleton occurs, transmitting cell traction forces (CTFs) to the ECM, which are strong enough to deform it.[25] Subsequent de-adhesion of the following edge of the myofibroblast from the ECM occurs.

This process is repeated, via continual waves of lamellipodia leading to the myofibroblast being pulled along the matrix. TGF-β1, TGF-β2 and TGF-β3 influence fibroblast migration.[26] TGF-β3 has a specific effect on the lamillopodium in which higher levels of TGF-β3 may lead to a greater number of directional changes,[27] having an effect on the level and composition of scarring on wound closure. Indeed, artificially elevated levels of TGF-β3 have been shown to reduce scarring in a rat model.[28]

Extracellular matrix generation and remodelling

Once at the wound site, influenced by the composition of the ECM, fibroblast migratory activities reduce and adhere to the fibrin clot and wound bed via multiple integrins located at the FAs.[20] They begin to proliferate and produce MMPs and other proteinases, such as seperinase, to remove denatured proteins and provisional matrix-associated material not required in the healed wound. These proteinases are tightly controlled by tissue inhibitors of metalloproteinases (TIMPS), which are also produced by fibroblasts.[29] Simultaneously, they also produce new ECM, initially relatively rich in collagen III, fibronectin and hyaluronic acid.[14] Collagen III is quick to produce, with the early matrix acting as a barrier to pathogens and to loss of serum and fluids. This is later degraded by proteases and remodelled by the fibroblasts to be replaced by type I collagen, which has a much higher tensile strength, but takes longer to deposit.[30]

Birk and Trelstad[31] observed that collagen fibrils are produced by the myofibroblasts. They found that procollagen was secreted and captured in vesicles on the exterior of the cell, where it was converted into collagen fibrils. These fibrils were further organised into a fibrous structure, which is the framework for epithelialisation. The myofibroblasts exert tension on the ECM via the FAs and stress fibre contraction, a force known as cell traction force (CTF),[32] remodelling the ECM and altered its three-dimensional (3D) structure and density. Evidence of this compaction has been demonstrated experimentally where a 40% increase in collagen density was observed in a closed system where no new collagen was being produced.[25]

Mechanoperception

The integrin–ECM binding is used by the cell to determine the microenvironment, a process referred to as mechanoperception.[10] This process directly influences myofibroblasts activity, affecting adhesion, receptor expression, gene expression and

protein synthesis, as well as influencing the cytoskeleton and cell motility.[10] In addition, the activity of the myofibroblasts affects the ECM composition in which they reside in a dynamic, reciprocal manner.[3] For example, when bound to fibronectin, the integrins transmit a signal into the cell to activate collagen synthesis,[10] which can be seen clinically as the presence of granulation tissue, with the altering levels of collagen consequently affecting the cells activity. This regulation of myofibroblast activity specific to the ECM was observed by Clark et al.[25] The necessity of TGF-β to stimulate collagen production was already known, with Clark et al. observing this *in vitro* on a plastic medium; however, once the myofibroblasts were introduced to an ordered collagen rich matrix, similar to one that would be observed toward the end of wound healing, the myofibroblasts no longer produced collagen even in the presence of TGF-β.

This evidence suggests the microenvironment can heavily influence the differentiation and activities of myofibroblasts. However, this knowledge is largely based on cell cultures and *in vitro* studies—the very action of placing them in these artificial environments may be effecting them in ways that do not occur *in vivo*, and this must be taken into consideration when applying these findings to clinical practice. More recently, 3D matrix models have been developed, which more accurately reflect the *in vivo* environment in which fibroblasts naturally reside,[33] and interestingly bring into question the accuracy of the evidence gathered in the two-dimensional (2D) models. The 3D models show differences in proliferation profiles of fibroblasts compared with 2D models,[34] with the same 3D models showing an impairment or barrier to cell migration in less elastic environments. Each model does not reflect a dynamic wound environment; therefore, it has been suggested that a range of models are required. These would represent pro-migratory and pro-contractive levels of growth factors, and high and low levels of tension, to understand the behaviour of fibroblasts in a number of simulated tissue environments.[35]

Wound contraction

A key feature of the fibroblasts and myofibroblasts is to provide the contractile forces that bring the wound edges together, which is observed in normal wound healing. The method of contraction is well discussed,[36] centring around, what were, two opposing theories. One theory was that the CTFs exerted by fibroblasts on the ECM during migration lead to compaction of the ECM and, therefore, wound closure.[32] The other theory hypothesised that myofibroblasts anchor to the ECM and contract in a manner similar to that of smooth muscle (SM).[21]

It is now understood that both processes may play a role in a phased approach.[10] First, the fibroblasts attach to the collagen fibrils and exert CTFs on the ECM, causing compaction in early healing; this compaction and alteration of the microenvironment also leads to differentiation into promyofibroblasts.[5] Stress fibres, further cell–matrix and cell–cell adhesions then form within the promyofibroblasts and, in the presence of TGF-β1, α-SMAs are expressed, typical of the myofibroblast cell type.[9] These cells proliferate and apply strong contractile forces across the wound bed, drawing the wound edges together. However the contraction does not follow the mechanism of SM contraction, as suggested by Gabbiani et al.,[21] because it is in fact slow, partially irreversible and achieves contraction by remodelling the ECM over relatively long periods of time, which is not the case for SM.[5]

Fibroblast activity post wound closure

Fibroblast levels decrease to normal levels by around day 14, when the new wound ECM has a similar tensile strength to the surrounding healthy tissue.[9] They undergo programmed cell death (apoptosis), which could be nitric oxide (NO) induced.[37] In a rat model, Desmoulière et al.[4] observed that increased myofibroblast apoptosis occured as the wound closed, suggesting this apoptosis triggers granulation tissue to evolve to scar tissue.

Discussion

Fibroblasts are key to the end state observed in healthy wound healing. If the fibroblasts are not activated, or sub-optimally migrate into the provisional matrix, then there will be reduced autolytic debridement of the denatured proteins and fibrin clot. When the myofibroblasts are present in the wound, the new matrix will be able to support angiogenesis and granulation (characterised by the light red and moist tissue, with the new buds or granules of capillary growth often visible from day 5 post injury).[38] Epithelialisation is also observed as new pink epithelial tissue grows from the wound edge as the keratinocytes migrate over the new granulation tissue. At this time, the tissue strength is sub-optimal, since the fibroblasts are laying down and remodelling the ECM;[30] this tissue also easily bleeds, so it is best practice to cover the wound with a protective dressing. The wound contracts by the action of the myofibroblasts and heals with a pink scar tissue that turns white over the proceeding weeks as it is remodelled. The fibroblasts are key to both laying down new material and wound contraction to close the wound; hence they have a major influence on the end state of healing. If the myofibroblasts remain present post normal healing it can lead to keloid, hypertrophic scarring or contracture (Fig 2).[39]

There are still gaps in our knowledge with regard to the origins of fibroblasts,[9] and understanding whether the origin of fibroblasts directly effects

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healing dynamics and the healed tissue end state could have significant implications for clinical understanding—particularly if the origins are influenced by patient comorbidities.

The area of fibroblast activation through disruption of the stress shielding in the ECM in uninjured tissue[10] is also worthy of future study to understand how the timing and spatial mapping of these traumatic events influences healing dynamics and outcomes—again, it is particularly interesting in those individuals who are susceptible to impaired healing and raises the question of whether their fibroblasts react to this loss of stress shielding in the same way as healthy fibroblasts.

The down regulation and apoptosis of fibroblast populations is still not fully understood[37] and is of critical significance to those suffering or susceptible to contracture, or keloid and hypertrophic scarring, all challenging clinical situations.[39] Another important area to consider is the effect of ageing; a trend for the developed and developing world has been shown to be an independent factor affecting healing,[40] with studies indicating this may be due to effects on fibroblast activity, affecting their response to chemokines[41] and their mobility.[42] his suggests that future studies, if they are to be related to clinical practice, should investigate whether effects are observed in both young and old fibroblast populations.

Conclusion

The presence and activities of fibroblasts in healthy wound healing is well established; however, it is not certain where the fibroblasts originate and there is still more to learn about the factors that influence their migration, differentiation, activity and clearance in the wound. Studies have demonstrated the key activities of the fibroblasts in contributing to ECM production and contraction in wound healing, with others demonstrating the control and regulation of their activity. The close regulation ensures effective wound closure via establishing granulation tissue and controlling wound contraction, followed by a return to pre-injury levels as the skins' integrity returns. ■

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