Iron and iron-dependent reactive oxygen species in the regulation of macrophages and fibroblasts in non-healing chronic wounds

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ABSTRACT

Chronic wounds pose a stern challenge to health care systems with growing incidence especially in the aged population. In the presence of increased iron concentrations, recruitment of monocytes from the circulation and activation towards ROS and RNS releasing M1 macrophages together with the persistence of senescent fibroblasts at the wound site are significantly enhanced. This unrestrained activation of pro-inflammatory macrophages and senescent fibroblasts has increasingly been acknowledged as main driver causing non-healing wounds. In a metaphor, macrophages act like stage directors of wound healing, resident fibroblasts constitute main actors and increased iron concentrations are decisive parts of the libretto, and – if dysregulated – are responsible for the development of non-healing wounds. This review will focus on recent cellular and molecular findings from chronic venous leg ulcers and diabetic non-healing wounds both constituting the most common pathologies often resulting in limb amputations of patients. This not only causes tremendous suffering and loss of life quality, but is also associated with an increase in mortality and a major socio-economic burden. Despite recent advances, the underlying molecular mechanisms are not completely understood. Overwhelming evidence shows that reactive oxygen species and the transition metal and trace element iron at pathological concentrations are crucially involved in a complex interplay between cells of different histogenetic origin and their extracellular niche environment. This interplay depends on a variety of cellular, non-cellular biochemical and cell biological mechanisms. Here, we will highlight recent progress in the field of iron-dependent regulation of macrophages and fibroblasts and related pathologies linked to non-healing chronic wounds.

1. Introduction

Chronic wounds, by definition, are indolent and do not heal over the course of three months even when subjected to state-of-the-art therapies. Chronic wounds account for 3–6% of total healthcare expenditure in developed countries conservatively estimated $28 billion per year in the American Medicare system [1]. The ongoing demographic change with a persistent increase in life-span [2] will, in perspective, significantly impact on the incidence and outcome of age-related wound healing pathologies [3].

In this review, we summarize recent findings on pro- and anti-oxidative mechanisms relevant for physiological and pathological wound healing involving macrophages and fibroblasts with special emphasis on their cellular and molecular connection to iron metabolism (Fig. 1). We will focus on the consequences of high concentrations of reactive oxygen species in diabetic ulcers and iron overloaded chronic venous leg ulcers with generation of oxidative stress and a hostile micro-environment, eventually breaking down cellular and tissue homeostasis.

The deleterious impact of reactive oxygen species (ROS) will be addressed, in particular of ROS at high concentrations which are major drivers for dysregulated expression and activity of growth factors and extracellular matrix (ECM)-degrading proteolytic enzymes in non-healing wounds. Also the strive and enthusiasm to identify biomarkers indicative of different phases of wound healing with currently little

Abbreviations: CVUs, chronic venous leg ulcers; DFO, deferoxamine; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; FeSOD2, iron SOD2; GPs, glutathione peroxidases; HSCs, hematopoietic stem cells; HIF-1α, hypoxia inducible factor-1 alpha; IFN-γ, interferon-gamma; IL-X, interleukin-X; LPS, Lipopolysaccharide; M1/2Φ, macrophage M1/2 subset; MMPs, matrix metalloproteinases; MnSOD2, manganese SOD2; MSCs, mesenchymal stromal cells; OXPHOS, oxidative phosphorylation; PU, pressure ulcer; RNS, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; TGF-β1, transforming growth factor 1; TNF-α, tumor-necrosis factor alpha

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success will be addressed. In addition, we introduced a chapter on the recently identified adaptive response of mesenchymal stem cells (MSCs) which holds substantial promise to be exploited for the therapy of difficult-to-treat wounds. Finally, we report on senolytic strategies to deplete senescent cells from aged tissues which may also help to improve healing of chronic wounds.

2. Iron - changing paradigms and its relation to tissue repair

2.1. Central role of labile and sequestered iron in physiological and pathological wound healing

2.1.1. Iron in the maintenance of tissue homeostasis

The trace element and transition metal iron is essential for many anabolic processes in cellular proliferation and differentiation [4] depending on the activation of different metalloenzymes [5] like ribonucleotide reductases and DNA glycosylases involved in DNA replication and in DNA repair [6]. Iron is also mandatory for protein synthesis and posttranslational modifications of procollagen essentially required for the stabilization of collagen molecules. In this context, iron – by its impact on the cross-linking prolyl/lysyl hydroxylases and oxidases [7] – is essential for the stability of all connective tissues. Importantly, hemoglobin iron in red blood cells is responsible for oxygen transport and its dissemination and supply to different tissues and organs [8].

Iron usually is sequestered by ferritin, a protein containing 24 subunits, which has the enormous binding capacity for up to 4500 iron atoms [9]. The view on transition metals like iron earlier focused on their ROS generating capacity. Iron-dependent ROS are generated via the Fenton reaction and exert their noxious action, severely damaging macromolecules like DNA, RNA, proteins and lipids. To avoid such deleterious damage, a strict control of free iron is indispensable. In addition to its established role as cofactors in DNA synthesis and repair enzymes, the paradigmatic view for iron [10] currently revamps to its impact on signaling. Interestingly, macrophage polarization from the pro-inflammatory M1 macrophages to the anti-inflammatory M2 macrophages, essential for the switch from the inflammatory phase to the granulation tissue formation phase of wound healing, intimately correlates with the metabolism of heme-iron in involved cells [11]. In skeletal muscle repair, iron recycling by macrophages occurs with iron uptake and storage in macrophages in early phases of wound repair and a ferroportin-mediated export of iron at later time points of tissue repair [12]. Interestingly, iron critically impacts on the differentiation of progenitor cells into myofibers instead of adipose cells.

So called “trigger molecules” most likely open iron-releasing cellular pores [9] and increase the free iron pool further driving the vicious cycle of detrimental tissue damage in non-healing chronic wounds. Although ferritin as iron-binding protein is mainly present within cells [13], iron-less serum ferritin may play a role in neutrophil mediated iron release. Of note, ferritin is destroyed by neutrophil elastase in airway epithelia [14]. Since the persistence of neutrophils in chronic wounds [15] results in increased elastase activity, this proteolytic activity further contributes to enhanced iron concentrations in...
Chronic wounds fail to proceed through the physiological phases of wound healing, but instead are stalled in the early inflammatory phase leading to a destructive biocidal, proteolytic, and oxidative microenvironment. Wound phases include hemostasis for a first provisional coverage of the wound to prevent bleeding and microbial invasion. Thereafter, in the inflammatory phase tissue debris and microbial infection are cleaned off by neutrophils and macrophages. In the granulation tissue phase angiogenesis with the formation of new blood vessels, proliferation of endothelial cells, fibroblasts, and keratinocytes with a permanent coverage of the wound bed by re-epithelialization takes place. During the granulation phase differentiated myofibroblasts contract the wound and finally, in the remodeling maturation phase myofibroblasts and the surplus of resident cells and extracellular matrix disappears by senescence and subsequent removal of senescent cells by the innate immune system. Fine-tuned proteolysis controls the excess of matrix deposition in this phase. Hence, the sequence of distinct wound healing phases in acute wounds is characterized by the activity of specific cell types with a characteristic gene expression profile. Chronic non-healing wounds, by contrast, are stalled in the early inflammatory phase with neutrophils and unrestrained activation of pro-inflammatory M1 macrophages. Chronic wounds fail to proceed to the next wound phases, and thus M1 macrophages cannot be replaced by anti-inflammatory M2 macrophages and cell types of the granulation and remodeling wound phase.

2.1.2. Iron and related oxidative stress

Oxidative stress is the result of an imbalanced generation of oxidants and their insufficient detoxification by enzymatic and non-enzymatic antioxidants [17]. During wound healing, microbicidal generation of oxidants meant to combat infection finally needs to be effectively counterbalanced to start regenerative, tissue-rebuilding cellular processes. Two main mechanisms are involved: First, the clearance of ROS- and RNS-generating cells from wound tissue, and secondly, enhanced activity of ROS-neutralizing enzymes. Here, superoxide dismutases (SOD), catalase and glutathione peroxidases (GPx) play a major role among other antioxidant enzymes [18]. Interestingly, lower organisms like bacteria and higher plants express an iron containing anti-oxidative SOD2. Intriguingly, Ganini et al. described the switch from the anti-oxidative manganese containing MnSOD2 to the iron-containing pro-oxidative FeSOD2 due to the excess of free iron in cell culture or in mice on high iron diet [19]. Even though currently speculation, it is possible that under the conditions of iron overload in chronic wounds like chronic venous ulcers (CVUs) [20–24], the balance between manganese and iron as the cofactor for the SOD2 might be disturbed resulting in enhanced pro-oxidative activity of the FeSOD2. For decades ROS have been approved as damaging oxidants that easily overwhelm the cellular anti-oxidative defense under specific conditions like photo-damage, cancer or aging. Recently, ROS are alternatively regarded as cellular “stress-elicited survival signals” to protect physiology and function at the cellular and molecular level [25]. Its primary function now relates to the induction of stress-response pathways to ensure cellular homeostasis sustaining signaling and effector pathways. It would be important to address what is the net impact of the intricate interplay of secreted factors and ROS, whether wound healing of the skin depends on ROS signaling like in amphibia [26,27], and whether these signals are transported from cell to cell over longer distances within tissues [28].

The impact of the iron-dependent Fenton reaction on fundamentally impairing cellular redox balance is now widely accepted in chronic wounds [22,29]. Direct targeting hydroxyl radicals due to their high reactivity and low diffusion distance [30,31] is difficult in vivo. Few attempts to scavenge hydroxyl radicals in non-healing wounds with dimethyl sulfoxide (reviewed in: [32]) revealed only limited improvement. As stated by Halliwell and Gutteridge [33], interfering with transition metals induced oxidative stress helps to address the redox state of metals, reduces the concentration of accessible metals, and/or quenches generated ROS. To successfully implement this strategy, a deeper knowledge of underlying biological and biochemical processes is urgently required. This would include understanding of time kinetics and localization of oxidative stress and the involved ROS occurring in chronic wounds. Furthermore, novel technologies are mandatory to reliably identify distinct entities of specific reactive oxygen and nitrogen species. Also, tissue distribution and resolution of the expression and activity of anti-oxidative enzymes in wounds still constitute unmet needs. Koskenkorva-Frank et al. reviewed mechanisms related to oxidative and nitrosative stress from iron in more detail [34]. In wound repair, targets of redox imbalance-induced signaling and deleterious pathways still need to be identified to successfully develop and employ therapies targeting redox imbalance.
3. Physiological and pathological wound healing

3.1. Distinct phases in physiological wound healing

In physiological wound healing, wound repair sequentially progresses through four partly overlapping phases including the phase of pro-oxidative inflammation, the phase of granulation tissue formation with a switch of pro-inflammatory M1 to anti-inflammatory M2 macrophages, angiogenesis, as well as proliferation of resident cells which contribute to replenish tissue defects, and the final phase of tissue remodeling. Healing proceeds in a time- and space-wise highly regulated process with cytokines, growth factors and adhesion molecules fine-tuning cell-cell- and cell-matrix-interactions to accelerate wound closure and to avoid infections [35,36] (Fig. 2).

Following initial blood clotting, in the early pro-oxidative inflammatory phase, inflammatory cells, like neutrophils and pro-inflammatory M1 macrophages invade into the damaged tissue to combat bacteria and other microbiota. Tissue debris is removed by proteolytic activity of different cells. Inflammatory neutrophil granulocytes recruited from the circulation and replenished by enhanced release from the bone marrow are the predominant cell type. Neutrophils act microbicidal by increased NADPH-oxidase activity with generation of superoxide anion radicals converted into hydrogen peroxide and hypochlorous acid as well as the formation of neutrophil extracellular traps referred to as expulsion of neutrophil DNA decorated with anti-microbial granules and enhanced phagocytosis of invading microorganisms [37]. As the wound site with disrupted blood vessels is hypoxic, neutrophils and M1 macrophages most likely depend on glycolysis for their energy demands. Thus, the enhanced release of ROS from neutrophils and macrophages rather depends on the membrane bound NADPH oxidase and not on the electron transport (OXPHOS). In general, the innate immune system aims at an efficient defense against pathogens, using recruitment of other immune and non-immune stromal cells during the inflammatory phase. Following neutrophils during the early pro-oxidative inflammatory phase, M1 macrophages significantly contribute to the microbicidal release of superoxide anion radicals and nitric oxide, and – by the generation of peroxynitrite – eventually killing bacteria. Thereafter, macrophages phagocytose bacteria, damaged tissue, and apoptotic neutrophils [38].

Macrophages are cells of the innate immune system that differentiate from circulating monocytes originating from hematopoietic stem cells (HSCs) of the bone marrow. Macrophages have been classified into M1 that are classically activated after stimulation with IFN-γ and LPS, and M2 alternatively activated macrophages after in vitro stimulation with IL-4 and IL-13. M1 macrophages reveal enhanced microbicidal capacity and secrete high levels of the pro-inflammatory cytokines TNF-α, IL-1, IL-6, and IL-23 and increased reactive oxygen radicals, as well as reactive nitrogen radicals to increase their killing activity. By contrast, M2 macrophages suppress pro-inflammatory cytokines, secrete components of the extracellular matrix, and may be essential for late phases of tissue repair [38].

The first response of pro-inflammatory, ROS-releasing M1 macrophages in physiological wound healing is the clearance of bacteria, dead cells and tissue debris by phagocytosis. Of note, phagocytosis of dead or apoptotic neutrophils results in a switch from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages which, in fact, initiates the phase of granulation tissue formation, and by the release of TGF-β1 from M2 macrophages promotes myofibroblast differentiation, matrix deposition and angiogenesis [39–41]. Under conditions of aberrant neutrophil phagocytosis by macrophages – as is the case of CD18 (common chain of β2 integrins) deficient mice and human Leukocyte adherence deficiency-1 patients – a severe delay of wound healing and severe infections occur [42]. TGF-β1, a major driver of myofibroblast differentiation and angiogenesis, apart from being released from M2 macrophages, can also be released from its binding Latency Associated Peptide (LAP) by reactive oxygen species. Accordingly, the phase of granulation tissue formation with myofibroblast differentiation and angiogenesis may also depend on moderate ROS release [43]. M2 macrophages attract and direct remodeling of stromal cells by the release of cytokines and chemokines like IL-6, IL-10, and TGFβ1 that triggers angiogenesis [44]. Under physiological conditions of wound healing the switch from M1 to anti-inflammatory M2 macrophages reliably occurs, while this switch is severely impaired in chronic wounds like diabetic foot ulcers, chronic venous leg ulcers and pressure ulcers, leaving these wounds in a chronic pro-inflammatory state with constant tissue break down and no tendency to heal (see Section 4.2 [22]).

Re-epithelialization of the epidermis with enhanced migration of keratinocytes from the edge of unwounded epidermis occurs during and after the phase of granulation tissue formation. Though unclear in human and mouse skin, taudples reveal enhanced ROS release in the epithelium driving overall wound healing at this stage [26]. Interestingly, enhanced ROS concentrations, particularly hydrogen peroxide and superoxide anion radicals, apparently drive re-epithelialization and stromal cell restoration. Lowering ROS levels, with pharmacological or genetic approaches, reduces cell proliferation and impairs tail regeneration. Sustained increase in ROS levels are required for Wnt/β-catenin signaling and Fibroblast Growth Factor 20 (FGF20) activation which is essential for tail regeneration in tadpoles. The increase in ROS concentrations during this phase was not due to inflammatory cells, at least not in the tadpole amputation model [26,27,45,46].

It remains to be seen, whether a transient increase in ROS concentrations in basal keratinocytes and epidermal stem cells – similarly to what is observed in the amputated tail tadpole model – drives a regenerative phenotype of wound healing via enhanced ROS concentrations in mammals.

The final remodeling and maturation phase with restoration of the physico-mechanical properties of the skin takes several months or years with remodeling of the injured tissue. The surplus of cells due to proliferation of the preceding wound phase is remodeled by the induction of transient cellular senescence. All senescent cells thereafter are removed by macrophages, and most likely also by natural killer cells very similar to remodeling during developmental processes. Thus, senescence serves as a mean to remodel wound tissue [47,48]. It has become clear that transient senescence plays an important role in physiological processes in tissue repair and development [49]. Demaria et al. [50] showed that senescent fibroblasts – as a key event of their senescence-associated secretory phenotype (SASP) – secrete the platelet-derived growth factor PDGF-AA which is responsible for myofibroblast differentiation and accelerated closure of skin wounds. Senescent fibroblasts only transiently occurred in skin wounds and PDGF-AA secretion was an early event [50]. Transient exposure to SASP factors generates a more pro-regenerative environment including induction of stemness and cellular plasticity in epithelial keratinocytes [51]. Interestingly, transient senescence also occurred in birds, mammals, amphibians and fishes in vertebrate development [49].

3.2. The pathophysiology of chronic wounds

In chronic venous leg ulcers, pressure ulcers and diabetic ulcers, representing the most prevailing non-healing wounds worldwide, the wound healing process is stalled and characterized by a persistent M1 macrophage dominated inflammatory phase [52,53] which generates a pro-oxidative hostile microenvironment at the wound site, eventually disturbing wound healing [35,54–56] (Fig. 2). The pathophysiology of chronic wounds is not fully understood. The gap of knowledge still constitutes a major hurdle for the efficient development of novel therapies. Also the identification of biomarkers for chronic wounds which indicate the failure of wounds to proceed through the normal sequence of wound phases is lacking behind the expectations of researchers, industry and clinicians. Even the application of state-of-the-art methods like transcriptomics [57], and proteomics [58] have not yet yielded reliable and valid biomarkers. Among other reasons, this might
be due to the complex and partly overlapping wound phases with the involvement of many different cell types. The development of single-cell analytical techniques for the proteome, methylome and transcriptome might help to solve this demand in future [59]. Systems biology approaches [60] most likely will add new knowledge to foster improved diagnostics and refined therapies.

The incidence of chronic wounds increases with age [3] mainly due to a compromised immune system [61] and aging of the connective tissue including – among other cells [62–64] – a significant accumulation of senescent dermal fibroblasts [65]. Impaired functions of senescent fibroblasts result in the secretion and activation of a variety of matrix degrading metalloproteases among several paracrine and autocrine pro-inflammatory factors which rather enhance inflammation and overall tissue degradation [66,67].

A consistently high concentration of iron was demonstrated in the non-healing CVU wounds or wound fluids as shown by complementary techniques including Prussian blue histochemical staining [22], atom absorption spectroscopy [23], and serum iron concentration [20]. Newly developed and improved methods for metal imaging like synchrotron-based X-ray fluorescence microscopy [68] might in near future allow to dissect specific tissue areas and cells with iron overload within chronic non-healing wound area with or without lipodermatosclerosis in relation to surrounding healthy tissue and cells with a high demand for iron in DNA-synthesis and proliferation. Neither underlying mechanisms nor the origin of iron excess in non-healing skin wounds are clarified in sufficient detail. Furthermore, erythrophagocytosis of damaged red blood cells by macrophages [69], direct release of iron from macrophages [70], oxidative damage or neutrophil elastase activity damaging ferritin molecules [14], and of lipofuscin [71] may contribute to a non-physiologic accumulation of iron, thus, sustaining the hostile microenvironment [56] which prevents healing.

4. Major cellular components and their pathogenic contributions to the hostile microenvironment of chronic wounds

4.1. The macrophage - introductory remarks

Circulating macrophages are immune cells of the innate immune system originating from the bone marrow. Hematopoietic stem cells (HSCs) stepwise differentiate into precursors and into monocytes which – in a final step of lineage decision – develop into circulating macrophages. Distinct tissue resident macrophages including skin dendritic cells, liver Kupffer cells, adipose tissue macrophages and brain microglia cells which originate from the yolk sac or the fetal liver, ultimately reside in different tissues. Macrophages constitute evolutionary ancient cells and are referred to under different names in invertebrates. Initially, they were described in most primitive multicellular organisms like sponges and cnidarians. A phagocytic function already exists in unicellular organisms like amebae [72]. During evolution, distinction between pro-inflammatory M1 “fight” and anti-inflammatory “repair” M2 macrophage was first observed in fish [72]. The complex adaptive immune system later on further developed in vertebrates (Fig. 3). In this regard, macrophages apparently are crucial for the regenerative capacity of organisms endowed with the capacity to fully regenerate lost limbs, like the aquatic salamander axolotl [73,74]. As described above, macrophages and the timely and spatially fine-tuned occurrence of distinct macrophage subpopulations are essential for physiological tissue repair. More subsets of macrophages have been reported during the last couple of years, however, their involvement in wound healing is less clear [75]. Seminal reviews on new macrophage subsets, their specific cell surface marker expression, transcription factors and distinct cytokine profiles have earlier been published [76]. In this review, we focus on M1 and M2 macrophages.

Fig. 3. Iron excess - through a self-perpetuating mechanism - fosters the prevalence of the pro-inflammatory M1 macrophage phenotype resulting in sustained inflammation and oxidative stress. M1 macrophages perpetuate the hostile microenvironment by the release of iron, ROS and a pro-inflammatory cytokine/chemokine profile.

4.1.1. Persistence of pro-inflammatory M1 macrophages causes impaired wound healing

Chronic wounds including diabetic foot ulcers, chronic venous leg ulcers, and pressure ulcers have all in common that they fail to proceed through the physiological sequence of wound phases, but remain entrapped in the early phase of inflammation. This is due to a lack of shifting from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages [22,54,77–79].

The underlying pathophysiology of persisting M1 macrophages in different wound conditions is complex. Hyperglycemia (increased glucose concentrations in the blood) in a diabetic state with inflammasome activation, venous insufficiency with enhanced iron deposition inside and outside M1 macrophages as well as persistent pressure and recurrent reperfusion ischemia injuries in pressure ulcers may add to the persistent and unrestrained activation of M1 macrophages [80].

4.2. Disease states due to unrestrained activation of M1 macrophages

4.2.1. Diabetic foot ulcers

M1 macrophages isolated from wounds of patients suffering from diabetes mellitus and from db/db mice, closely reflecting hyperglycemia in human type 2 diabetes, revealed unrestrained inflammasome activity and reduced expression of endogenous inflammasome inhibitors. Soluble factors in the microenvironment of diabetic wounds activate the inflammasome in M1 macrophages [81]. In fact, following exposure of macrophages to supernatants of diabetic wounds an enhanced ROS release from M1 macrophages and, subsequently, the induction of caspase-1, a key driver of inflammasome activation, was observed, and, in consequence, the release of inflammation driving interleukins like IL-1β and IL-18 was significantly enhanced. Inhibition of the inflammasome activity in wounds of db/db mice by topical application of pharmacological inhibitors reversed delayed wound healing as a consequence of an enforced switch from pro-inflammatory M1 macrophages to healing-associated M2 macrophages. Of note, bone marrow–transfer experiments from knock-out mice of NLRP-3, a key
protein of the inflammasome, or caspase-1 to db/db mice provide direct evidence that blocking inflammasome activity in bone marrow cells is sufficient to improve healing. This finding supports the notion that – apart from the hostile microenvironment at the diabetic wound sites – possibly bone marrow cells further add to the propensity for the development of unrestrained M1 macrophage activation and their persistence at the wound site of diabetic ulcers.

Yan and colleagues investigated the impact of the diabetic microenvironment in bone marrow on the function and differentiation capacity of HSCs-towards macrophages [82]. Interestingly, HSC-derived monocytes not only give rise to circulating macrophages that are recruited to wound sites, HSCs also differentiate into a variety of macrophage subsets such as hepatic stellate cells, skin dendritic and Langerhans cells, and brain microglia [83]. However, so far the detailed contribution of these macrophage subsets at the wound site is not completely explored. Yan et al. now have set out to systematically analyze the macrophage differentiation from HSCs in a type 2 diabetes mellitus (T2DM) chronic wound healing mice model [82]. To further explore the role of differentiation into distinct macrophage subsets, they lethally irradiated wild type mice and transplanted bone marrow HSCs from T2DM db/db mice with a spontaneously mutated Leptin receptor gene closely mirroring the major pathology of diabetic patients. Wounds of db/db bone marrow transplanted wild type mice revealed profoundly reduced wound repair with significant skewing towards the M1 macrophage phenotype, similar to the prevalence of M1 macrophages in human T2DM. In T2D bone marrow, due to enhanced activity of the NADPH oxidase NOX2, oxidative stress induced expression of the microRNA Let-7d-3p upregulated the DNA methyltransferase 1. Intriguingly, knockdown of DNA methyltransferase 1 reconstituted the differentiation capacity of HSC towards macrophages and impaired wound healing of T2DM was at least in part rescued. Of note, the induction of hyperinsulinaemia associated with insulin resistance in vivo reduced the differentiation capacity of human HSCs into macrophages very much resembling the results from the T2DM db/db murine model in vivo. These findings underline the impact of the bone marrow resident HSCs and their differentiation capacity into M1 or M2 macrophages as newly recognized cellular “hubs” in wound repair. Of major interest is a recent paper which provides indirect evidence that iron is also involved in pressure induced diabetic wounds. In fact, topical application of deferoxamine attenuates impaired healing in diabetic mice [84]. Disruption of the activity of hypoxia inducible factor-1 alpha (HIF-1α), a transcription factor which under normal conditions controls neovascularization at the wound site, leads to suppressed neovascularization in diabetic mice. The function of HIF-1α is disrupted by a high glucose-induced and reactive oxygen species-mediated modification of its coactivator p300 in diabetic wounds. This, in consequence, results in impaired HIF-1α transactivation. This paper provides causal evidence that local enhancement of HIF-1α activity profoundly improves diabetic wound healing. For this purpose the authors employed a transdermal drug delivery system containing DFO, an iron chelator that earlier has been shown to enhance HIF-1α transactivation in diabetes by preventing iron-catalyzed reactive oxygen stress [85]. Of note, DFO-treated diabetic wounds demonstrated enhanced neovascularization, and suppression of free radicals. These results imply that iron – most likely by enhancing oxidative stress – impairs HIF-1α, and transdermal delivery of DFO accelerates diabetic wound healing and even prevents ulcer formation.

4.2.2. Chronic venous leg ulcers

Chronic venous leg ulcers (CVUs) represent the final outcome of chronic venous insufficiency due to non-closing valves of veins which drain the de-oxygenized blood from legs [86]. Thus, the blood flow normally directed towards the heart and lungs for re-oxygenation – due to non-functional venous valves – is partly disrupted and is mainly flowing back into the veins of the lower extremities. Therefore, venous valve insufficiency contributes to a high blood pressure in the veins of the lower limbs. CVUs fail to proceed through the physiological phases of wound healing, but instead, remain in a chronic inflammatory state with little signs of healing [56,86–89]. We previously employed a complementary approach investigating biopsies from patients with chronic venous leg ulcers, a model disease for macrophage-driven chronic inflammation, and in addition, established a mouse model closely mirroring the pathogenesis of human CVU. Similar to other investigators, we found that due to enhanced blood pressure in veins of human subjects erythrocytes (red blood cells) are pressed out of the veins into the surrounding skin tissue (extravasation of red blood cells). We observed that macrophages in CVUs and the iron overload mouse model contain high amounts of Prussian blue-positive iron [22,90,91], most likely due to enhanced engulfment of extravasated erythrocytes. After erythrocyte phagocytosis, iron is released and bound to intracellular ferritin within macrophages and gradually changes its structure to hemosiderin [92]. This results in an up to 20-fold higher iron concentration in the lower limbs of CVU patients than in the upper arms of the same patients [93]. In line with our observation [22] iron overload in tissue and wound exudates of CVUs, but not of acute wounds, with significant oxidative damage was confirmed in 2 independent studies [23,24]. In the presence of hydrogen peroxide released by activated macrophages and neutrophils, iron promotes the generation of highly toxic hydroxyl radicals via the Fenton reaction. Intriguingly, we observed that iron overload of macrophages induced a subset of macrophages with an unrestrained pro-oxidative and pro-inflammatory M1 activation state in CVUs as well as in iron overload wounded mice. Via enhanced hydroxyl radical, peroxynitrite and TNF-α release, this macrophage population perpetuated inflammation and induced a p16INK4a-dependent irreversible senescence program in resident fibroblasts at the ulcer site, which in addition to the state of unrestrained ROS release and pro-inflammation profoundly impaired wound healing (Fig. 3). Of note, dereroxamine, a proficient iron chelator, when injected after iron overload into mice, completely abrogated these vicious events, and even attenuated the persistence of pro-inflammatory M1 macrophages and fibroblast senescence. This study provided fundamental insight into the role of an iron-induced macrophage population in CVUs in vivo [22]. Targeting this population may hold promise for the development of novel therapies for chronic inflammatory diseases such as chronic venous leg ulcers (see Section 7).

The identification of enhanced iron accumulation in tissue and macrophages as the prime event in the pathogenesis of CVUs eventually driving persistent inflammation and tissue damage is also of major relevance for other chronic inflammatory disorders with enhanced iron accumulation, like repetitive reperfusion injuries in pressure ulcers and possibly neurodegenerative diseases [92,94,95].

4.2.3. Pressure ulcers

Pressure ulcers (PUs) constitute a serious complication of multimorbidity and lack of mobility. Patients with spinal cord injury represent a high risk group for PUs. Similarly, patients at the intensive care unit suffering from severe disorders including multiple trauma, or burns are at high risk (for review see [96]). PUs are driven by a variety of pathogenetic causes including disturbed skin microcirculation and repetitive ischemia-reperfusion injury. Ischemia-reperfusion injury is referred to tissue damage occurring in response to returning blood flow after a period of ischemia or lack of oxygen. In fact, the absence of oxygen from blood during the ischemic period results in the damage of vessels and promotes leakage of red blood cells into the tissue which, subsequently, enhances unrestrained inflammation and oxidative stress by the iron-driven Fenton and Haber-Weiss chemistry. Of note, in a pig model for PU, administration of DFO attenuates impaired wound healing and even prevents the occurrence of PU [95], implying that iron does play a critical role in PU. Even though macrophage subsets have not been studied in human PU, it is most likely – that similar to CVU – unrestrained activation of M1 macrophages may play a critical role in the pathogenesis of PU. Interestingly, similar to CVUs, senescent
fibroblasts are detectable in pressure ulcers [97]. In addition, fibroblasts cultured under increased pressure in vitro depict premature aging [98]. However, the role of iron in the development and maintenance of pressure ulcers has not been addressed in full detail.

5. Cellular and molecular details revisited

5.1. Senescent fibroblasts induced by persisting M1 macrophages further impair wound healing

The capacity of connective tissue fibroblasts to synthesize and organize the extracellular matrix and to communicate with adjacent cells and tissues of distinct histogenetic origin makes them a central component in organ homeostasis. Cellular senescence was first described as the stable proliferative arrest of fibroblasts in cell culture in vitro [99]. Senescence is a hallmark of aged tissues [25] and is now acknowledged as a main driver of aging associated diseases.

Senescence is induced by disturbed cellular homeostasis, and oxidative stress plays an important role [100]. Exposure of fibroblasts to oxidative stress – as occurring in chronic wounds with unrestrained M1 macrophage activation – drives them into cellular senescence. Nitric oxide, superoxide anions and peroxynitrite damage DNA which leads to an installment of an irreversible, p16INK4A mediated senescence. Senescence of resident wound fibroblasts, in consequence, adds and perpetuates the hostile microenvironment by secreting the senescence-associated secretory phenotype (SASP) of paracrine mediators responsible for chronic inflammation and tissue destruction [101]. Further SASP factors released by senescent fibroblasts include matrix-metalloproteinases [102], while the deposition of extracellular-matrix proteins is severely reduced, implying the development of tissue atrophy [103] (Fig. 4). Of note, mitochondrial oxidants are responsible for about 50% of all cellular oxidants [104]. Age-related defects in mitochondria [105–108] contribute to dysfunctional oxidative phosphorylation (OXPHOS) with increased generation of ROS which feed into the iron-dependent Fenton reaction and thus perpetuate oxidative stress in injured or aged skin. In addition to the mitochondrial-dependent production of oxidants, lipofuscin-bound iron has been described as being responsible for oxidative stress in senescent fibroblasts [71]. Lipofuscin, also named “aged pigment” represents a mixture of highly aggregated proteins and lipids combined with sugar residues in higher aged cells. About 2% of this conglomerate is composed of metals like iron and other transition metals. In senescent cells, lipofuscin is visible by autofluorescence. Apart from enhanced release of ROS from disrupted mitochondria, energy supply of cells is hampered in phases of enhanced energy demands during tissue repair.

Although the skin is often exposed to natural and artificial ultraviolet radiation (UVR) of tanning devices, which enhance ROS generation, our knowledge on the influence of UVR on wound healing is rather scant. At least, epithelialization of skin wounds is impaired following pretreatment of the skin with UVR [109]. From this in vivo and in vitro data, it is clear that the OXPHOS-related and -unrelated generation of ROS in disease and aging has an important role in dermal tissue repair in old adults.

Cellular senescence was first interpreted as a tumor-suppressing mechanism [110]. Recently, cellular senescence is reported as an important molecular mechanism in physiological wound healing and development [111,112]. Under conditions of chronic wounds, cellular senescence persists most likely due to a failure to be removed by macrophages and Natural Killer cells perpetuating the hostile microenvironment. The current concept is that the pro-oxidative and pro-inflammatory hostile environment of non-healing wounds generates profound DNA damage and even mutations, and the induction of senescence prevents cancer formation at the expense of gradual tissue decline. As mentioned above, irreversible senescence of fibroblasts at the site of chronic wounds – by the adoption of the senescence-associated secretory phenotype (SASP) and the release of pro-inflammatory cytokines – amplifies and perpetuates the non-healing state of chronic wounds. Clinicians for a long time have surgically removed tissue from chronic wounds occupied with senescent cells. Intriguingly, such surgical interventions with removal of senescent cell and tissue from non-healing wounds can switch chronic wounds to acute wounds with a good tendency to heal [113].

5.2. Extracellular matrix and its degradation by matrix-metalloproteinases in chronic wounds

Matrix metalloproteinases (MMPs) are main players in matrix reorganization and regeneration. MMPs are secreted as pre-forms and processed extracellularly. Their activity is strictly regulated by the tissue inhibitors of metalloproteinases (TIMP). In chronic wounds, excessive MMPs’ activity – by unbalanced proteolysis – hinders tissue restoration and ECM deposition [114,115]. The family of the zinc-dependent matrix metalloproteinases now comprises more than 20 members involved in remodeling of the connective tissue by addressing different extracellular matrix molecules. MMPs like interstitial collagenase (MMP-1) are highly expressed and activated in chronic wounds.

Protease inhibitors, specifically targeting MMPs, have earlier been acknowledged as promising therapeutic strategy for the treatment of chronic wounds [116,117]. Unfortunately, efforts to develop MMP inhibitors so far were not very successful in the past, possibly because they are degraded in chronic wounds [39,118].

Therefore, the focus has now shifted to the development of new small molecule inhibitors with higher stability, specificity and selectivity. These synthetic peptides inhibit MMPs by targeting the zinc-binding group responsible for the activity of MMPs. Most of the new developments actually were inspired from cancer research and rely on data that several and distinct MMPs are critical for the dissemination and metastasis of cancer cells [118].
As MMPs are significantly induced and activated by oxidative stress [119], and given that the activity of MMPs is enhanced in non-healing wounds, the control of the redox state in wounds is crucial to improve tissue repair in chronic wounds. By contrast to the delicately regulated redox homeostasis inside of the cell, ECM proteins are relatively unprotected against oxidative stress. Thus, due to a variety of pro-oxidative mechanisms, ECM is particularly prone to damage with subsequent loss of its function [120].

6. Increased iron concentrations impair wound healing and contribute to chronic wound pathophysiology

6.1. Excess iron present in chronic wounds promotes functional impairment in macrophages and senescent fibroblasts

Interestingly, macrophages are also central in the control of free iron in the organism [121]. We previously reported that ferrous iron reacts in the Fenton reaction with hydrogen peroxide to give rise to the highly toxic hydroxyl radical and this sequence of events is responsible for the development of CVUs [22]. Impaired erythrophagocytosis, as seen in senescent fibroblasts and, more importantly, in overfed macrophages, in conjunction with increased free ferrous iron levels in chronic wounds enhance the Fenton reaction driven ROS generation and promote the hostile microenvironment in chronic wounds [56]. Previously, we showed that iron induces expression and activity of MMPs in vitro, and this can be interrupted by the iron chelator deferoxamine. This finding served as a proof of principle that iron drives a hostile microenvironment [23] and was further supported by in vivo studies, employing an iron overload murine model which closely reflects major pathogenic events in chronic venous leg ulcers [22]. In fact, after deferoxamine injection, we could fully abrogate the delay in wound healing in iron loaded mice. We provided also evidence that following injection of deferoxamine, pro-inflammatory M1 macrophages undergo a shift towards anti-inflammatory M2 macrophages [22]. Of note, deferoxamine also improves healing for diabetic ulcers in a mouse model in vivo [84]. These data imply that iron may also play a role in diabetic foot ulcers and other iron related conditions. Iron-dependent modulation of epigenetics in diabetic and other chronic wounds may causally add to the missing switch from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages and might open further opportunities for the treatment of chronic wounds [86]. Notably, replicative senescent fibroblasts in vitro, as a consequence to their changed cellular metabolism, accumulate iron in their cytoplasm [122,123] with 20 fold higher iron concentrations when compared to young fibroblasts. This iron accumulation in senescent cells results from lysosomal dysfunction with an increase in iron-storing ferritin and from reduced ferroptosis of senescent murine fibroblasts in vitro. Ferroptosis is referred to as a form of regulated cell death which is characterized by the iron-dependent accumulation of lethal concentrations of lipid hydroperoxides. In the liver of 30 months old mice, 10 times more senescent cells were found, and this correlates with a 2.6 fold increase in iron and 10 fold increase in ferritin molecules in vivo. Due to reduced ferroportin expression, the iron efflux is reduced and dysregulated with iron uptake leading to the accumulation of iron in senescent fibroblasts. However, in the post-senescent state, in conjunction with increased ferritin levels [123], senescent fibroblasts are resistant against erastin-induced ferroptosis. The underlying mechanisms for this resistance are not fully understood. In addition, senescent fibroblasts reveal reduced autophagy. This is most likely due to an impaired turnover of ferritin by the proteasomal and lysosomal degradation systems [124]. By contrast to the persistence of senescent fibroblasts at the wound site of non-healing wounds, senescent cells in acute wounds and during development are phagocytotically removed by macrophages and Natural Killer cells [125,126]. For chronic wounds it is currently not known, (i) whether senescent cells accumulate iron intrinsically, (ii) whether persisting high numbers of M1 macrophages in chronic wounds cannot be sufficiently removed by phagocytosis, and (iii) whether M1 macrophages which are overfed with iron [22] cannot undergo ferroptosis and thus may hinder the polarization into M2 macrophages. In addition, it is unclear whether the macrophage phenotype impacts on enhanced iron uptake in M1 macrophages and on increased iron release from M2 macrophages by modulating ferroportin and ferritin expression. Alternatively, it is possible that the iron concentration directly impacts on the differentiation into M1 and M2 macrophages [70]. It is, however, likely that responsible mechanisms are distinct under different microenvironmental conditions of acute versus chronic wounds. For tissue remodeling, Recalcati et al. hypothesized that iron release from M2 macrophages supports proliferation of stromal cells such as fibroblasts as well as of microbiota [127]. This at least in part contradicts the view that free iron mainly, due to ROS generation, exert microbicidal function in wound healing. Recently, Youssef et al. reported that iron-overload induced high erythrophagocytosis and, in addition, enhanced the turnover of splenic red pulp macrophages. Accordingly, enhanced ferroptosis and a subsequent increase in oxidative stress and lipid peroxidation was reported by the same authors [69]. There is some evidence that connective tissue resident fibroblasts have evolutionarily developed countering strategies to cope with high iron concentrations in the tissue. These strategies most likely depend on their state of quiescence, proliferation, or senescence.

Nevertheless, cell-selective and local accumulation of iron in senescent fibroblasts and chronic wound tissue was shown by different authors [122,123]. The relation between aged connective-tissue, iron homeostasis and ferroptosis, and wound healing is very complex. We are now only beginning to understand cell-cell interactions induced by disturbed iron metabolism in wound healing and related pathologies. Using comparative cell and molecular biology, a better understanding might in future allow to address the role of iron diagnostically and therapeutically in more detail in the context of personalized medicine.

6.2. The role of iron in hematopoiesis and its impact on stem cell behavior

Cell-cell and cell-matrix-interactions including the spatially and timely controlled delivery of pharmacological compounds, biologicals and stem cells is now a hot research topic for the advanced development of more efficient therapies for chronic wounds [128,129]. Delivery of compounds is dependent on the context in the chronic wounds that is currently not sufficiently understood. Since chronic wounds fail to proceed into the granulation phase, but instead remain in the phase of chronic inflammation, this may hamper the renewal capacity of mesenchymal stem cells and may upon constant pro-oxidative inflammation drive MSCs into apoptosis. MSCs have the potential to differentiate into different lineages, but, more importantly in the context of tissue repair, they modulate and suppress unrestrained inflammation [130]. Thus, they sense and shape their neighborhood at the wound site and act as an “adaptive drug store” to enhance tissue repair and homeostasis [131]. The specific effect of severely disturbed iron homeostasis on MSCs is not known in detail. Nevertheless, there is compelling evidence that redox homeostasis and ROS at least regulate stem cell behavior of hematopoietic stem cells and their bone marrow niche [132]. Redox regulation plays a significant role in self-renewal, mobilization, differentiation, and proliferation of stem cells [133–135].

Patients with hematological disorders such as bone marrow failure or anemia, require constant blood transfusion resulting in iron overload. This labile iron pool leads to damage of the hematopoietic system with organ dysfunction and significant tissue damage [136]. These detrimental effects affect the generation, differentiation and migration of bone marrow derived hematopoietic stem cells (HSC) progenitors as well as effector blood cells important in wound healing. Following deferoxamine treatment, many parameters indicative of stem cell integrity and function substantially improve, as reported for serum ferritin levels, colony-forming capacity in vitro and reduction of enhanced ROS levels [136]. Whether oxidative stress fully depends on iron under
in vitro investigated the effect of iron overload on the differentiation capacity of MSCs. Balogh et al. investigated the effect of iron overload on the differentiation capacity of MSCs in vitro [150]. Accordingly, bone osteoprogenitor cells from murine bones were exposed to increased iron concentrations and the differentiation capacity into the osteogenic, chondrogenic and adipogenic lineages was tested. Only the osteogenic differentiation was inhibited when compared to the other two lineages. Supplementing iron-binding ferritin, unexpectedly, mimicked impairment of osteogenic lineage differentiation. The authors speculate that ferritin might have a pro-oxidant effect. Ferritin treated bone osteoprogenitor cells, in fact, revealed increased ROS levels. This impairment of osteogenic differentiation was mediated by down-regulation of the key transcription factor Runx2 which is responsible for osteogenesis. Borriello and colleagues investigated the effect of high iron concentrations on proliferation, ECM-deposition, and mineralization potential of human bone marrow derived MSCs [151]. Increasing iron concentrations, similar to iron overload diseases, resulted in a dose-dependent increase in proliferation of bone marrow derived MSCs. Similarly, supplementation of BM-MSC medium with iron resulted in a 1.8-fold increase in cell numbers. In addition, upon iron exposure higher cell numbers in S-phase were detected by FACS when compared to untreated control cells. This increase in proliferation was associated with a significantly enhanced intracellular iron and ferritin concentrations. Interestingly, using the dye 2′,7′-dichlorofluorescein diacetate, no significant increase in ROS was detected. Increase in iron concentrations led to a significantly impaired osteogenic differentiation potential towards osteoblasts, while osteocalcin and collagen type-I deposition was remarkably reduced. Iron overload may impair the ability of MSCs to differentiate into osteoblasts and this, in consequence, may contribute to the development of osteoporosis and the susceptibility for bone fractures observed in iron overload patients [151]. Whether physiological wound healing of acute and chronic wounds depends on MSCs or other stem cells mobilized from the bone marrow or predominantly rely on resident MSCs or a combination thereof is unclear. Are these processes modulated by distinct iron concentrations? What is the impact of disturbed DNA and heme synthesis in iron overload conditions in comparison to the Fenton-related generation of ROS on chronic wounds?

7. Clinical applications

7.1. Manipulation of pro-inflammatory macrophages – Failures and successes

A variety of approaches to manipulate macrophages and to enforce a shift from M1 to M2 macrophages have been reported in the last 5 years, and – due to its clinical relevance – constitutes a very active research area [75,76,152]. Understanding the underlying drivers responsible for the persistence of M1 macrophages in distinct disease states inspired several therapeutic modalities.

Among many agonists, glucocorticoids apparently promote anti-inflammatory macrophage populations [153]. Of note, treatment of CVUs with topical corticosteroids was beneficial for 79% of patients suffering from chronic wounds [154].

In an alternative approach, the transcription factor IRF5 has been shown to be causally involved in the polarization of macrophages towards a pro-inflammatory phenotype that perpetuates inflammation and, in consequence, disrupts wound repair. Therefore, strategies to suppress IRF5 activity hold promise to increase the number of anti-inflammatory and pro wound-healing macrophages, as was suggested in preclinical models of obesity and insulin resistance [155].

Importantly, topical application of pharmacological inhibitors suppressing inflammasome activity in wounds of diabetic db/db mice significantly improved delayed wound healing as a consequence of an enforced switch from pro-inflammatory M1 macrophage to healing-associated M2 macrophages [80]. Depletion of macrophages resulted in excessive scar generation by enhanced activity of the fibroblast-derived lysyl-oxidase which is responsible for crosslinking of collagen molecules. Interleukin-4 receptor alpha activated macrophages induce profibrotic collagen cross-links. Thus, the reliable switch from M1 to M2 anti-inflammatory macrophages is an essential prerequisite for wound healing. Administration of in vitro M2-polarized pro-repair macrophages expressing arginase-1 and YM-1 with reduced ROS release, however, did not result in enhanced or even attenuated wound healing in diabetic mice [156]. The authors exclude a reversed polarization from M2 back to M1. Instead, they assume that the injection of M2 macrophages at day one after wounding might have disrupted the balance between pro-inflammatory M1 and anti-inflammatory M2 macrophages resulting in an even further delay of wound healing. By contrast, application of purified macrophages onto pressure ulcers led to significantly improved wound healing in the treated compared to the control group.

By contrast, administration of mesenchymal stem cells (MSCs) into acute or chronic iron-overload wounds resulted in a significant acceleration of wound healing by an enhanced switch from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages [148,157]. It is most likely that progenitors or stem cells are much more adaptive to the microenvironment when compared to in vitro pre-polarized macrophages. In fact, mesenchymal stem cells have been referred to as “adaptive drug stores”, which sense and shape their neighborhood at the wound site [144]. Of note, in response to distinct environmental cues at the wound site MSC profoundly reprogram their transcriptome to efficiently meet the requirement of the wounds [144,146,157]. Finally, we identified TNFα as an important M1 macrophage effector which profoundly impairs healing of wounds in iron overload mice [22]. In fact, release of high TNF-α concentrations from the pro-inflammatory M1 macrophages perpetuated inflammation. Interestingly, topical application of Infliximab, a chimeric IgG1 monoclonal antibody that binds to the soluble and transmembrane TNF-α, led to a substantial acceleration of healing in 12 of 14 patients suffering from previously therapy-resistant CVUs [158].

7.2. Antioxidant approaches

There is causal evidence that local enhancement of HIF-1α activity
profundely improves diabetic wound healing. Employing a transdermal drug delivery system containing the FDA-approved deferoxamine (DFO) significantly enhanced HIF-1α transactivation in diabetes [84]. Employing antioxidants rescue redox homeostasis in fibroblasts eventually resulting in improved wound gap closure in the in vitro wound healing scratch assay [159]. Treatment of old mice in vivo with the mitochondria-targeted antioxidant SkQ1 revealed improved wound healing of the skin as measured by granulation tissue formation and re-epithelialization in skin wounds of old mice [160]. Remarkably, topical application of DMSO, a hydroxyl radical scavenger, or of allopurinol which inhibits xanthine oxidase, an enzyme generating high superoxide anion radical concentrations under the hypoxic conditions occurring in CVUs, distinctly improved healing in a series of 133 studied CVU patients [161]. Still, the underlying mechanisms and consequences on tissue-specific or systemic antioxidant strategies are poorly understood.

7.3. Senolytic approaches

Clinicians for a long time and based on their empirical observation, have surgically removed tissue from chronic wounds and thus got surgically rid of senescent cells in CVUs. Intriguingly, such surgical interventions of tissue removal switch chronic wounds towards acute wounds with a good tendency of healing [113]. Depletion of senescent cells by genetic approaches using induced suicide of p16INK4a-positive cells led to a significant improvement of aging related pathologies [162]. In addition, in recent years pharmacological approaches have been developed to deplete senescent cells tissues via induction of apoptosis (for review: [100,163]). Improvement of immunosurveillance and clearance of senescent cells by the immune system is another promising approach. Further refinement of these strategies, will advance the therapeutic benefit in chronic wound healing in near future.

7.4. Mesenchymal stem cell based therapies

Mesenchymal stem cells are endowed with the unique capacity to coordinate histogenetically distinct cell species involved in different phases during wound healing in a variety of preclinical murine models [148,157,164] and in impaired human wounds [165,166], eventually leading to accelerated and scar reduced tissue repair. Most likely, MSCs exert their beneficial effect by their unique ability to sense and shape their neighborhood at the wound site. Due to these beneficial effects, MSC-based therapies are currently assessed in clinical phase I/IIa studies to improve wound healing with accelerated wound closure, suppression of inflammation and scar reduced healing [166,167]. Recently, we and others provided evidence that MSCs can switch unrestrained activation from M1 macrophages into anti-inflammatory M2 macrophages [76,157,168]. This unique ability of MSCs will be refined and exploited for the therapy of non-healing wounds.

8. Perspectives

The demand for improving human health-span in elderly individuals applies – among other aging-associated disorders – particularly for non-healing chronic wounds. This goal can only be achieved by the development of innovative therapies which can be afforded by health care providers. Iron is essential for many physiological processes and iron homeostasis affects all steps of physiological wound healing ranging from stem cell lineage identity, distinct effectors of the innate immune system to the regenerative capacity of connective tissue. In depth understanding of cellular and molecular causes and the role of iron-overload will help to develop new therapeutic strategies for difficult-to-heal wounds (Fig. 5). Currently, the development and refinement of stem-cell based therapies possibly also through priming of stem cells for specific wound conditions hold substantial promise to be successfully introduced into clinical routine [106]. Murine models for wound healing reveal some differences from healing of human wounds.

Fig. 5. Iron induced M1 macrophages and senescent fibroblasts play a causal role in tissue repair disorders like chronic venous leg ulcers and pressure sores. Most pathogenic insight on the role of iron has been generated in chronic venous leg ulcers. (a) Due to venous valve insufficiency the blood pressure is high in lower leg veins. This is responsible for enhanced extravasation of erythrocytes into the skin tissue of chronic venous leg ulcers. Clinically, it becomes apparent by a brownish pigmentation of the skin. Macrophages ingest iron and thereby get persistently activated and causally contribute to persisting inflammation and the induction of fibroblast senescence in their neighborhood. Senescent fibroblast via SASP factors perpetuate the vicious cycle and spread senescence, eventually preventing chronic venous leg ulcers to heal. (b) A similar sequence of events applies for pressure sores and ulcers. Erythrocyte extravasation occurs due to repetitive ischemia reperfusion injury. When the perfusion and the blood supply is disrupted, tissue and cells undergo at least in part damage and necrosis. In case blood flow is reestablished, the blood leaks out of damaged vessels into the interstitial skin tissue. Thereafter, erythrocytes are removed by engulfment through macrophages with a similar sequence of pathogenic events as described for tissue break down in chronic venous leg ulcers (a). Based on this insight, novel therapies will be developed (see Section 7).

This and differences in the immune system, requires critical caution when translating findings from murine models to human wounds. Nevertheless, many mechanisms of iron-dependent cellular and molecular changes in chronic wounds share similar mechanisms between organisms. Comparative biology addressing evolutionary conserved mechanisms will help to decipher the role of iron in the animal kingdom as suggested by Theodosius Dobzhansky in his famous essay “Nothing in Biology Makes Sense Except in the Light of Evolution” [169]. Novel strategies and any advance distinctly depend and profit from the combination of in vivo models with in silico approaches employing systems biology in the light of evolution. Multidisciplinary approaches with clinicians, basic research centered scientists and scientists from industry will promote meaningful progress.
9. Concluding remarks

Employing model systems and human wound biopsies to deepen insight into cellular and molecular processes of wound healing will help to differentiate between species-specific “private” mechanisms in contrast to species-independent “public” mechanisms. This knowledge on mammalian regeneration and disturbances in wound healing can eventually be exploited to pave new avenues for advanced diagnostics and therapies in the management of non-healing wounds. Further approaches and investigations into the role of iron levels and its cellular location as well as molecular pathways linked to iron signaling in wound repair and regeneration will allow to define conditions and to design mechanism-based therapies within the context of personalized medicine in the near future.

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