FORUM ARTICLE

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Senescence Programs Shape the Chronic Wound Microenvironment

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Significance: Chronic wounds such as diabetic foot ulcers, venous leg ulcers, and pressure ulcers are characterized by impaired healing and persistent inflammation. Cellular senescence, defined as irreversible growth arrest with a pro-inflammatory secretory phenotype (senescence-associated secretory phenotype), has emerged as a potential driver of these nonhealing states. While transient induction of senescence may aid acute repair, chronic accumulation of senescent cells is thought to disrupt tissue regeneration, promote extracellular matrix degradation, and sustain inflammation.
Recent Advances: Single-cell RNA sequencing and spatial transcriptomics have revealed diverse cell states in chronic wounds, including senescent subsets. Studies in diabetic, venous, and pressure ulcers implicate senescent fibroblasts and immune cells in impaired remodeling, often triggered by oxidative stress, hyperglycemia, or ischemia–reperfusion injury. Therapeutic strategies targeting senescent cells in delayed wound healing have demonstrated promise in preclinical models; however, interventions must be timed and targeted precisely.
Critical Issues: Despite emerging evidence, the identity, abundance, and location of senescent cells in chronic wounds remain poorly defined. Reliance on nonspecific markers such as p21 or SA-β-gal complicates interpretation. Senescence appears to play context-dependent roles, with beneficial effects during acute healing but harmful persistence in chronic wounds, presenting challenges for therapeutic targeting.
Future Directions: More studies using single-cell RNA sequencing, spatial transcriptomics, and longitudinal profiling are needed to define senescent subpopulations, map their spatial distribution, and track dynamics during wound progression. These approaches will help distinguish transient from persistent senescence. A deeper understanding of interactions with immune, epithelial, and stromal components will guide precisely timed, cell type–specific interventions to improve outcomes.

Keywords: chronic wounds, cellular senescence, senescence-associated secretory phenotype, fibroblast senescence, wound healing

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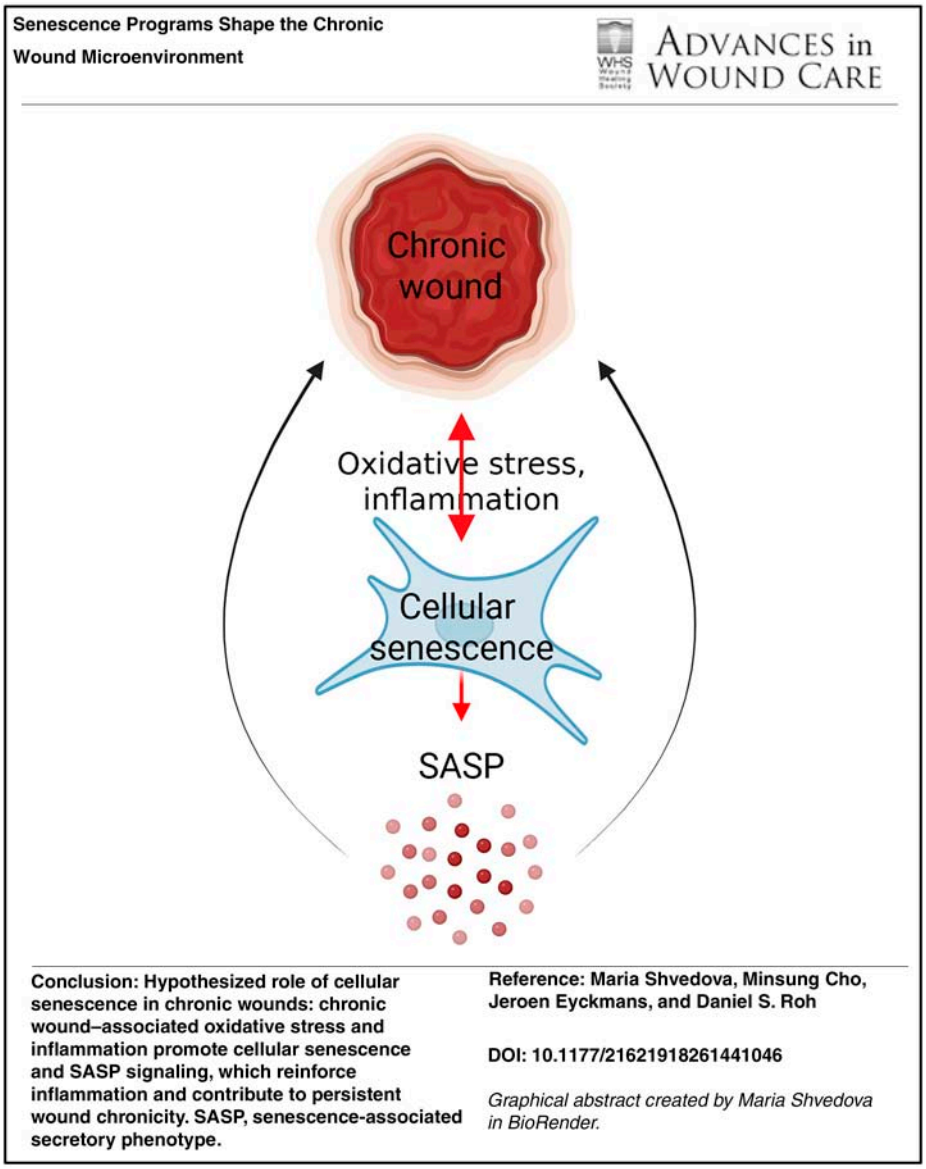


SCOPE AND SIGNIFICANCE

This review examines the role of cellular senescence in chronic wounds, with a focus on diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), and pressure ulcers (PUs). While senescence is hypothesized to play a beneficial, transient role in acute wound healing, its chronic persistence may drive pathological inflammation, extracellular matrix (ECM) degradation, and impaired regeneration.[1–3] We synthesize current evidence from human studies and preclinical models, emphasizing the need for precise characterization of senescent cell types and functions. By integrating recent advances in single-cell and spatial transcriptomics, this review aims to clarify unresolved questions and guide the development of senescence-targeted interventions for chronic wound treatment.

TRANSLATIONAL RELEVANCE

Emerging technologies such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have enabled the identification of senescent states within human chronic wound tissues. These tools are beginning to link specific senescent cell populations with impaired healing phenotypes *in vivo*, providing a more physiologically relevant framework than earlier *in vitro* studies. Translating these insights into therapies requires understanding the dynamic, context-dependent effects of senescence. Preclinical studies using senescence-targeting approaches have demonstrated promising results, highlighting the potential to modulate senescence burden and improve tissue repair. This review integrates translational findings that can inform future biomarker and therapeutic development.

CLINICAL RELEVANCE

Chronic wounds are a major cause of morbidity and health care costs, particularly in aging and diabetic populations.[4,5] Cellular senescence contributes to impaired healing by disrupting tissue remodeling and perpetuating inflammation.[1–3] Understanding the role of senescent cells in chronic wound pathology may enable the development of novel diagnostics and targeted treatments. Clinical interventions that modulate senescence could complement existing wound care strategies, especially in cases resistant to standard therapies. This review provides clinicians with a mechanistic overview of how senescence impairs healing and highlights emerging therapeutic opportunities grounded in recent human and animal studies.

BACKGROUND

Chronic wounds, encompassing conditions such as DFUs, VLUs, and PUs, represent a significant clinical and economic burden[5] due to their persistent failure to progress through the normal stages of healing, often becoming arrested in a prolonged inflammatory state.[4,6–8] This challenge is particularly prominent in elderly and diabetic populations, where the prevalence of these nonhealing wounds is notably high.[9]

While the direct clinical evidence regarding the contribution of cellular senescence to the pathophysiology of chronic wounds is still limited, a substantial body of literature has emerged proposing that chronic wounds are characterized by an accumulation of senescent cells (Graphical Abstract).[1,3,10–16] Senescent cells are metabolically active cells that have permanently exited the cell cycle and secrete a complex mix of pro-inflammatory mediators, growth factors, and proteases known collectively as the senescence-associated secretory phenotype (SASP).[3,14,15,17] Through the release of cytokines, proteases, and growth factors, chronic senescent cells are hypothesized to alter the local wound microenvironment and interfere with tissue repair mechanisms, resulting in delayed wound healing and chronic wounds.[17–19]

Many prior studies investigating the role of senescence in chronic wounds have based their conclusions predominantly on *in vitro* findings or examined senescence markers in a limited or descriptive manner in human tissues. There remains a significant gap in comprehensive analyses rigorously examining the presence, specific cell types and their phenotypes, and functional contributions of senescent cells directly *in situ* or *in vivo* within the complex microenvironment of human chronic wound tissues. The extent to which *in vitro* observations accurately reflect the intricate cellular interactions and pathological processes occurring in chronic wounds in patients is mostly an unanswered question that is only recently being addressed.

It is recognized that senescence plays a nuanced role in wound biology.[2,20] A transient presence of cells expressing senescence markers during acute cutaneous injury may be beneficial, contributing to tissue remodeling and limiting fibrosis.[21,22] However, it is important to distinguish this acute, transient accumulation from the persistent, chronic accumulation observed in nonhealing wounds, which contributes to sustained inflammation, ECM breakdown, and impaired epithelialization, thereby delaying healing. Validating this hypothesis and fully understanding the context-dependent functions of senescent cells in human chronic wounds necessitates moving beyond primarily *in vitro* models.

Recent technological advances, such as scRNA-seq and spatial transcriptomics, offer advanced opportunities to dissect the cellular composition and transcriptional dynamics of human wound tissue at high resolution. While scRNA-seq provides detailed gene expression profiles of individual chronic wound cells, spatial transcriptomics adds another critical layer by revealing the location of these cells and their gene expression within the existing tissue architecture. These approaches are revealing the true heterogeneity of cell populations, including potentially senescent subtypes, and uncovering disrupted intercellular communication networks directly within chronic wound environments. These approaches have demonstrated distinct cellular subpopulations[23] and disrupted intercellular communication networks in chronic wounds compared with normal healing wounds.[24] Large-scale analyses utilizing these technologies are now providing deeper, albeit still evolving, insights into the specific senescent signatures and aberrant signaling pathways present *in situ*, offering a more physiologically relevant context to explore the cellular dysfunction underlying nonhealing wounds and address the limitations of earlier *in vitro* studies.[24–27] It is important to note, however, that while scRNA-seq and spatial transcriptomics have advanced our understanding of chronic wound heterogeneity, both approaches have limitations when used in isolation.

Gene expression alone does not fully capture senescent cell identity, which may be regulated at

the epigenetic, translational, or metabolic level. Integration with additional single-cell modalities such as scATAC-seq (chromatin accessibility), single-cell proteomics, and metabolomics can provide complementary information on regulatory landscapes, protein activity, and metabolic shifts characteristic of senescence. For example, coupling scRNA-seq with scATAC-seq enables linking transcriptional programs to underlying enhancer–promoter accessibility changes, while spatial proteomics can validate SASP factor localization *in situ*. In addition to transcriptomic profiling, proteomic and metabolomic approaches provide complementary insights into chronic wound biology by capturing molecular changes at the protein and metabolic levels. Proteomic analyses of chronic wound exudates and dressing-derived biomaterial have identified distinct alterations in inflammatory mediators, proteases, keratinocyte activation markers, and extracellular vesicle–associated stress proteins that distinguish healing from nonhealing wounds and reflect underlying immune activation and tissue remodeling processes.[28] Metabolomic profiling of chronic wound biopsies and exudates has revealed coordinated shifts in amino acid metabolism, central carbon metabolism, and energy pathways, as well as associations between metabolic states and microbial colonization, indicating extensive metabolic reprogramming within the chronic wound microenvironment.[29,30] However, these studies have been performed at the bulk tissue or wound exudate level and have not been used to directly identify or characterize senescent cell populations, leaving the proteomic and metabolomic features of senescent cells in chronic wounds largely unexplored at this time, representing an important opportunity for future investigation.

Conceptual framework

A central challenge in chronic wound biology is the absence of a unifying mechanistic explanation linking the major pathological features of these wounds, including persistent inflammation, ECM degradation, impaired epithelialization, and defective angiogenesis. These processes are usually described separately, yet the cellular programs that stabilize this nonhealing state remain poorly defined. Cellular senescence provides a potential unifying framework because it combines stable growth arrest with a pro-inflammatory and matrix-remodeling secretory phenotype that can propagate dysfunction through paracrine signaling (Fig. 1). Importantly, pathological conditions characteristic of chronic wounds, including hyperglycemia, oxidative stress, iron overload, and ischemia–reperfusion injury, are established triggers of senescence-associated pathways. Recent advances in single-cell RNA sequencing, spatial transcriptomics, and other multiomic approaches now allow these programs to be examined directly within

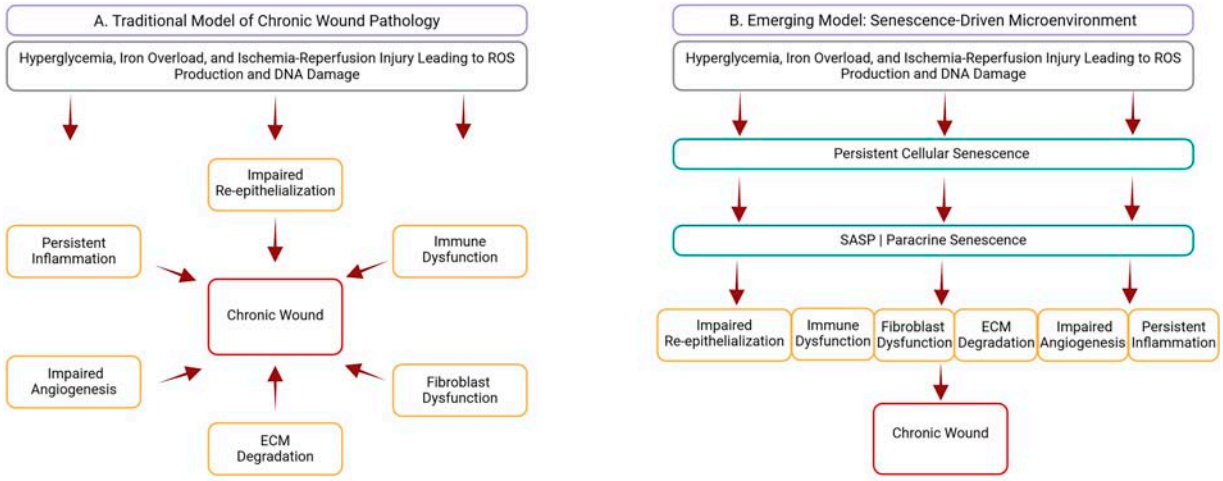


Figure 1. Conceptual model of chronic wound pathology. **(A)** Traditional model of chronic wound pathology. Chronic wounds have historically been explained as the result of multiple parallel pathological processes triggered by underlying stressors such as hyperglycemia, iron overload, and ischemia–reperfusion injury leading to reactive oxygen species (ROS) production and DNA damage. These processes contribute to impaired re-epithelialization, persistent inflammation, immune dysfunction, impaired angiogenesis, fibroblast dysfunction, and extracellular matrix (ECM) degradation, which together promote the development and persistence of chronic wounds. **(B)** Emerging model of the chronic wound microenvironment centered on cellular senescence. Pathological stimuli including hyperglycemia, iron overload, and ischemia–reperfusion injury induce persistent cellular senescence. Senescent cells produce a senescence-associated secretory phenotype (SASP) and propagate senescence through paracrine signaling. These processes drive key pathological features of chronic wounds including ECM degradation, immune dysfunction, impaired angiogenesis, persistent inflammation, and impaired re-epithelialization, thereby stabilizing the chronic wound microenvironment and contributing to sustained wound chronicity.

human wound tissues. These technologies enable identification of senescence-associated cellular states, their spatial distribution, and their interactions with surrounding cell populations. Together, they support an emerging view of chronic wounds as tissue environments shaped by persistent senescence-associated cellular programs.

DISCUSSION

Molecular mechanisms of cellular senescence in wound healing

Cellular senescence: Defining features and inducing stimuli. Studies examining cellular senescence in chronic wounds are predominantly descriptive, relying on expression of markers such as p16, p21, and SASP factors, as well as transcriptomic and spatial profiling, to identify senescent cell populations and characterize their molecular features within the wound microenvironment. In contrast, functional studies are more limited and involve pharmacologic or genetic interventions, such as senolytic agents, to directly assess the causal impact of senescent cells on wound healing.

Characteristics and Biomarkers. Cellular senescence is a stable state of cell-cycle arrest triggered by damaging stimuli (in particular, DNA damage) and telomere attrition, which is characterized by the expression of cyclin-dependent kinase inhibitors p16 (CDKN2A) and p21 (CDKN1A), increased senescence-associated β-galactosidase (SA-β-gal) activity, and secretion of inflammatory and matrix-degrading factors called SASP.[31,32] Morphologically, senescent cells *in vitro* often appear enlarged and flattened and exhibit an increased lysosomal compartment.[33] They also tend to resist apoptosis by upregulating anti-apoptotic proteins, particularly members of the BCL-2 family.[34] Given the heterogeneity of senescent states, no single marker of cellular senescence is absolutely sensitive or specific[35]; therefore, researchers typically rely on a combination of features, including cell-cycle arrest markers, SA-β-gal activity, upregulated DNA damage markers, and lack of expression of proliferative markers, to detect and characterize senescence.[36,37] A comprehensive overview of senescence markers, including their functional categories and context-specific considerations, is available in several recent reviews, and readers are referred to these resources for detailed summaries.[38–40] To support interpretation in the context of this article, Table 1 summarizes the commonly used general markers of senescence, whereas Table 2 outlines those identified in chronic wounds.

Senescence-Associated Secretory Phenotype. Despite cell-cycle arrest, senescent cells remain metabolically active and release a diverse array of signaling molecules, collectively termed SASP.[11] This phenotype includes interleukins (*e.g.*, IL-1β, IL-6), tumor necrosis factor-α (TNF-α), transforming growth factor (TGF), chemokines such as C-X-C motif chemokine ligands (CXCLs), and matrix metalloproteinases (MMPs).[45,56,57] SASP factors not only reinforce senescence within the secreting cell through autocrine signaling but also influence neighboring cells through paracrine mechanisms.[57–60] These signals can amplify

Table 1. *General markers of cellular senescence (compiled from[38–40])*

Marker	*Type/Function*	*Notes*
$p16^{INK4a}$	Cell cycle inhibitor, RB pathway	Stable marker, often accumulates with age
$p21^{CIP1/WAF1}$	Cell cycle inhibitor, downstream of p53	Early/inducible marker, sometimes transient
p53	DDR effector, transcriptional regulator	Triggers p21, part of senescence entry
Hypophosphorylated RB	Cell cycle checkpoint	Maintains arrest via E2F repression
SA-β-gal activity	Lysosomal enzyme activity	Classic histochemical assay; indirect
Lamin B1 loss	Nuclear envelope protein	Robust in multiple contexts; loss indicates chromatin remodeling
HMGB1 loss (nuclear)	Chromatin-binding protein	Relocalization or loss used as marker
γH2AX foci/DNA-SCARS	DDR foci	Indicates DNA damage
SAHF (heterochromatin foci)	Chromatin remodeling	Seen in some models, not universal
ROS accumulation	Oxidative stress	Reinforces senescence
Lipofuscin accumulation	Lysosomal aging pigment	Observed in aging tissues
SASP factors (IL-6, IL-8, TNF-α, TGF, MMPs, etc.)	Secretory phenotype	Functional hallmark, varies by cell type
Surfaceome changes (*e.g.*, DPP4, uPAR)	Cell surface proteins	Emerging class of senescence markers
Mitochondrial dysfunction	Metabolic	Altered membrane potential, increased ROS
Pro-survival signaling (BCL-2 family)	Apoptosis resistance	Allows persistence of senescent cells

Adapted from references.[38–40]

IL, interleukin; MMP, matrix metalloproteinase; RB, retinoblastoma; ROS, reactive oxygen species; SA-β-gal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha.

Table 2. *Reported senescence markers across wound types*

Marker	*Context*	*References*
$p16^{INK4a}$	DFU: fibroblasts, *in vitro*, murine models and human biopsy	41–43
	DFU: macrophages, *in vivo* (mouse wounds, paracrine senescence)	44
	VLU: macrophages, murine model, human biopsies	45
$p21^{CIP1/WAF1}$	DFU: fibroblasts, keratinocytes, *in vitro* + *in vivo*	46–48
	DFU: bulk tissue RNA-seq at ulcer edge	49
	PU: fibroblasts, wound biopsies ($p21^{+}/PCNA^{-}$)	50
p53	DFU: fibroblasts, *in vitro* and *in vivo* rat wounds	46,47
SA-β-gal	DFU: fibroblasts, keratinocytes (*in vitro* + animal models)	46,48
	VLU: fibroblasts, wound biopsies and cell culture	51,52
γH2AX	DFU: fibroblasts, *in vitro* (oxidative stress–induced DNA damage)	46
SASP cytokines and chemokines	DFU: IL-1A, CXCL8, MMPs, SERPINE1 (bulk tissue)	49
	DFU: macrophage SASP with CXCR2 ligands (propagating fibroblast senescence)	44
	VLU: TNF-α, IL-1β, TGF-β1 in wound fluid and fibroblast culture	53,54
	PU: TGF-β1, plasmin, and PAI-1 in wound fluid and fibroblast culture	54
Terminin	PU: fibroblasts isolated from wound center (*in vitro* + tissue sections)	55

DFU, diabetic foot ulcer; PU, pressure ulcer; SASP, senescence-associated secretory phenotype; VLU, venous leg ulcer.

inflammation, degrade ECM components, and impair regenerative processes, contributing to the persistence of chronic wounds.[12,57,61] While SASP generally promotes a pro-inflammatory environment, its effects can vary depending on tissue context. In some cases, such as tumor-associated senescence or liver injury, components of the SASP can instead stimulate regeneration by promoting anti-inflammatory, M2-type macrophage polarization.[62,63]

Triggers of Cellular Senescence. Common inducers of cellular senescence include DNA damage from oxidative stress, telomere attrition, oncogene activation, and mitochondrial dysfunction, although the last two factors are often hard to disentangle from the DNA damage response itself, which commonly accompanies mitochondrial dysfunction (due to increased reactive oxygen species [ROS] production) and oncogene activation (since the latter is often concurrent with DNA damage and causes increased uncontrolled proliferation further exacerbating DNA damage).[36,39,64] Therefore, the DNA damage response (including at the telomere sites as a result of telomere attrition) is the major inducer of cellular senescence.[65–70] In the context of chronic wounds, persistent oxidative stress and inflammatory signaling are the major drivers (Fig. 2).[71–73] ROS, for instance, can induce the DNA damage response and elevate p16 expression in cutaneous cells.[17,74] Repeated

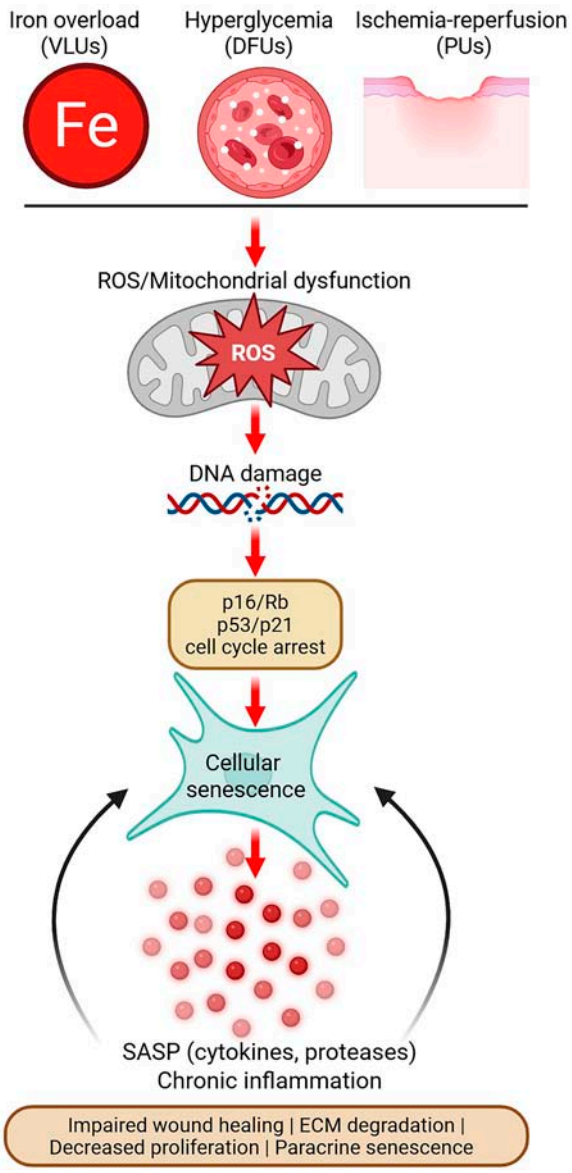


Figure 2. Triggers of cellular senescence in chronic wounds. Pathological stimuli, such as iron overload in venous leg ulcers (VLUs), hyperglycemia in diabetic foot ulcers (DFUs), and ischemia–reperfusion injury in pressure ulcers (PUs), cause oxidative stress and mitochondrial dysfunction, resulting in DNA damage. This activates canonical senescence pathways through p16/Rb and p53/p21 signaling resulting in cell-cycle arrest. The resulting senescent cells secrete SASP factors that promote chronic inflammation and ECM degradation, creating a reinforcing loop that sustains cellular senescence and impairs wound healing.

tissue injury and inflammatory cytokines can also activate p53-dependent pathways, shifting cells toward a senescent phenotype rather than reversible quiescence or programmed cell death.[75] Moreover, classical SASP components, IL-6 and IL-8, have been demonstrated to reinforce the senescent state in an autocrine way and propagate cellular senescence in a paracrine manner.[76]

Temporal and context-dependent dual role of senescence.

Physiological Role of Senescence Signaling in Acute Wound Healing. The heterogeneity of senescent cell populations prompted the idea of classifying them into "Deleterious" and "Helper" types based on their influence on tissue repair.[77] Following cutaneous wounding, senescent cells emerge rapidly and contribute to closure as in a murine model where senescent cells were selectively eliminated, wound closure was delayed.[22] Senescence also plays an anti-fibrotic role during repair. The matricellular protein CCN1, which is induced during healing, promotes fibroblast senescence through integrin signaling and ROS generation, leading to activation of p53 and p16 pathways.[21,78] This response promotes expression of anti-fibrotic genes. In mice lacking CCN1's ability to induce senescence, wounds developed excessive scar tissue, whereas topical application of CCN restored both senescence induction and normal scarring patterns.[21,79] Senescent fibroblasts were also found to support re-epithelialization and collagen deposition in radiation-induced skin injury through the secretion of IL-33, which promotes pro-repair macrophage polarization.[80] Removal of p16+ senescent cells or neutralization of IL-33 impaired healing, highlighting a context-dependent beneficial role of senescent fibroblasts through immune modulation.[80] During physiological wound repair, cells expressing senescence markers are transient and are ultimately removed by immune surveillance.[81] Anti-inflammatory macrophages have been proposed to mediate this clearance once re-epithelialization and tissue closure are achieved. This removal of cells expressing senescence markers leads to resolution of SASP-driven inflammation, allowing the transition into the remodeling phase of healing.[12] These data indicate that the timely appearance of senescent myofibroblasts helps regulate ECM deposition and fibrosis, promoting effective resolution of wounds (Fig. 3).

The Detrimental Role of Persistent Cellular Senescence in Chronic Wounds. When senescent cells are not cleared effectively, as is often the case in chronic wounds where they may accumulate due to increased oxidative stress causing the DNA damage response, their continued presence contributes to pathological outcomes (Fig. 3).[20] Wounds in aged or diabetic individuals may exhibit a buildup of senescent cells that secrete SASP factors over extended periods.[16] This chronic SASP exposure perpetuates inflammation, upregulates ECM breakdown, and maintains a growth-inhibitory environment. In particular, cells in human chronic VLUs and diabetic wounds often display a phenotype characterized by high matrix-degrading enzyme expression and suppressed proliferation.[82] Rather than supporting transient matrix remodeling, these cells contribute to ongoing degradation of collagen and other ECM components, leading to structural instability. Furthermore, they can attract immune cells without enabling effective clearance, resulting in an unresolved inflammatory state.[16,82] However, these studies did not do a comprehensive examination of senescence and relied on nonspecific markers. It is important to note that a combination of markers is required to definitively determine that a cell is senescent because other cell states and particular non-senescent cell types can express some of the markers temporarily; for example, quiescent cells express cell-cycle arrest markers[83] but not SASP components, and macrophages have been demonstrated to express p16 and SA-β-gal reversibly as a part of the normal immune response.[84] Experimentally, the role of chronic senescence in delayed wound healing has been supported by our model where dermal injection of senescent fibroblasts caused delayed wound healing in young mice.[85]

Single-cell and spatial transcriptomic profiling of human acute wounds revealed that pro-inflammatory macrophages and fibroblasts support re-epithelialization in a sequential, phase-specific manner through dynamic interactions with migrating keratinocytes.[23] In chronic wounds, this coordination is disrupted: migratory keratinocytes are scarce, pro-inflammatory macrophages are deficient or dysfunctional, and expression of CXCL1, EGF ligands, and HGF is blunted, highlighting impaired immune-epithelial and fibroblast signaling as key barriers to healing.[23] Crucially, our analysis of this publicly available single-cell dataset[23] revealed fourfold higher percent of p16+ cells in human venous ulcers compared with nonwounded skin; this analysis also confirmed very high baseline p21 expression, which appears to be nonspecific to senescence in this context (at least at the messenger RNA [mRNA] level), especially compared with p16 (Fig. 4). Detailed information regarding current knowledge on senescent cells in different chronic wound types is presented in the following sections.

Reinforcing Feedback Between Senescence and Inflammation. In chronic wounds, senescence and inflammation are hypothesized to form a self-perpetuating vicious cycle.[15,17] SASP molecules, including IL-1, IL-6, and TNF-α, maintain a pro-inflammatory immune environment that inhibits the progression to the proliferative phase of

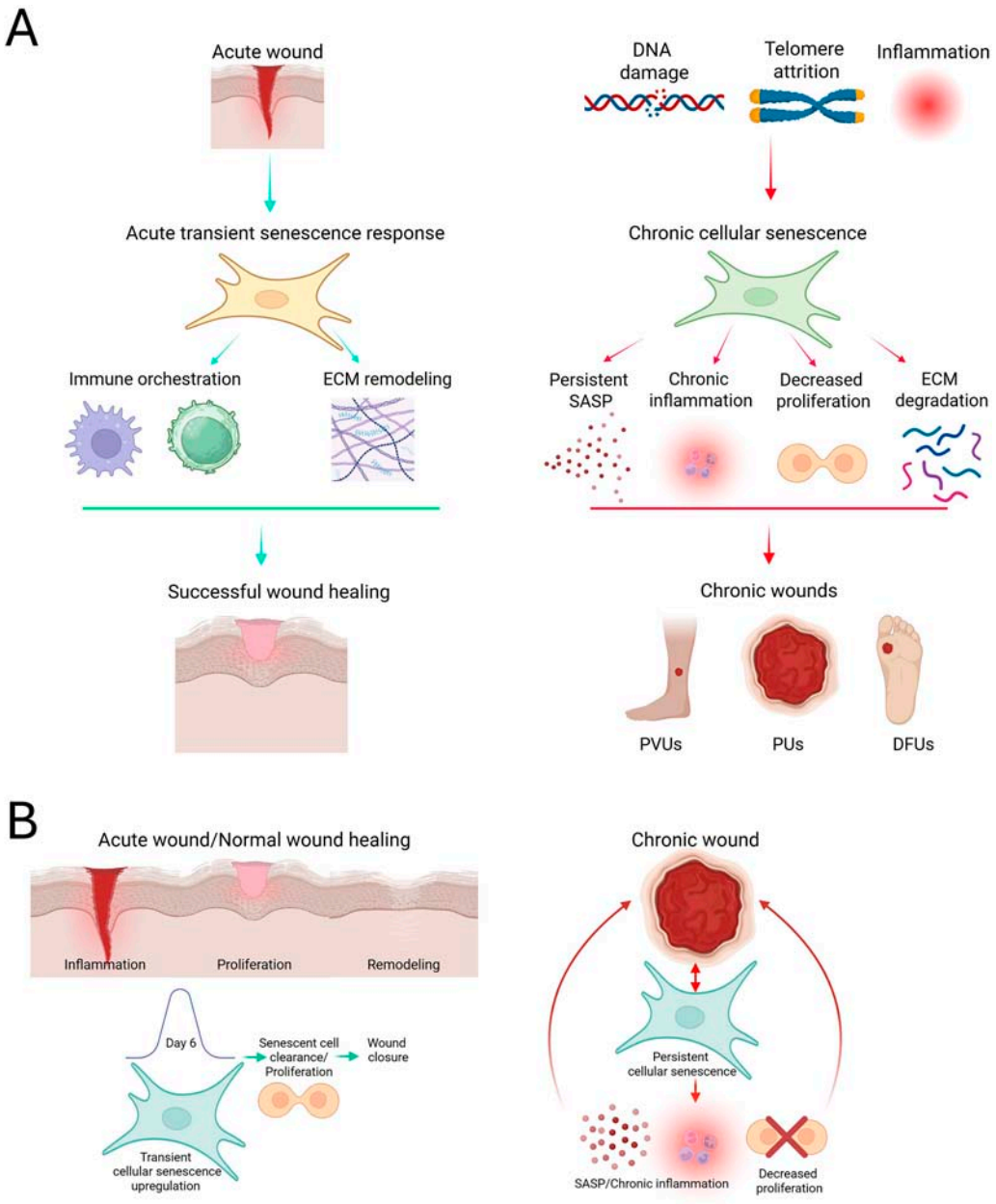


Figure 3. **(A)** Dual role of cellular senescence in wound healing. *Left panel*: Following acute injury, a transient senescence response promotes wound resolution through immune orchestration and ECM remodeling, resulting in successful healing. *Right panel*: Chronic stressors such as DNA damage and inflammation, as well as telomere attrition, induce persistent senescence, leading to sustained SASP production, chronic inflammation, reduced proliferation, and ECM degradation. Chronic cellular senescence contributes to the development of chronic wounds, including VLUs, PUs, and DFUs. **(B)** Dynamics of cellular senescence during normal versus chronic wound healing. *Left panel*: During normal wound healing, transient upregulation of cellular senescence peaks around day 6, coordinating tissue remodeling and modulating inflammatory response. Senescent cells are subsequently cleared, enabling fibroblast proliferation and wound closure. *Right panel*: Chronic wounds exhibit persistent senescent cell accumulation, resulting in sustained SASP signaling, chronic inflammation, and suppressed cellular proliferation, leading to impaired tissue regeneration and delayed healing.

healing.[15,17] Persistent immune infiltration, in turn, can reinforce senescence by releasing factors that promote further senescent conversion in neighboring cells. For example, in murine models of wound healing in aging and diabetes, macrophages expressing p16 have been identified, which secrete CXCR2 ligands that induce senescence in adjacent cells, thus amplifying the cycle of cellular dysfunction.[44] Although macrophages can express p16 reversibly,[86] this finding was validated in primary human dermal fibroblasts *in vitro*, where expression of CXCR2 triggered paracrine induction of p21 expression.[44]

Percent-Dependent Impact of Senescent Cells on Healing Outcomes. Emerging evidence suggests a quantitative relationship between senescent cell burden and wound healing impairment. One study

reported that when approximately 15% or more of cells within venous ulcers were senescent, wounds were more likely to become chronic.[51] Subsequent histological analyses of various chronic wound types, such as DFUs, VLUs, and PUs, demonstrated that regions with high senescent cell density (measured only by SA-β-gal staining) exhibited significantly reduced collagen content.[82] Another article demonstrated that fibroblasts from human PU specimens are mostly nonproliferative and positive for p21 immunostaining and negative for PCNA, a finding which, while not necessarily confirming senescence *per se*, still indicates that either senescence or hypoproliferative/quiescent cellular state contributes to chronic wound persistence.[50] Accelerated wound closure was associated with a reduction in p21-positive cells and a concomitant increase in PCNA-labeled cells.[50] A recent analysis of 79 human patients with chronic wounds confirmed that higher p21 expression is predictive of longer time to heal and demonstrated higher p16 staining in diabetic compared with nondiabetic wounds.[87] It is important to note that while these findings associate elevated p21 expression with delayed healing, it remains unclear whether this reflects bona fide senescence or alternative growth-arrest states. p21 mRNA is frequently elevated in nonsenescent, quiescent, or stressed cells, complicating its use as a definitive senescence marker. Overall, these findings underscore that both the abundance and persistence of senescent cells may play important roles in poor healing outcomes, and their measurement may hold prognostic value in chronic wound management.

Senescence marker limitations and interpretation in tissue studies

Identifying senescent cells in tissues presents significant challenges because all commonly used markers are not absolutely sensitive nor specific when used individually (Box 1). Markers such as SA-β-gal activity and expression of the cyclin-dependent kinase inhibitors p16 (CDKN2A) and p21 (CDKN1A) are frequently used to identify senescence; however, these features can also occur in nonsenescent contexts including transient stress responses, inflammation, differentiation states, or reversible cell-cycle arrest. As a result, detection of a single marker alone does not establish the presence of bona fide senescent cells.[40]

Box 1. Identifying Senescence in Tissues

No single biomarker is absolutely sensitive or specific to definitively identify senescent cells in tissues. Current guidelines recommend relying on a combination of markers that include multiple hallmarks of senescence, such as cell-cycle inhibitors (p16 and/or p21), absence of proliferation markers (*e.g.,* Ki67), and additional features including DNA damage markers or senescence-associated secretory phenotype (SASP) factors. Senescence should therefore be interpreted using multiparameter approaches rather than any single marker.[40]

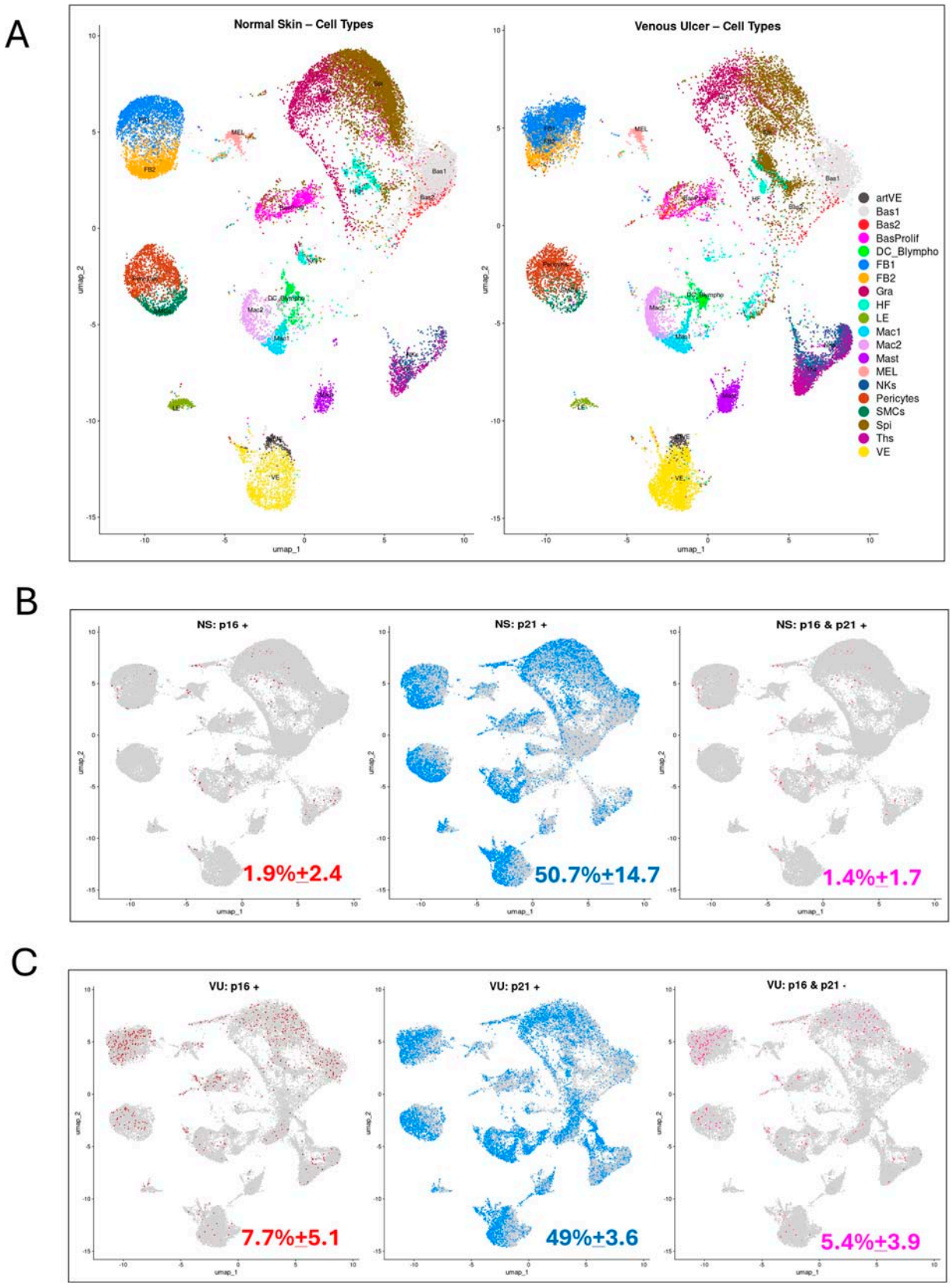
Current consensus recommendations emphasize the use of multimarker approaches that combine evidence of stable cell-cycle arrest with additional molecular or functional features of senescence, such as DNA damage signaling, absence of proliferation markers, or expression of SASP factors.[40] Throughout this review, findings suggesting senescence in chronic wound tissues are therefore interpreted cautiously and, when possible, in the context of multiple complementary markers.

Cellular senescence in DFUs

Diabetic ulcer pathophysiology and induction of senescence. Evidence supporting the involvement of senescence in DFUs derives from several complementary sources, including human biopsy studies, single-cell transcriptomic analyses, animal models, and *in vitro* mechanistic experiments.

Chronicity of Diabetic Wounds. DFUs result from hyperglycemic changes, resulting in a complex interplay of pathophysiological factors, including peripheral neuropathy, which decreases sensation and delays wound detection, peripheral arterial disease leading to ischemia, and increased vulnerability to infection.[88] The hyperglycemic milieu contributes directly to wound chronicity. Elevated

Figure 4. Cellular identity and senescence marker expression in normal skin and venous ulcers. **(A)** UMAP (Uniform Manifold Approximation and Projection) of single cells from normal skin (NS, *left*) and venous ulcer (VU, *right*) tissues colored by annotated cell type. Major cell populations include fibroblasts (FB1, FB2), keratinocytes (Bas1, Bas2, BasProlif), endothelial cells (VE, artVE, LE), immune cells (Mac1, Mac2, DC_Blympho, Mast, NKs, Ths), and other stromal or rare types (Pericytes, SMCs, MEL, Spi). Cell type identities were assigned using curated metadata and verified by canonical markers. **(B)** UMAPs of NS samples highlighting cells expressing senescence-associated genes: CDKN2A (p16) >0 (*left*, *red*), CDKN1A (p21) >0 (*middle*, *blue*), and both markers >0 (*right*, *magenta*). Corresponding VU plots are shown in **(C)**, with visibly higher density of p16+ and double-positive (p16+ and p21+) cells. Percent of CDKN2A+, CDKN1A+, and co-expressing (CDKN2A+ CDKN1A+) cells shown for each sample with mean ± standard deviation. Data were obtained from GEO accession GSE265972, profiling chronic wound tissues from four VU patients and five NS samples by 10× single-cell RNA-seq—Liu et al.[23]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NS: p16 +
NS: p21 +
NS: p16 & p21 +
1.9%±2.4
50.7%±14.7
1.4%±1.7
C
VU: p16 +
VU: p21 +
VU: p16 & p21 -
7.7%±5.1
49%±3.6
5.4%±3.9

glucose levels promote the formation of advanced glycation end-products, increase oxidative stress through ROS, and impair innate immune responses.[89,90] Bulk RNA-seq of tissue on the edges of human DFUs identified elevated expression of markers associated with cellular senescence, such as CDKN1A, IL1A, CXCL8, IGFBP2, MMP10, SERPINE1, and TGFA, with concurrent TP53 suppression[49]; however, these findings do not definitively identify senescence since analysis of bulk tissue cannot definitively distinguish senescence from other cellular states (see the "Senescence Marker Limitations and Interpretation in Tissue Studies" section). Protein interaction network analysis revealed disrupted senescence-associated intercellular signaling, highlighting p53 and p21 as potential contributors to chronic wound pathology.[49] However, there were no specific cells identified as being a bona fide source of senescence.

Hyperglycemia-Driven Senescence Mechanisms. Sustained high glucose exerts direct pro-senescent effects on skin cells. It induces mitochondrial dysfunction and oxidative DNA damage, activating the p53/p21 signaling pathway, which enforces proliferative arrest.[91] Fibroblasts isolated from DFUs frequently exhibit features of premature senescence elicited through p53/p21-dependent pathway, suggesting that stress-induced senescence predominates over replicative exhaustion in this context, a finding validated *in vitro*, *in vivo* rat wounding model, and in human samples.[46,47] In addition to fibroblasts, hyperglycemia has also been demonstrated to trigger senescence in endothelial cells and keratinocytes identified by SA-β-gal activity, p21, and proliferative arrest, a finding validated in multiple cells across multiple species.[48,92] These cellular alterations contribute to the persistent inflammatory state observed in diabetic wounds, characterized by elevated levels of cytokines and matrix-degrading enzymes that prevent progression to the proliferative phase of healing.[93–95]

Senescent and dysfunctional cell types in DFUs.

Fibroblast Dysfunction and Senescence in DFUs. Among cell types implicated in DFUs, fibroblasts have been the most extensively characterized. Nearly 30 years ago, fibroblasts isolated from diabetic wounds were reported to exhibit a senescent phenotype, with impaired proliferation and abnormal morphology.[96] Subsequent studies confirmed these observations, demonstrating that DFU fibroblasts demonstrate reduced proliferative capacity, diminished migration, and a failure to differentiate into contractile myofibroblasts—an essential process for wound contraction.[41,97–100] Premature senescence in these cells has been validated by numerous markers, including elevated expression of p16, p21, and p53; increased SA-β-gal activity; and secretion of inflammatory mediators consistent with the SASP profile.[41–43]

Functionally, fibroblasts and myofibroblasts derived from DFUs are characterized by altered ECM profile[101] and exhibit other changes in secretory phenotype. Compared with fibroblasts from healthy tissue, they express lower levels of key chemokines and cytokines such as IL-8, MCP-1, and SDF-1, and upregulated CXCL1 in senescent fibroblasts, which compromises communication with immune cells and disrupts angiogenesis.[102–105] A single-cell analysis of DFUs uncovered two fibroblast subtypes with distinct distributions across wound states: fibroblasts enriched in healing ulcers (HE-Fibro) were characterized by elevated ECM remodeling activity and pro-repair signaling, whereas fibroblasts in nonhealing DFUs demonstrated increased expression of MMPs and inflammatory mediators overlapping with components of SASP, as well as complement activation pathways.[27] These deficits collectively impair tissue remodeling and contribute to wound chronicity. Mechanistic studies have identified pathways involved in altered fibroblast phenotype in DFUs; for instance, pyruvate dehydrogenase kinase 4 (PDK4) has been implicated in fibroblast senescence under hyperglycemic conditions.[43] Inhibition of PDK4 in both the mouse wound model and the human fibroblast culture model was found to reverse senescence features and promote healing, suggesting it as a potential therapeutic target.[43] These findings suggest that a subset of fibroblasts in chronic wounds may adopt a stable transcriptional state characterized by inflammatory signaling and matrix degradation, which may contribute to maintaining the nonhealing wound microenvironment.

Keratinocyte Dysfunction. DFUs typically feature a thickened wound edge composed of keratinocytes that proliferate but fail to migrate, contributing to a persistent open wound.[106,107] Although the involvement of fibroblast senescence is well established, the specific contribution of keratinocyte senescence in diabetic wounds remains underexplored. However, recent scRNA-seq has uncovered unique keratinocyte subtypes within DFU tissues.[108] This study identified a population termed "diabetes-associated keratinocytes," characterized by downregulation of differentiation-related transcription factors and

upregulation of genes involved in inflammation.[108] Another basal layer keratinocyte subset, labeled BC-2, demonstrated transcriptional signatures enriched in oxidative stress and apoptotic regulators such as TXNIP and IFITM family genes.[108] Although these findings do not definitively indicate senescence, they reflect transcriptional dysregulation consistent with impaired barrier repair. This abnormal balance of persistent proliferation coupled with defective differentiation and migration disrupts epithelial regeneration and perpetuates the ulcer chronicity.

Immune Cell Senescence. The persistent inflammation in diabetic wounds also affects immune cell phenotypes, particularly macrophages. In a normal healing environment, macrophages transition from a pro-inflammatory (M1) to a reparative (M2) phenotype during the resolution phase.[109] In contrast, diabetic wounds accumulate macrophages with features distinct from either classical phenotype. These macrophages express p16 and secrete a SASP enriched in CXCR2 ligands,[44] which can propagate senescence to surrounding cells, including fibroblasts, through p53-dependent pathways.[60] Experimental inhibition of CXCR2 signaling in diabetic mouse wounds was found to reduce senescence burden and enhance healing, underscoring the deleterious role of this feedback loop.[44]

scRNA-seq has enabled high-resolution mapping of cellular diversity within DFUs, highlighting key differences between healing and nonhealing wounds. In a large-scale study profiling over 170,000 cells from DFU wound beds, surrounding skin, and blood, Theocharidis *et al*. identified unique cell clusters associated with the diabetic wound niche. Notably, their analysis revealed a paradoxical increase in M1-polarized macrophages in healing DFUs relative to nonhealing DFUs, suggesting that a finely tuned inflammatory response is important in the context of wound healing.[27] Therefore, although chronic inflammation is widely implicated in diabetic wound pathology,[110,111] these scRNA-seq data[27] challenge the notion that M1 macrophages are solely detrimental, suggesting that early pro-inflammatory responses may be required for effective resolution. It is important to note that although macrophage activation has historically been described using the M1/M2 polarization framework, this binary classification is now recognized as an oversimplification, as macrophages exhibit a spectrum of activation states shaped by the wound microenvironment.[112,113] However, the M1/M2 framework remains useful for describing broad functional polarization states.

Beyond macrophages, emerging data suggest broader immune alterations in the diabetic cellular environment. For example, peripheral blood analyses from individuals with prediabetes revealed an increase in senescent $CD8^+$ T cells, identified by $CD28^-$ $CD57^+$ (indicating replicative exhaustion), characterized by impaired glucose metabolism, elevated pro-inflammatory cytokine expression, and increased oxidative stress.[114] While their exact role in DFU pathogenesis remains unclear, these findings suggest that systemic immune senescence may contribute to impaired wound responses in diabetes since chronic hyperglycemia, oxidative stress, mitochondrial dysfunction, and persistent inflammatory signaling—the key pathophysiological mechanisms in diabetes—promote DNA damage responses and pro-inflammatory cytokine production, which are mechanisms implicated in both senescence induction and delayed wound repair. However, direct identification and characterization of senescent immune cell populations within diabetic chronic wounds remain limited, and most evidence is derived from peripheral blood analyses, *in vitro* models, or indirect marker expression. Further studies using cell type–specific and functional approaches will be necessary to define the contribution of immune cell senescence to impaired healing in diabetic wounds.

Endothelial Cell Dysfunction and Impaired Angiogenesis. Deficient neovascularization is a hallmark of impaired healing in DFUs.[115,116] This has been substantiated by both bulk and scRNA-seq studies of human dermal microvascular endothelial cells (HDMECs) derived from DFU tissue. Compared with endothelial cells from healthy individuals, DFU-HDMECs exhibited severely reduced angiogenic potential, including diminished tube-forming capacity and downregulation of pro-angiogenic gene sets.[117] Pathway enrichment analyses from independent reevaluation of the scRNA-seq data revealed that vascular endothelial cells from healing DFUs displayed increased activity of the NF-κB signaling pathway,[118] a central regulator of inflammation and a key modulator of the SASP.[119] These findings indicate a more nuanced role of NF-κB, potentially contributing to beneficial immune activation rather than persistent inflammation, although the downstream consequences on senescence and wound repair remain to be fully clarified. Additional analyses of endothelial cells from DFU scRNA-seq datasets revealed that nonhealing wounds

exhibit significant suppression of genes involved in inflammation-mediated angiogenesis, such as CCND1, ENO1, HIF1α, MMP2, and SERPINE1, relative to healing counterparts.[118] In addition, pathways associated with ECM–receptor interactions and IL-17 signaling, a factor implicated in wound chronicity, were also inhibited in vascular endothelial cells from nonhealing DFUs.[118,120] These findings point to a dual dysfunction in inflammatory and angiogenic signaling that likely underlies the poor vascular response and delayed healing seen in chronic diabetic wounds. However, despite these transcriptomic and functional abnormalities, endothelial cell senescence has not been definitively demonstrated in chronic wounds, as most studies have not directly assessed canonical senescence markers or performed functional validation specifically within endothelial cell populations, highlighting a critical gap and an important opportunity to define the role of endothelial senescence in impaired wound repair.

Overall, the evidence suggests that senescence in DFUs involves multiple cell types and is characterized by markers such as p16, p21, p53, SA-β-gal, pro-inflammatory cytokines (*e.g.*, IL-1A, CXCL8), matrix-degrading enzymes, and impaired proliferative capacity, although definitive cell-specific identification remains limited in many studies. While these findings support the involvement of fibroblast, keratinocyte, and potentially endothelial and immune cell senescence in DFUs, most evidence is derived from correlative markers, often without multimarker confirmation. Functional validation *in vivo*, such as senolytic or senomorphic interventions or lineage tracing, is limited, and causality remains to be established.

Cellular senescence in VLUs

Evidence for senescence in VLUs includes observations from human biopsy studies, transcriptomic analyses of human wound tissues (Fig. 4), experimental animal models, and *in vitro* studies of fibroblast dysfunction.

Venous ulcer pathophysiology and senescence-inducing mechanisms. VLUs typically arise from chronic venous insufficiency, a condition marked by sustained venous hypertension resulting from dysfunctional venous valves and retrograde blood flow.[121,122] This prolonged elevation in venous pressure leads to microvascular injury, and the associated endothelial dysfunction and increased vascular permeability facilitate leukocyte adhesion and infiltration into the skin.[123] Once recruited, these immune cells, along with activated endothelial cells, release pro-inflammatory mediators and proteolytic enzymes, such as MMPs, which degrade ECM components and compromise tissue integrity.[121,124,125]

Simultaneously, venous stasis and capillary leakage promote the extravasation and degradation of red blood cells, leading to local iron accumulation. Iron overload in VLU tissue has also been implicated in immune dysregulation and impaired wound healing in both human studies and murine wounding models of iron overload.[45,126] For example, iron-mediated activation of M1 macrophages can result in excessive production of TNF-α and hydroxyl radicals, further reinforcing inflammatory signaling and promoting senescence in neighboring fibroblasts through p16-dependent pathways.[45] The finding that unrestrained pro-inflammatory M1 macrophages with iron accumulation are present in VLUs was mechanistically validated in a murine model of iron overload, which connected iron overload-induced senescence with impaired wound healing.[45]

This iron acts as a catalyst for ROS generation, intensifying oxidative stress and sustaining inflammation within the tissue.[45,121] The result is a chronically inflamed, protease-rich environment that disrupts the normal wound healing trajectory and prevents tissue regeneration.[82] Elevated oxidative stress is in fact a hallmark of VLU pathology, driven by persistent inflammation and vascular congestion causing iron deposition followed by ROS generation through the Fenton reaction. Excessive ROS production leads to cumulative cellular damage, including DNA damage, which can drive dermal fibroblasts and other resident cells into a senescent state.[65,69,127,128] Rather than undergoing apoptosis or repairing damaged tissue, these senescent cells remain metabolically active but nonproliferative, contributing to a dysfunctional healing environment.[10,51]

Thus, the convergence of oxidative damage, iron deposition, and chronic inflammation plays a central role in promoting senescence and maintaining the nonhealing state characteristic of VLUs.

Senescent fibroblasts and their role in impaired healing in VLUs. VLUs were among the first chronic wound types where fibroblast senescence was identified as a contributing factor to poor healing. Fibroblasts isolated from the margins of VLUs exhibit markedly reduced proliferative capacity compared with cells derived from adjacent, unaffected skin in the same patient.[52] These cells tend to be enlarged and display elevated SA-β-gal activity. In addition, they abnormally produce matrix components such as cellular fibronectin, which is

now considered part of the senescence signature.[52,129] As ulcer duration increases, the burden of senescent fibroblasts in the wound bed also rises, implicating a link between prolonged wound existence and progressive accumulation of senescent cells, as was demonstrated by studies of human biopsies, which relied on SA-β-gal staining to identify senescent cells.[51] Clinically, the bases of VLUs often appear fibrotic, a condition likely driven by persistent SASP activity, which promotes proteolytic breakdown and inhibits effective tissue regeneration. Mendez *et al*. demonstrated that venous ulcer wound fluid and pro-inflammatory cytokines, particularly TNF-α and TGF-β1, reduce proliferation and increase SA-β-gal activity in neonatal fibroblasts, suggesting that ulcer-associated inflammatory mediators can induce a senescence phenotype.[53] The same group demonstrated that fibroblasts from the distal lower limbs of patients with venous reflux, even without ulcers, exhibited increased SA-β-gal activity, elevated fibronectin protein and mRNA, and reduced proliferation compared with proximal fibroblasts, suggesting region-specific senescence precedes ulcer formation.[130,131]

Fibroblasts in venous ulcers not only lose their ability to deposit organized ECM but also actively contribute to its degradation. Instead of producing healthy collagen, they generate high levels of matrix-degrading enzymes, tipping the balance away from tissue repair.[132] Evidence from chronic human wound studies, including those involving VLUs, reveals an inverse relationship between senescent cell prevalence based on SA-β-gal staining and collagen density in the dermis: in wounds with elevated SA-β-gal activity, collagen content was significantly diminished.[82] This study utilized second-harmonic generation (SHG) imaging to assess collagen architecture in VLU tissue and paired this with senescence detection using SA-β-gal staining. Areas with sparse collagen fiber networks, as visualized by SHG, overlapped with regions of intense X-gal staining, suggesting spatial co-localization of reduced matrix integrity and senescent cell presence. The authors proposed this combined imaging strategy as a noninvasive means of identifying senescent cell-rich wound regions, potentially offering predictive value regarding healing outcomes without requiring biopsy.[82]

Immune dysfunction in VLUs. Innate immune dysregulation also plays a role in venous ulcer pathology. Neutrophils within VLU tissue have been reported to persist for prolonged periods of time and undergo excessive degranulation.[133] Elevated levels of neutrophil-derived enzymes, such as neutrophil elastase and various MMPs, have been detected in VLU wound fluid, implicating these cells in sustained tissue damage.[134–136] These enzymes not only degrade ECM proteins but can also inactivate critical growth factors such as VEGF.[137] In these settings, chronic neutrophil activity drives continuous proteolysis and inflammation, preventing the wound from transitioning to a reparative state and contributing to the chronicity of the ulcer.[134–136]

Overall, the evidence for specific senescence in VLUs is the strongest among chronic wound types and includes markers such as SA-β-gal activity, p16, p21, p53, fibronectin expression, and altered secretory profiles (*e.g.*, elevated plasmin, plasminogen activator inhibitor-1, and TGF-β1), with validation across human biopsy studies, fibroblast culture models, and mechanistic murine experiments. However, although VLUs have some of the strongest evidence for fibroblast senescence among chronic wound types, many studies still rely on single-marker approaches, which should be interpreted cautiously as discussed in the "Senescence Marker Limitations and Interpretation in Tissue Studies" section. Functional evidence directly linking senescent cell clearance to improved healing in patients with VLU is lacking, and research aimed at further functional confirmation is needed.

Cellular senescence in PUs

Current knowledge regarding senescence in PUs derives from human tissue analyses, single-cell transcriptomic studies, and *in vitro* investigations.

Pathophysiological mechanisms and senescence triggers in PUs.

Ischemia–Reperfusion Injury and Oxidative Stress. PUs develop when sustained mechanical pressure over bony prominences, such as the sacrum or heels, restricts local blood flow, leading to prolonged ischemia.[138] Once the pressure is alleviated, the reintroduction of oxygenated blood initiates a burst of ROS. This ischemia–reperfusion process, particularly with repeated cycles, leads to mitochondrial damage and the release of cytochrome c, promoting oxidative stress and DNA damage.[139,140] Such conditions create a strong pro-senescent environment through cumulative mitochondrial dysfunction and the DNA damage response.

Neutrophil Involvement and Systemic Aging Factors. The cellular injury in PUs often extends beyond the epidermis into deeper tissue layers.[141] Necrotic cells resulting from ischemia release danger signals that attract neutrophils, which intensify

the inflammatory response.[140,142] When this inflammation becomes chronic (due to ongoing pressure or secondary infection), it may drive surviving cells in the wound bed into senescence.[15] In addition, PUs are more frequent in elderly patients, who are predisposed to impaired healing due to systemic aging-related changes such as immune dysregulation (immunosenescence), nutritional deficiencies, and elevated baseline levels of senescent cells in skin tissue.[15,143–145] These age-related factors further exacerbate the local pro-senescent wound environment and hinder recovery.

Senescent fibroblasts in PUs. Direct evidence of senescent fibroblasts in PUs was reported by Vande Berg *et al*., who isolated cells from the center of chronic PUs and found them incapable of proliferation *in vitro*, exhibiting fewer than 0.5 population doublings over the course of a week.[55] These fibroblasts also expressed "terminin," a cytoplasmic protein associated with senescence, both in culture and within tissue sections, confirming their senescent status.[55,146] In contrast, fibroblasts obtained from the ulcer margins or nearby intact skin retained proliferative ability, indicating that senescence is spatially concentrated in regions of maximal tissue damage.[55] This pattern resembles observations in venous ulcers, where chronic injury leads to a core region of nonproliferative, senescent fibroblasts within the wound bed.[147] Another study demonstrated that fibroblasts from PU beds exhibited significantly reduced replicative capacity, increased SA-β-gal positivity, and elevated secretion of plasmin, plasminogen activator inhibitor-1, and TGF-β1 compared with fibroblasts from adjacent normal skin, indicating a senescent phenotype with an altered proteolytic profile.[54]

The extent of fibroblast senescence appears to vary between patients, with some ulcers containing a higher proportion of senescent cells than others, potentially reflecting differences in systemic contributors such as age, immune status, or comorbidities.[55] In a broader analysis of chronic wounds across different etiologies, Lim *et al*. quantified senescent cell burden using SA-β-gal staining and evaluated its association with collagen deposition. Their findings revealed a consistent inverse relationship across PUs, DFUs, and VLUs: wounds with a greater number of senescent cells contained less collagen and exhibited poorer healing.[82] While the limitation of this study is reliance on SA-β-gal staining only to detect senescent cells, these results suggest that fibroblast senescence may be a common pathological feature underlying impaired regeneration in diverse types of chronic wounds.

Melanocyte-driven inflammation in PUs. Recent scRNA-seq analysis comparing acute wounds and chronic PUs has revealed profound alterations in intercellular signaling dynamics at the wound edge of PUs. One notable finding is the heightened activity of protease-activated receptor (PAR) pathways.[24] In the chronic wound context, both proteases and their corresponding receptors are substantially upregulated, emphasizing that PUs represent a distinct inflammatory environment rather than simply a stalled acute wound. These aberrant signaling patterns suggest that PUs involve active and pathogenic remodeling of cellular crosstalk mechanisms, with PAR-associated axes emerging as key contributors to sustained inflammation.[24]

An unexpected outcome of this study was the identification of melanocytes as active participants in chronic wound inflammation. Unlike their typical role in melanin production for ultraviolet protection, melanocytes in PUs were found in increased numbers and displayed a pro-inflammatory secretory profile. Specifically, they produced cathepsin G (CTSG), a protease more commonly linked to neutrophil function.[24] Melanocyte-derived CTSG activates PAR-2 on neighboring cells, initiating downstream inflammatory signaling that leads to cytokine release and tissue stress.[148–151]

Although melanocytes in these wounds have not been definitively characterized as senescent, their inflammatory phenotype aligns with features of the SASP, where cells that are typically homeostatic adopt a pathological, tissue-damaging role. The CTSG–PAR-2 axis driven by melanocytes exemplifies how chronic injury may reprogram otherwise noninflammatory cells into amplifiers of wound pathology.[24,152] Given the established role of excessive protease activity and inflammation[152] in PU progression, this pathway presents a potential therapeutic target. Modulating melanocyte-derived proteolytic signaling could help reduce the inflammatory burden and support more effective wound healing. Further studies are warranted to determine whether these melanocytes also express canonical senescence markers and to explore strategies aimed at modifying their secretome. Importantly, while not in the context of wounding, senescent melanocytes, identified by p16 expression, HMGB1 loss, and telomere dysfunction, have been demonstrated to impair keratinocyte proliferation through CXCR3-mediated ROS and contribute to epidermal thinning in 3D human skin equivalents.[153] These senescent melanocytes exhibit specific SASP profile (RANTES and IP-10 [CXCL10] were significantly increased,

while Gro-α and VEGF were significantly downregulated compared with nonsenescent controls), and senolytic removal of senescent melanocytes prevented this paracrine inhibition of keratinocyte growth, supporting a functional role for melanocyte senescence in skin homeostasis. Although these findings have not yet been confirmed in chronic wound tissue, they suggest that melanocyte senescence could influence re-epithelialization through similar paracrine mechanisms.[153]

Melanocytes reside within a vascularized stromal niche and are influenced by signals from endothelial, immune, and stromal cells. Impaired vascular function, inflammation, and ECM alterations in chronic wounds may therefore also influence melanocyte function through shared microenvironmental mechanisms. However, the extent to which vascular and stromal dysfunction directly contributes to melanocyte alterations in chronic wounds remains unclear and represents an important area for future investigation.

Overall, the evidence for senescence in PUs is limited and has not definitively demonstrated senescence marker expression beyond reduced proliferative capacity and indirect indicators such as SA-β-gal staining and terminin, without confirmation of canonical markers such as p16 or p21. No studies to date have functionally tested whether targeting these cells improves healing in PU models; therefore, their causal role remains to be determined.

Clinical implications of senescence in chronic wounds

Although the concept of cellular senescence has generated considerable interest in wound biology, its clinical interpretation remains challenging. One major limitation is that the markers most commonly used to identify senescent cells, including senescence-associated β-galactosidase (SA-β-gal), p16 (CDKN2A), and p21 (CDKN1A), lack specificity when used individually. These markers are frequently interpreted as indicators of senescence in tissue studies, yet each can also be expressed in nonsenescent contexts such as transient stress responses, inflammation, quiescence, or differentiation. Consequently, detection of any single marker alone does not establish the presence of bona fide senescent cells.[40]

SA-β-gal activity is widely used because senescent cells exhibit increased lysosomal content, which produces strong β-galactosidase activity detectable at pH 6. However, this enzymatic activity is not unique to senescence and can also occur in other conditions characterized by increased lysosomal activity or cellular stress, limiting its specificity as a standalone marker.[40]

Similarly, the cyclin-dependent kinase inhibitors p16 and p21 are commonly used indicators of senescence because they enforce cell-cycle arrest. Nevertheless, these proteins are also expressed in a range of physiological and pathological contexts unrelated to senescence, including immune activation and transient cell-cycle arrest states. As a result, increased p16 or p21 expression alone cannot unambiguously identify senescent cells.[40]

For these reasons, current consensus in the senescence field emphasizes the need for multiparameter approaches that combine several lines of evidence, such as cell-cycle arrest markers, DNA damage indicators, altered chromatin structure, and SASP factors, rather than reliance on a single biomarker.[40]

From a clinical perspective, this limitation has important implications. At present, senescence markers cannot be used as diagnostic tools or treatment selection criteria in chronic wound care. Most available evidence remains correlative and derived from experimental models or descriptive tissue studies. However, emerging technologies such as scRNA-seq, spatial transcriptomics, and integrated multiomic analyses are beginning to provide higher-resolution insights into senescence-associated cellular states within human wound tissues. As these approaches mature, they may help identify biologically distinct wound microenvironments and potentially enable biomarker-guided therapeutic strategies targeting senescence-associated pathways.

In the future, companion diagnostic approaches may help translate these insights into clinical practice. Quantification of senescence burden in wound tissues, for example, through spatial assessment of p16-positive cells or approaches combining senescence markers with structural imaging methods such as SHG-based collagen mapping, could potentially aid in identifying wounds enriched for senescence-associated pathology. In addition, monitoring soluble biomarkers associated with the SASP in wound fluid may provide a means to track treatment response in therapies targeting senescence pathways. Integration of such parameters with routine clinical tissue sampling or wound fluid analysis may ultimately enable stratification of patients and guide the application of senescence-modulating therapies.

Potential therapeutic approaches

Senolytics: Preclinical data in wound healing models. Senolytics are agents that selectively eliminate senescent cells by disrupting the survival mechanisms that protect them from apoptosis.[154] In aged mouse skin, topical navitoclax (ABT-263

—BCL-2 family inhibitor) reduced senescence markers and primed the tissue for improved repair, with accelerated wound closure following pretreatment.[155] Similar benefits were observed in diabetic mouse models, where pharmacologic clearance of senescent cells enhanced re-epithelialization and closure.[42] In a recent preclinical DFU model, fisetin (a naturally occurring flavonoid targeting multiple senescence-associated pathways such as BCL-2, PI3K/AKT, p53, and NF-κB[154]) improved healing and modulated inflammatory and oxidative pathways in diabetic rats, although senescence markers and the senolytic action of fisetin were not evaluated in these studies.[156,157] Although wound-specific clinical trials of systemic senolytics are not yet available, dasatinib, quercetin, and fisetin have entered randomized clinical testing for other indications and have reduced senescence burden markers in early human studies.[158] Interestingly, while dasatinib plus quercetin (BCL-2 family inhibitors) accelerated epigenetic aging in humans, the addition of fisetin ameliorated this effect, indicating substantial differences in the mechanistic action of different senolytics and, therefore, different clinical outcomes and effects on biomarkers of aging.[159] Together, these data support the feasibility of senolytic approaches while underscoring the need for studies aimed at elucidating the differential effects of distinct senolytic molecules and wound-focused clinical studies.

Senomorphic agents in wound healing. Senomorphics are agents that reduce the inflammatory and other SASP-associated effects of senescent cells without eliminating the cells themselves.[154] Recent studies have identified metformin and resveratrol as topical senomorphic agents capable of enhancing cutaneous wound healing in young rodents, with metformin exerting more pronounced effects on epidermal regeneration, follicular architecture, and collagen deposition.[14] These compounds can be classified as senomorphics since they attenuate the deleterious effects of SASP without eliminating senescent cells, thereby promoting tissue repair while preserving homeostatic functions of transient induction of senescence. Rapamycin, a well-known mTOR inhibitor, also exhibits senomorphic properties; in addition to extending lifespan, short-term treatment in elderly individuals has been shown to activate type I interferon signaling and improve immune responses,[14] and rapamycin has been demonstrated to reduce senescent dermal fibroblasts in human skin presumably through inhibiting SASP production.[160] While most of the current evidence linking senescence to chronic wounds is correlative, intervention studies using senotherapeutic agents provide functional support that senescent cells can play a causal role in impaired healing. Collectively, these data support the role of senomorphics as a potential therapeutic strategy to modulate chronic senescence signaling and enhance wound healing outcomes in aging. However, the dual nature of senescence in wound biology demands more precise mechanistic dissection.

Delivery strategies, timing of intervention, and future directions. While some of the studies on senotherapeutics in wound healing focused on systemic administration, the local delivery route is a promising strategy for chronic wounds because it concentrates drugs at the pathology site and limits systemic exposure.[161] Effective targeting of senescent cells in chronic wounds presents unique delivery challenges, as senescent fibroblasts, keratinocytes, and immune cells reside within a complex and poorly vascularized tissue environment. Systemic administration may result in limited drug penetration into the wound bed and increased risk of off-target effects. Local delivery strategies therefore offer a potential advantage by enabling sustained exposure within the wound microenvironment, where senescent cells contribute to impaired healing through persistent SASP signaling.

Senotherapeutics have been incorporated into hydrogels and nanoparticle-reinforced dressings that enable sustained, moisture-balanced release and protection from proteolytic wound fluids. For example, navitoclax can be loaded into polymer–nanoparticle hydrogels with selective senescent cell clearance *in vitro*,[162] and metformin has been delivered using conductive or thermo-responsive hydrogels and nanofiber dressings to enhance angiogenesis and re-epithelialization in diabetic and burn wounds.[163,164]

Hydrogel-based delivery systems may be particularly suitable for targeting senescence-associated mechanisms in chronic wounds because their highly hydrated polymer networks enable sustained local release and prolonged retention of therapeutic agents within the wound bed.[165] In addition to controlled drug delivery, hydrogels provide a biocompatible microenvironment that supports cell adhesion, migration, and proliferation by mimicking key structural and biochemical features of the ECM.[165] This scaffold-like function may be especially relevant in chronic wounds, where senescent fibroblasts and keratinocytes

exhibit impaired proliferative capacity and contribute to ECM degradation and persistent inflammation. Hydrogels can also maintain a moist wound environment, promote angiogenesis, and reduce inflammatory signaling, all of which may help restore tissue repair processes disrupted by senescence-associated dysfunction. Furthermore, their ability to conform closely to wound contours enhances tissue contact and local therapeutic exposure while minimizing systemic distribution.[165] However, despite these advantages, the extent to which specific hydrogel-based delivery systems can selectively target senescent cell populations or modulate defined senescence-associated pathways remains unclear and represents an important area for future investigation.

From a therapeutic perspective, the timing of senescence-targeting interventions may be critical. In acute wounds, senolytic therapy may be undesirable because transient senescence can support tissue remodeling and limit fibrosis during normal repair.[22] In contrast, in established chronic wounds, where senescent cells are thought to persist and contribute to sustained inflammation, matrix degradation, and impaired regeneration, senolytics may be more beneficial. In wounds with delayed healing or at risk of chronicity, senomorphic approaches may be preferable because they could suppress deleterious SASP signaling while preserving potentially beneficial short-term functions of senescence. Although this framework remains hypothetical and requires experimental validation, it may help guide future studies aimed at optimizing the timing of senescence-targeting interventions in wound healing. Rigorous biomarker-guided trials are needed to optimize these parameters.

Future research should focus on delineating how senescence programs vary across fibroblast and immune subtypes, and how they evolve dynamically during different wound stages and influence the wound matrix. Spatial mapping technologies and longitudinal single-cell profiling may help resolve whether senescent cell depletion or rescue of their signaling function is more appropriate in specific contexts. Furthermore, distinguishing between acute, programmed upregulation of senescence markers in the context of wound healing and pathological senescence may guide interventions toward temporally appropriate modulation rather than blanket inhibition. Ultimately, therapeutic approaches will require precision modulation of this pathway to retain its beneficial effects while mitigating chronic inflammation and tissue damage. In addition, spatial transcriptomic analyses can reveal where senescence-associated cellular states accumulate within the wound bed, which may

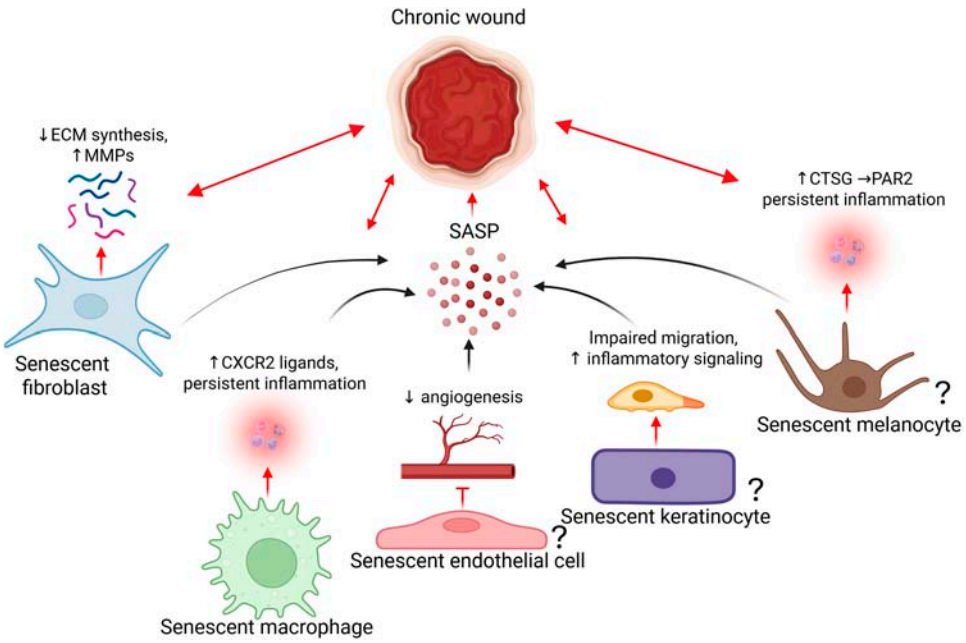

Figure 5. Senescent cell types and their functional impact in chronic wounds. Senescent fibroblasts, macrophages, and potentially keratinocytes, endothelial cells, and melanocytes accumulate in chronic wounds and contribute to a sustained SASP. Senescent fibroblasts exhibit reduced ECM synthesis and increased MMP production; senescent macrophages secrete CXCR2 ligands that reinforce sustained inflammation; potentially senescent keratinocytes are characterized by impaired migration and upregulated inflammatory signaling; hypothesized senescent endothelial cells impair angiogenesis; melanocytes secrete, which activates PAR-2 and drives inflammation. SASP factors from senescent cells drive a feedback loop that maintains chronic inflammation and tissue degradation, perpetuating nonhealing wounds.[38–40]

eventually help guide targeted interventions such as selective debridement of pathological tissue.

SUMMARY

Cellular senescence plays a nuanced and context-dependent role in wound healing. Transient accumulation of cells expressing senescence markers can support regeneration by contributing to tissue remodeling and immune orchestration, particularly in young organisms.[22,143] However, in chronic wounds and aging, persistent accumulation of senescent cells leads to a pro-inflammatory and degradative tissue microenvironment that impairs healing.[10,11] Fibroblasts and myeloid cells are the primary senescent populations implicated in delayed wound closure; however, other senescent cell types may also contribute to chronic wound persistence, although direct validation is needed (Fig. 5).

Despite the significant progress made, several knowledge gaps remain, and future research directions are clearly indicated. Across chronic wound types, the majority of studies rely on correlative or indirect markers of senescence, often in limited sample sets, and few employ functional assays to establish causality. Greater use of multimarker panels in conjunction with senolytic or senomorphic interventions, lineage tracing, and *in vivo* clearance assays will be critical to determine whether these cells are drivers, bystanders, or context-dependent modulators of impaired healing.

A more comprehensive characterization of senescent cell heterogeneity across different types of chronic wounds and in human versus animal models is needed. Longitudinal single-cell studies that track the evolution of senescent cell populations during the transition from acute to chronic wounds would provide invaluable insights into the dynamics of senescence in this context. Such studies may also enable functional classification of senescent cells based on their secretory profiles, matrix remodeling activity, or immunomodulatory effects, allowing researchers to distinguish pathogenic versus potentially beneficial subtypes and guide targeted interventions. The integration of multiomics approaches, combining scRNA-seq with techniques such as scATAC-seq, proteomics, metabolomics, and spatial transcriptomics, will be crucial for a holistic understanding of the epigenetic regulation and spatial organization of senescent cells within the wound. A key challenge will be defining robust, context-specific senescence signatures from these complex datasets. Machine learning approaches, including supervised classifiers trained on experimentally validated senescent cell populations and unsupervised clustering to identify novel senescent states, hold promise for distinguishing transient from persistent senescence. Integrative frameworks such as multimodal deep learning can merge transcriptomic, epigenomic, proteomic, and metabolic data to refine senescence classification, predict functional consequences, and inform cell type–specific therapeutic targeting in chronic wounds.

Finally, understanding the signaling crosstalk between senescent cells and their microenvironment, including immune cells, ECM components, and epithelial cells, using single-cell analysis, is

KEY FINDINGS

- Senescent fibroblasts and immune cells are consistently associated with impaired healing in DFUs, VLUs, and PUs.
- Enhanced SASP activity (*e.g.,* MMPs, cytokines) correlates with ECM degradation and chronic inflammation across wound types.
- Functional interventions (*e.g.*, senolytics and senomorphics) in preclinical models demonstrate proof-of-concept for cellular senescence causality in impaired healing.
- Multiomic integration and machine learning approaches are emerging tools to distinguish pathologic versus reparative senescence, informing future therapeutic timing and specificity.

TAKE-HOME MESSAGES

- Senescent cells stop dividing but remain metabolically active, releasing inflammatory and tissue-degrading mediators known as the SASP.
- Emerging evidence suggests that chronic wounds such as DFUs, VLUs, and PUs are associated with persistent cellular senescence.
- While the short-term senescence response is hypothesized to be a physiological mechanism facilitating normal wound healing, its prolonged presence disrupts tissue repair and promotes chronic inflammation.
- Fibroblasts and immune cells are the primary senescent cell types identified to date, contributing to impaired healing in chronic wounds.
- Single-cell and spatial transcriptomics have revealed senescent states and disrupted cellular communication in chronic wound tissue.
- Therapies that reduce or modulate senescent cells improve healing in animal models of diabetic and aged wounds.
- Better tools to identify and target harmful senescent cells may lead to more effective therapeutic approaches for chronic wounds.

essential for understanding why senescent cells persist in these wounds and how the immune system might be harnessed to promote their clearance and facilitate healing.

INNOVATION

Despite substantial advances in wound care, the specific role of cellular senescence in chronic nonhealing wounds remains poorly understood. Our review integrates *in vitro* and *in vivo* data and emerging single-cell and spatial omics with functional senotherapeutic studies to clarify the contribution of senescent cells across DFUs, VLUs, and PUs. We highlight preclinical evidence where senolytic and senomorphic interventions ameliorated senescence-induced healing deficits. This synthesis offers a mechanistic framework with direct clinical relevance for future biomarker-driven, targeted wound therapies.

AUTHORS' CONFIRMATIONS

All authors have reviewed and approved the final version of this article. Each author contributed to the conception, drafting, and revision of the work.

AUTHOR DISCLOSURE AND GHOSTWRITING STATEMENT

The authors declare no competing interests. The content of this article was written solely by the authors listed. No ghostwriters were involved in the preparation of this article.

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Abbreviations and Acronyms

CXCL = C-X-C motif chemokine ligand
CTSG = cathepsin G
DFU = diabetic foot ulcer
ECM = extracellular matrix
HDMECs = human dermal microvascular endothelial cells
IL = interleukin
mRNA = messenger RNA
MMP = matrix metalloproteinase
p21 = cyclin-dependent kinase inhibitor 1A (CDKN1A)
p16 = cyclin-dependent kinase inhibitor 2A (CDKN2A)
PAR = protease-activated receptor
PDK4 = pyruvate dehydrogenase kinase 4
PU = pressure ulcer
ROS = reactive oxygen species
SASP = senescence-associated secretory phenotype
SHG = second-harmonic generation
scRNA-seq = single-cell RNA sequencing
TGF = transforming growth factor
TNF-α = tumor necrosis factor alpha
UMAP = Uniform Manifold Approximation and Projection
VLU = venous leg ulcer