Wound chronicity and fibroblast senescence – implications for treatment

Keith G Harding, Keith Moore, Tania J Phillips

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ABSTRACT

A proportion of chronic wounds fail to heal in response to standard therapy. For venous leg ulcers, a correlation exists between longer duration before treatment initiation and poor healing response to compression therapy. Differences identified between the healing wound microenvironment and that of the non healing chronic wound suggests that many potential mechanisms exist to impair healing. One contributory mechanism may be inhibition of fibroblast proliferation and induction of a stress-induced premature senescence phenotype by the continuing inflammation found in chronic wounds. Senescent fibroblasts exhibit an extracellular matrix degradative phenotype that contributes to wound chronicity. Accumulation of greater than 15% senescent fibroblasts has been described as a threshold beyond which wounds become hard to heal. The ratio of senescent : non senescent cells is therefore critical to determining response to treatment, and adjunctive therapies that modulate this ratio in favour of non senescent cells are likely to enhance therapeutic healing rates. A number of tissue-engineered dermal replacements contain non senescent fibroblasts and can donate cells to the wound environment additional to releasing growth factors and reversing the antiproliferative activity of chronic wound exudate. Recognition of the role of fibroblast senescence in wound chronicity may allow for identification of those wounds that will respond positively to these products.

Key words: Chronic wound ● Fibroblast ● Oxidative stress ● Senescence ● Treatment

Key Points

- in the absence of complicating factors, wound healing of the skin is a rapid and efficient process leading to restoration of barrier function
- healing is slower in the aged but still effective; however, many conditions associated with ageing such as vascular disease can lead to the development of chronic wounds
- the most prevalent chronic wound types are venous leg ulcers (VLU), diabetic foot ulcers and decubitus ulcers

INTRODUCTION

In the absence of complicating factors, wound healing of the skin is a rapid and efficient process leading to restoration of barrier function. Healing is slower in the aged but still effective. However, many conditions associated with ageing such as vascular disease, diabetes, immobility and other comorbidities can lead to development of chronic wounds that do not follow a normal healing trajectory and may persist for months and in some cases many years. The most prevalent chronic wound types, venous leg ulcers (VLU), diabetic foot ulcers and decubitus ulcers, incur significant costs in terms of health care, loss of earning potential and quality of life to the many patients involved.

Standard therapy for VLU comprises of the application of graduated compression bandaging combined with an appropriate wound-contact dressing to maintain an optimal level of moisture and promote healing. This regime can induce healing within 24 weeks for a number of VLU. However, a significant proportion of ulcers are slow to respond or unresponsive to compression therapy and require further interventions.

While all VLU are initially treated with compression therapy, poor or non responders may then be treated with recently introduced adjunctive treatments such as recombinant growth factors (1), tissue-engineered skin equivalents (2) or aggressive surgical debridement under local or general anaesthesia (3) with the intention of converting the chronic

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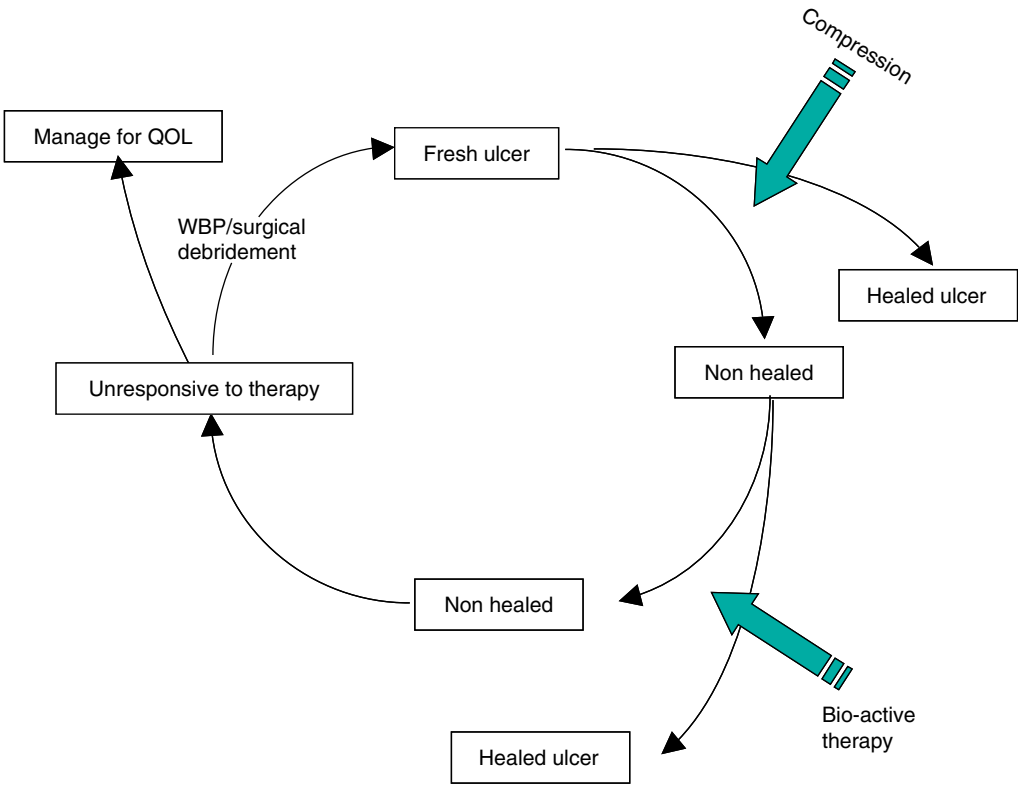

Figure 1. The cycle of treatment options for venous leg ulcers

Key Points

- standard therapy for VLU comprises of the application of graduated compression bandaging combined with an appropriate wound contact dressing to maintain an optimal level of moisture and promote healing
- while all VLU are initially treated with compression therapy, poor or non responders may then be treated with recently introduced adjunctive treatments such as recombinant growth factors on tissue-engineered skin
- evaluation of a number of putative clinical prognostic factors for VLU has demonstrated a consistent relationship between size and duration of ulcers and healing outcome

wound to one that more closely resembles a healing acute wound (Figure 1). As this cycle of treatment options progresses, the total treatment cost increases.

WOUND DURATION AND NON HEALING

Evaluation of a number of putative clinical prognostic factors for VLU has demonstrated a consistent relationship between size and duration of ulcers and healing outcome (4). Ulcers that are less than 10 cm^2 in area and less than 12 months old at first visit have a 71% chance of healing by 24 weeks of compression therapy while those ulcers greater than 10 cm^2 and 12 months duration only have a 22% chance of healing. It has been suggested that the effect of wound duration on outcome is the greatest as the wound initially ages and then diminishes over time. This effect may be linked to temporal changes in the wound microenvironment, so that eventually accumulated changes reach a critical point beyond which healing is less likely to occur (5).

Analysis of the chronic wound microenvironment demonstrates many physiological differences compared with a normally healing wound (6). Failure to re-epithelialise can be a consequence of a number of factors, including prolonged inflammation, an imbalance of regulatory growth factors and cytokines, defective extracellular matrix (ECM) that fails to support keratinocyte migration, modified fibroblast function and defective capillary function leading to inadequate tissue oxygenation. In essence, the normal time frame of healing is arrested at an inflammatory stage preventing progression towards the proliferative and subsequent re-epthelialising phases. The possibility exists that the longer a wound remains in the inflammatory phase so an accumulation of cellular defects may render it less responsive to treatment.

Poor healing in ulcers of long duration may be a consequence of more severe venous disease, but wound fibroblast senescence may also be a contributory factor (7). The dermal fibroblast plays a central role in healing. It is essential for production of ECM to allow keratinocyte migration from the wound margin to achieve wound closure and for subsequent matrix remodelling to achieve maximal

Key Points

- ulcers that are less than 10 cm^2 in area and less than 12 months old have a 71% chance of healing by 24 weeks
- ulcers greater than 10 cm^2 and 12 months duration only have a 22% chance of healing
- analysis of the chronic wound microenvironment demonstrates many physiological differences compared with a normally healing wound
- poor healing in ulcers of long duration may be a consequence of more severe venous disease
- there is a growing body of evidence to suggest that premature fibroblast senescence may be implicated in the delayed healing of VLU
- on the basis of evidence for the role of fibroblast senescence in non healing of VLU, we suggest that therapeutic delivery of non senescent cells to the wound may enhance the healing rates of hard to heal ulcers
- replicative senescence is a process that ensures normal cells have the ability to undergo a limited number of cell divisions
- chronic wound fluid inhibits the proliferation of fibroblasts by inhibiting DNA synthesis
- the ratio of non senescent cells may influence treatment outcome, so that a shift towards senescence contributes to compromised healing
- tissue-engineered products comprising viable neonatal non senescent fibroblasts in a bioresorbable matrix have been used to treat VLU like ulcers
- this demonstrated *in vitro* that these products can counteract the inhibitory effects of chronic wound fluid on fibroblasts *in vitro*
- for those wounds of longer duration, repeated applications of fresh fibroblasts or chemoattractant would be required to determine whether a threshold level of non senescent cells has to be achieved before healing is initiated

strength of the healed wound. There is a growing body of evidence to suggest that premature fibroblast senescence may be implicated in the delayed healing of VLU. For example, a clinical correlation has been demonstrated between the number of senescent cells identified in a wound biopsy and time to healing. It has been suggested that greater than 15% of senescent cells identified in a population of wound fibroblasts may be the critical point at which an ulcer becomes hard to heal (8).

On the basis of the evidence for the role of fibroblast senescence in non healing of VLU, we suggest that therapeutic delivery of non senescent cells to the wound may enhance the healing rates of hard-to-heal ulcers.

CELLULAR SENESCENCE AND WOUND CHRONICITY

Replicative senescence is a process that ensures normal cells have the ability to undergo a limited number of cell divisions. This finite replicative life span is thought to be a mechanism protective against tumourigenesis (9). As cells reach the end of their replicative life span, they become resistant to apoptotic death and accumulate as senescent cells with an altered phenotype. Senescent fibroblasts produce, by comparison to presenescent cells, elevated levels of the proteolytic enzymes collagenase, elastase, stromelysin and decreased levels of the metalloproteinase inhibitors TIMP-1 and TIMP-3 (9). This switch from an ECM synthesising to a degrading phenotype is particularly relevant to VLU where the same degradative environment can be demonstrated by analysis of chronic wound exudates (10). The possibility exists therefore that accumulation of senescent fibroblasts may contribute to wound chronicity by virtue of their functional modifications.

Evidence for this is provided by the positive relationship between higher levels of fibroblasts with a senescent phenotype identified in VLU and a poor prognosis (8,11,12). The aberrant fibroblast phenotype characterised in these studies may not, however, be a result of genetically programmed replicative senescence. Replicative senescence is characterised by telomere shortening at each successive cell division *in vitro*, and a recent study has demonstrated that fibroblasts isolated from VLU do not exhibit this feature (13). However, telomere shortening may not be a requirement for development of senescence *in vivo* (14) which can develop as a consequence of environmental stress. The phenotype of stress-induced premature senescence may be generated *in vitro* by chronic exposure of non senescent cells to stressors such as oxidative stress (15) or pro-inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α (16). These same stressors can be identified in the chronic wound environment (6), and culture of non senescent human skin fibroblasts in the presence of VLU exudate induces a senescent-like phenotype (17). Chronic wound fluid inhibits the proliferation of fibroblasts by inhibiting DNA synthesis (17) with arrest in the G0/G1 phase of the cell cycle (18) mediated via Ras-dependent signalling (19).

Given that senescent cells do not undergo apoptotic death, continuing exposure of cells within VLU to stressors derived from the chronic inflammatory microenvironment may generate an increasing proportion of senescent fibroblasts with longer ulcer duration. Once a critical threshold has been achieved, this population of cells may contribute to the increasing difficulty found in achieving healing as wounds age.

TREATMENT STRATEGIES

The ratio of non senescent to senescent cells may influence treatment outcome, so that a shift towards senescence contributes to compromised healing. By corollary, healing of VLU undergoing compression therapy may be improved by adjunctive therapies that increase the ratio of senescent to non senescent fibroblasts in wound tissue.

Tissue-engineered products comprising viable neonatal non senescent fibroblasts in a bioresorbable matrix have been used to treat VLU (20,21), and it has been demonstrated *in vitro* that these products can counteract the inhibitory effects of chronic wound fluid on fibroblasts *in vitro* (22). Their normalising effect on chronic wound healing is thought to be a consequence of delivery of growth factors and ECM to the wound environment. The donated fibroblasts have been demonstrated to survive in the wound bed for 6 months after application to allow a persisting counter senescence effect (2). An alternative source of fibroblasts may be within adjacent dermis that could be induced to

migrate into the ulcer by use of cytokines as chemoattractants (23). However, the danger here is that fibroblasts derived from dermis adjacent to VLU may have a senescent phenotype before entering the wound as a consequence of the stress-induced premature senescence induced by venous hypertension (24).

The precise relationship between wound duration and percent fibroblasts with a stress-induced premature senescence phenotype remains to be elucidated, but it may be assumed that there is an increase with wound duration. The greater the wound duration and, hypothetically, the greater the proportion of senescent fibroblasts, the more non senescent fibroblasts that would need to be supplied to the wound in order to initiate healing. For those wounds of longer duration, repeated applications of fresh fibroblasts or chemoattractant would be required to determine whether a threshold level of non senescent cells has to be achieved before healing is initiated. It may be that a non linear relationship exists between duration and per cent senescent cells and that healing/non healing thresholds exist (8). Such thresholds would create windows of therapeutic opportunity for different treatment modalities, as chronic wounds progress from treatment responsive to treatment non responsive.

A prerequisite for treatments designed to combat stress-induced premature senescence is that the cellular microenvironment of the wound bed be one that does not continue to induce stress-induced premature senescence. One of the major sources of pro-inflammatory cytokines and oxidative stress in chronic wounds is the inflammatory response resulting from the presence of colonising or infecting bacteria (6). It is thus imperative to ensure that the treated wounds have minimal bacterial contamination before initiating fibroblast therapy.

Fibroblast containing tissue-engineered products has been demonstrated to reverse the growth inhibitory effects of chronic wound fluid (22), and it has been suggested that by acting as a source of fibroblasts with a dermal phenotype, they will improve healing outcome (25). Exploration of a hypothesis that the healing rate of VLU may be improved by therapeutic manipulation of the non senescent : senescent fibroblast ratio would allow design of treatment strategies to optimise use of these products. In this way, a rationale may be developed to identify those wounds most likely to respond to treatment and the optimal dosing frequency required.

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Key Points

- one of the major sources of pro-inflammatory cytokines and oxidative stress in chronic wounds is the inflammatory response resulting from the presence of colonizing or infecting bacteria
- it is thus imperative to ensure that the treated wounds have minimal bacterial contamination before initiating fibroblast therapy
- fibroblast containing tissue-engineered products has been demonstrated to reverse the growth inhibitory effects of chronic wound fluid; it has been suggested that by acting as a source of fibroblasts with a dermal phenotype, they will improve healing outcome

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