Submitted: 09/22/2022

Accepted: 03/10/2023

Published: 03/26/2023

Review Article

[1]Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada

Keywords

Iron, wound healing, hepcidin, skin infection, chelators

Email Correspondence

idia.boncheva@mail.mcgill.ca

https:// doi.org/10.26443/msurj.v18i1.188

Idia Boncheva[1]

The Role of Iron in Epidermal Healing and Infection

Abstract

In recent years, the field of iron studies has expanded into sub-domains that investigate the regulation of this metal in various tissues including the heart, mucosal surfaces, tumors, and the skin. Iron homeostasis in the skin and the role of other non-hepatic cells in the regulation of iron are currently incompletely understood. This paper summarizes the role of iron in wound healing, highlights the importance of maintaining iron concentrations within an intermediate range to avoid toxicity or defects; and integrates the antimicrobial role, interactions, and regulation of various cell types. Notably, the autoregulation of hepcidin secretion by keratinocytes and recruited myeloid cells is described. Additionally, the potential therapeutic role of iron chelators in infection control and their mechanisms of action are explored. This paper aims to elucidate the relevance of local iron control in epidermal infections. Although some of the molecular details underlying this condition remain unclear, published data suggest that iron-regulating therapies are a promising treatment for the eradication of skin infections due to their wound-healing and immune-modulating potential.

Introduction

Iron plays a central role in the housekeeping processes in our cells and organs, making it one of the most essential components of our diet. Its functions are so vital that our bodies have evolved distinct ways to recycle iron rather than excrete it, as they have for other nutrients[1]. A healthy individual ingests approximately 10-20 mg of iron daily[1]. However, only 10% is absorbed into the circulation, while the rest is lost as waste[2]. Additionally, around 1-2 mg of iron is shed daily in bodily fluids and through skin desquamation[1]. The absorption of iron from our dietary intake is accomplished in the upper part of the digestive tract via various import proteins such as divalent metal transporter-1 (DMT-1) and heme carrier protein 1 (HCP1) on the surface of enterocytes[2]. The details of this step vary with the ionic form and the protein-association state of iron[2].

Subsequently, iron is transported in the blood via plasma transferrin[2]. Transferrin is an iron-binding protein which delivers the vital metal to the liver and the spleen for storage, and to all other cells in our body for their survival[2]. Iron is vital because it is located within internal structures such as the iron-sulfur clusters of complexes within the electron-transport chain (ETC) in the mitochondrial membrane[3]. These iron-containing structures are required for the proteins to generate an electrochemical gradient which can subsequently be used to produce ATP—the predominant energy source in cellular metabolism[3]. Unbound iron is found in the body only at very low levels, as most of it is associated with ferritin and hemosiderin which are intracellular iron-storage proteins[1]. Transferrin will also deliver iron to the bone marrow for erythropoiesis[1]. Around 20 mg of iron is used up every day in the bone marrow for the formation of red blood cells[1]. Such an investment is made because iron is an essential component of hemoglobin, the protein that binds, transports, and delivers oxygen through the blood to the entire body[2]. Once red blood cells die, the iron that is held within them is processed by macrophages and brought back to the bone marrow to resume the cycle (Figure 1)[1,2,4].

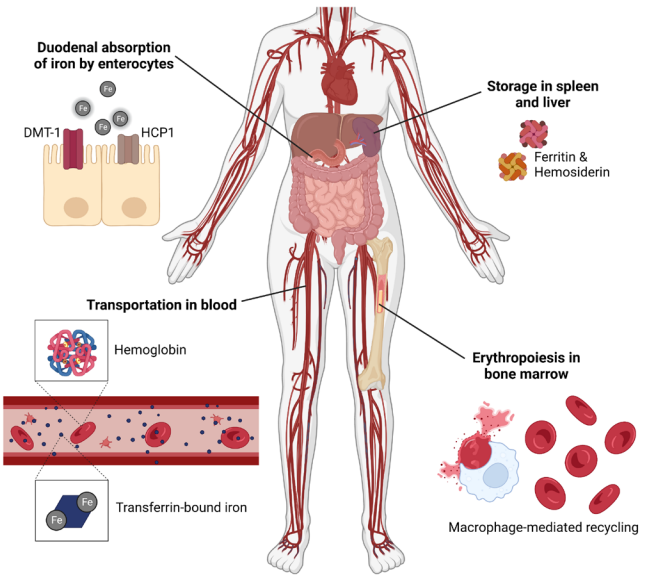

Figure 1. Simplified schematic of iron cycling in the body 1,2.

Despite its abundance in nature, iron has low bioavailability as it is predominantly found in its insoluble ferric form (Fe^{3+})[5]. This has made it a highly coveted metal by all life forms, including microorganisms. In fact, bacteria have developed specialized iron uptake mechanisms to acquire this metal from the environment[4]. For example, siderophores are small organic molecules that diffuse out of bacteria, tightly bind extracellular iron, and deliver the metal to microbes through the reabsorption of iron-siderophore complexes[5]. Different bacterial species have evolved other enzymatic or receptor-mediated iron-uptake systems to optimize nutritional scavenging in their particular niches[5]. In the context of infection, these microbes will try to acquire iron from our cells. In response, our body will set off an immune defense mechanism that will sequester iron from the environment and stunt the proliferation of the invaders[5]. In a phenomenon called hypoferremia of inflammation, individuals who develop an infection see their plasma iron levels decrease within hours[5]. This is one of the many mechanisms our bodies use to fight against microbes.

With predictions of increasing antibiotic resistance, there is an increased need for alternative antimicrobial protocols to treat infections. This review paper summarizes selected topics relating to the role of iron in wound healing and pathogen control in local skin infections. Additionally, it evaluates the potential therapeutic use of iron chelators on topical wounds. This review aims to gather and integrate the current knowledge and evidence in this emerging field to facilitate the modulation of iron homeostasis in the treatment of infections.

The Role of Iron in Skin Wound Healing

Even in the absence of infection, iron has a complex influence on our skin's health and healing. Upon tissue damage, the skin must undergo a series of temporally coordinated, dynamic, and locally controlled repair mechanisms[1]. For instance, blood clotting, inflammation, immediate vascular responses, re-epithelialization, glandular tissue formation, and angiogenesis are a few of the vital steps involved in cutaneous wound healing[1]. Many cells, such as keratinocytes, which are the primary cell type composing our skin, fibroblasts, and innate immune cells such as macrophages, are involved in this phenomenon[6]. This becomes even further convoluted when we consider chronically disturbed wound healing where the role of iron is heterogeneous[6].

In healthy individuals, the concentration of iron in the skin, as measured by X-ray fluorescence, is around 10.22.5 µg per gram of dry weight, but this can greatly vary amongst the sites measured[7]. For instance, neutron activation analysis (NAA) of the abdomen epidermis records iron concentrations of 90.245.2 µg g-1 in groups of healthy individuals[7]. There is also a wide variation in skin iron concentrations for different skin disease conditions and between individuals with the same disease[7]. Regardless of these differences, analysis of iron functions suggests that extreme concentrations can be damaging; high iron levels can cause cell death, whereas low levels impair wound healing.

At one end of this spectrum, iron can be toxic to cells due to reactive oxygen species (ROS) generated via the Fenton reaction[1]. In this reaction, ferrous iron (Fe^{2+}) reacts with hydrogen peroxide to generate ferric iron (Fe^{3+}), hydroxide, and a hydroxyl radical[8]. Subsequently, ferrous iron can be regenerated via the superoxide-driven Fenton reaction[8]. Thus, low amounts of iron can power the Fenton reaction via this redox cycling (Figure 2)[8,9]. The combination of high iron and abundant ROS can drive ferroptosis, a non-apoptotic programmed cell death that triggers the release of inflammatory immunogenic intracellular molecules and induces necroinflammation[10]. Mitochondria are a major source of ROS, as altered mitochondrial DNA upon cell damage increases the production of these small molecules[11]. In fact, it was found that mitochondrial alterations within wound fibroblasts can impede the healing process by affecting nuclear transcription events, motility, and growth[11]. Although ROS can be beneficial in combatting invading microbes, they are detrimental to the host at high concentrations upon prolonged periods as this perpetuates a non-healing state[1,11]. Thus, local iron regulation is vital for homeostasis. To avoid the buildup of this waste product, labile iron can be released from keratinocytes[10]. The iron released into the extracellular environment minimizes intracellular oxidative damage[1]. Additionally, intracellular iron storage proteins such as ferritin, and iron-regulatory proteins (IRPs), which are transcriptional regulators of iron-associated proteins, can regulate labile iron availability independently of systemic iron control such as to avoid toxicity[12].

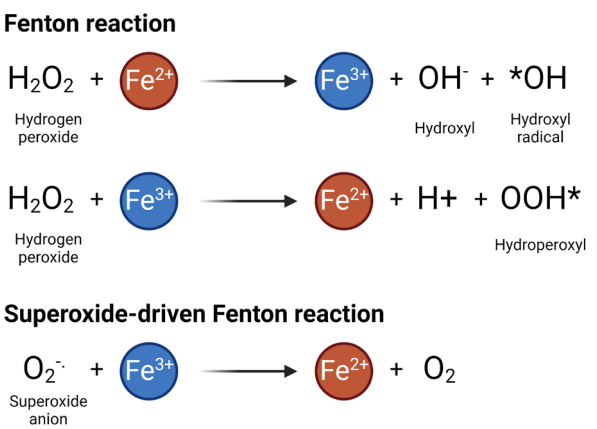

Figure 2. Generation of ROS via the Fenton reaction and the related superoxide-anion driven Fenton chemistry 3,4.

At the other end of the spectrum, there is evidence suggesting that low iron levels can also be detrimental to skin regeneration[1]. For example, in a comparative study, Sprague-Dawley rats were made anemic by weekly bleeding for 6 weeks and were fed a low-iron diet[13]. These rats were wounded by laparotomy incisions and the wound tensile strength was assessed 7 days later by the Howes method, which measures the force required to pull apart a segment of wound[13,14]. It was found that the healing rate in iron-supplemented rats was on average twice as strong than in the low-iron group, as measured by wound tensile strength[13]. Additionally, there is evidence suggesting that increased local iron levels can be beneficial in wound healing[15]. For instance, lactoferrin is a glycoprotein that binds iron when it is released by glandular epithelial cells into various body fluids such as maternal milk, saliva, tears, and mucosal secretions[15]. When lactoferrin is present in infected tissues and pus, it locally concentrates iron which raises the initial levels of inflammation after injury, increases cell proliferation and recruitment, and enhances fibroblast-mediated collagen contraction[15]. Similarly, it was also found that iron concentrations were enhanced in animal wound-healing models compared to baseline[16]. Lewis rats were subjected to dorsal biopsy punctures, and the levels of iron were measured at various time points after injury via inductively coupled plasma mass spectrometry (ICP-MS) to assess the levels of iron at different healing stages[16]. The levels of iron recorded peaked during the proliferation phase which involves keratinocyte migration to the surface of the wound, granulation by fibroblasts, and neovascularization[16]. Thus, in some cases, iron can be an essential element for healing.

The studies described in this section indicate that neither iron-overload nor iron-deficiency is beneficial to our skin. The evidence summarized in this section is somewhat contradictory as elevated iron levels can potentially be toxic to cells due to ROS, but normal wound healing in rodents depends on iron abundance. This suggests an optimal level of iron must be maintained in the body under normal conditions and upon injury to promote healing.

Hepcidin Control of Myeloid Cells in Infection

To better understand how the iron levels in the body are balanced, outlining its regulators is vital. Hepcidin is the iron-regulating hormone. This peptide is encoded by the *HAMP* gene, and it is mainly secreted by hepatocytes[2]. Hepcidin engages different interference mechanisms to promote the accumulation of iron inside cells and reduce iron export. It can either downregulate the expression of ferroportin—the only known iron-exporting membrane protein—and stimulate its degradation, or at higher plasma concentrations, hepcidin can directly block the efflux of iron through ferroportin[5,17]. These functions have established hepcidin as an integrative regulator of iron in the body. For instance, upon microbial exposure, the cytokine-rich environment resulting from infection will promote systemic hepatocyte-derived hepcidin production[5]. Under high hepcidin concentrations, iron will be sequestered inside the cells and its availability to invading pathogens in extracellular fluids will be reduced[4].

Hepcidin is also starting to be understood as an important molecule on a local scale, particularly in the skin. For instance, keratinocytes have been established as local modulators of hepcidin and as immunomodulatory cells during skin infections[18]. Indeed, histological staining of cross-sectional human skin biopsies has shown that keratinocyte production of hepcidin is increased in patients with cutaneous Group A *Streptococcus* (GAS) infection, compared to healthy patients[18]. Infections with GAS are the most common cause of necrotizing fasciitis (NF), which has a 35% mortality rate, so investigating the effect of iron on immune responses is of particular interest for this condition[18]. In a pioneer study done by Malerba et al. (2020), GAS NF was used as a model of skin infection to investigate the control of iron upon microbial attack[18]. Mutant mouse models with a keratinocyte-specific knockout in the *HAMP* gene were engineered. The mutant mice showed unchanged systemic iron parameters compared to normal mice, indicating that keratinocyte-derived hepcidin does not play a role in systemic iron control, which is mainly controlled by hepcidin-derived hepatocytes. However, the mutant mice did not secrete hepcidin in the infected tissue, whereas wild-type mice did. This result demonstrates that hepcidin stains in skin tissue of patients infected with GAS are a prod

uct of keratinocyte secretion rather than systemic production by hepatocytes. Additionally, local hepcidin injection in the infected mice prevented the progression of a systemic disease, further emphasizing this peptide's antimicrobial role. Thus, hepcidin may be a marker for NF as it is upregulated in patients with this condition, and this iron-regulation hormone may also have protective roles against pathogens in skin infections as its presence is associated with a lower disease burden[18].

Interestingly, the therapeutic effects of hepcidin are lost in mice with knockouts in the CXCL1 gene, the functional homologue of human IL-8[18]. This is because hepcidin prevents the dissemination of GAS infection via a chemokine-dependent pathway[18]. The IL-8/CXCL1 chemokines are known as chemotactic factors that recruit neutrophils and other granulocytes[18]. The pro-inflammatory cytokine CXCL1 is secreted by keratinocytes upon hepcidin binding to the corresponding surface receptor ferroportin[18]. Subsequently, this chemokine recruits and activates myeloid cells that play an essential role in the innate immune response upon infection or injury (Figure 3)[18-20]. A defect in chemokine secretion by keratinocytes results in a failure to limit the spread of a microbe from a localized infection to a systemic one[18]. Additionally, both neutrophils and macrophages in subcutaneous compartments can secrete hepcidin upon recognition of microbial antigens in a TLR-4 dependent manner[19]. Indeed, mice mutants in this pattern recognition receptor cannot induce hepcidin production upon exposure to GAS 19. The details of this relationship have not yet been characterized, but it appears that keratinocytes and myeloid cells can protect our body from serious infections via a hepcidin-mediated regulatory feedback loop (Figure 3).

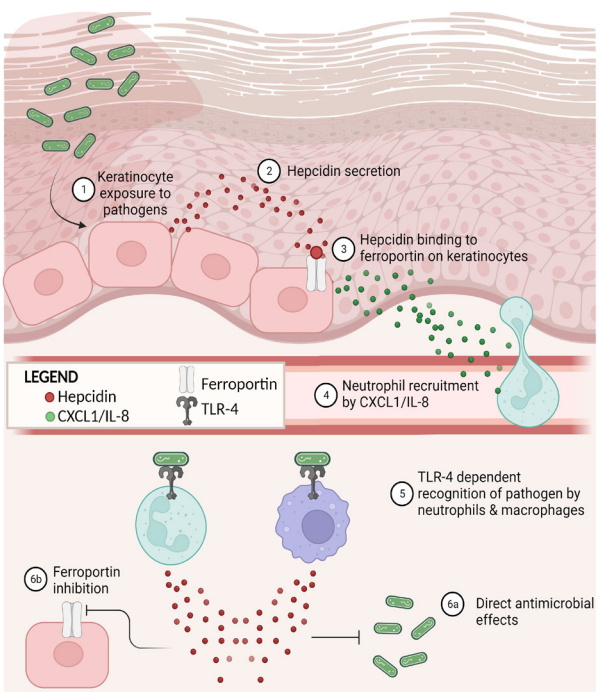

Figure 3. Regulatory feedback loop of hepcidin production and myeloid cell recruitment 5-7.

It is also possible that the relationship between iron and innate immune cells is more complex due to the presence of various iron-binding factors. For instance, the genetic deletion of ferroportin on macrophages will induce the retention of intracellular iron and affect various steps of skin homeostasis and repair such as stromal cell proliferation, angiogenesis, and fibrogenesis[21]. This is hypothesized to be a result of defective iron redistribution to neighboring cells, highlighting the importance of macrophages in skin regeneration[21]. Iron is also essential for the tissue repair functions of macrophages as the accumulation of this metal will induce the differentiation of macrophages into an M2 pro-healing phenotype that produces a high level of wound-healing chemokines[22]. Additionally, neutrophils have been shown to depend on the iron-binding lactoferrin protein for their characteristic oxidative bursts[23]. This functional response releases potent antimicrobial ROS into the environment, contributing to host defense[23].

Overall, hepcidin is an interesting candidate for the treatment of infections such as NF because it can provide protective effects against microorganisms by interacting with the surface receptors of immune cells and because hepcidin is able to camouflage itself from the invading microbes which lack a direct defense against hepcidin. It is also important to not overlook the importance of other iron-binding proteins and cell types, as these can greatly contribute to both innate immune modulation and tissue repair. Although more research must be done to characterize the self-regulatory mechanism of hepcidin in peripheral tissues, it can be hypothesized that the metabolism of iron in the skin is controlled by proximal cells, such as neutrophils, macrophages, and keratinocytes, and by locally secreted factors such as hepcidin, CXCL1/IL-8, and iron-associated factors.

Iron Chelators as Therapeutic Agents in Skin Infections

Iron chelators are synthetic or microbe-derived molecules that strongly bind iron via various chemical interactions[4]. There are multiple iron chelators that have long been approved for clinical use—Desferrioxamine (DFO), Deferiprone (DFP), and Deferasirox (DFX) are a few examples[4]. These diversely structured molecules will sequester the iron metal and enable its excretion through urine or feces[24]. Iron-chelation therapy is primarily used to treat patients with iron-overload diseases which can be either genetic or acquired[24].

In clinical applications, the rationale for iron chelator therapy is analogous to how our bodies naturally mount a biological defense upon infection. Chelators will bind labile iron and reduce its accessibility to the invaders in the extra-cellular environment[4]. For example, DFX is a bidentate oral chelator with protective effects against *Candida albicans* infection[4]. Alternatively, the tridentate chelator DFP was shown to have beneficial effects in wound healing upon topical application in rodents, and in treating biofilms on surgical wounds of *in vitro* models of multi-drug resistant bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*[25,26]. A synthetic hydroxypyridinone-containing anti-microbial polymer called DIBI is another example of a promising iron chelator when tested in vivo. In fact, it was found to have a dose-dependent attenuation of *S. aureus* infection[27]. This bacterium accounts for 84% of wound infections, half of which are methicillin-resistant staph aureus (MRSA)[27]. When applied to the skin, DIBI reduced total bacterial titer and overall inflammation. This compound reduces the availability of iron to the pathogen and is particularly potent at fighting infections by enhancing the activity of the antibiotic it is combined with[27].

However, not all iron-chelators are good candidates: DFO—a hexadentate chelator—is not ideal for infection treatments, as it is derived from the *Streptomyces* bacterium, and it can be exploited by pathogens to favor their proliferation[4]. Furthermore, iron chelators are not yet used on a large scale for infection treatment because they are potentially toxic to cells and require high physiological concentrations to exert their therapeutic effects[27]. Additionally, it is hypothesized that local iron chelation can have an impact on the immune capacity of tissues by impeding ROS production 4. As previously established, reduced ROS can be either beneficial in tissue healing or disadvantageous in pathogen killing. There is great debate over the effect of chelators on the susceptibility to infections, especially after intravenous administration due to potential tissue toxicity[28]. In general, topical application of any therapy reduces toxicity compared to systemic administration. Thus, local treatment of skin infection using iron chelators could present fewer risks, but the effects remain unclear.

Iron chelation therapy has great potential for its alternative application in infection control due to its indirect antimicrobial capacity and its anti-inflammatory potential. However, before it can be implemented as a standard treatment, we must first establish the adverse effects of the diverse chelators upon topical application and their chemical interactions with

different species of bacteria. Until then, it is recommended that the clinical use of iron chelators is done in a patient-specific and time-sensitive manner such as to minimize the dosage and avoid adverse effects[28].

Conclusion

The complex relationship between global iron homeostasis and local iron control is not entirely understood. Although the liver is responsible for the systemic control of iron levels in cells and fluids via hepcidin secretion, it does not account for local adaptive changes. The major research contributions described in this paper highlight the significance of local iron regulation in epidermal wound healing, the role of keratinocytes and myeloid cells in infection control via hepcidin and chemokine secretion, and the potential anti-microbial use of topical iron chelators. Analysis of these selected topics reveals that maintenance of iron levels within an intermediate range is essential for the homeostasis of tissue and organs, and that many innate immune cell functions are affected by iron and its associated factors. The vast role of iron in physiological functions renders it a critical subject of investigation with respect to skin conditions.

References

1. Wright, J. A., Richards, T. & Srai, S. K. The role of iron in the skin and cutaneous wound healing. *Front. Pharmacol.* **5**, 156 (2014). https://doi.org/10.3389/fphar.2014.00156

2. Waldvogel-Abramowski, S. et al. Physiology of iron metabolism. *Transfus. Med. Hemother.* **41**, 213-221 (2014). https://doi.org/10.1159/000362888

3. Merrill, J. F. et al. Iron deficiency causes a shift in AMP-activated protein kinase (AMPK) subunit composition in rat skeletal muscle. *Nutrition & Metabolism.* 9, **104** (2012). https://doi.org/10.1186/1743-7075-9-104

4. Scott, C., Arora, G., Dickson, K. & Lehmann, C. Iron Chelation in Local Infection. *Molecules.* **26**, 189 (2021).

5. Ganz, T. Iron and infection. *Int. J. Hematol.* **107**, 7-15 (2018). https://doi.org/10.1007/s12185-017-2366-2

6. Eming, S. A., Martin, P. & Tomic-Canic, M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci. Transl. Med.* **6**, 265sr266 (2014). https://doi.org/10.1126/scitranslmed.3009337

7. Bagshaw, A. P. & Farquharson, M. J. Simultaneous determination of iron, copper and zinc concentrations in skin phantoms using XRF spectrometry. *X-Ray Spectrometry.* **31**, 47-52 (2002). https://doi.org/https://doi.org/10.1002/xrs.535

8. Messner, D. J., Murray, K. F. & Kowdley, K. V. in Physiology of the Gastrointestinal Tract (Fifth Edition) (eds Leonard R. Johnson et al.) 1507-1527 (Academic Press, 2012).

9. Meng, X. et al. Fenton reaction-based nanomedicine in cancer chemodynamic and synergistic therapy. *Appl. Mater. Today.* **21**, 100864 (2020). https://doi.org/https://doi.org/10.1016/j.apmt.2020.100864

10. Vats, K. et al. Keratinocyte death by ferroptosis initiates skin inflammation after UVB exposure. *Redox Biol.* **47**, 102143 (2021). https://doi.org/10.1016/j.redox.2021.102143

11. Janda, J., Nfonsam, V., Calienes, F., Sligh, J. E. & Jandova, J. Modulation of ROS levels in fibroblasts by altering mitochondria regulates the process of wound healing. *Archives of Dermatological Research.* **308**, 239-248 (2016). https://doi.org/10.1007/s00403-016-1628-9

12. Sandnes, M., Ulvik, R. J., Vorland, M. & Reikvam, H. Hyperferritinemia-A Clinical Overview. *J. Clin. Med.* **10** (2021). https://doi.org/10.3390/jcm10092008

13. Bains, J. W., Crawford, D. T. & Ketcham, A. S. Effect of chronic anemia on wound tensile strength: correlation with blood volume, total red blood cell volume and proteins. *Ann. Surg.* **164**, 243-246 (1966). https://doi.org/10.1097/00000658-196608000-00009

14. Crawford, D. T., Bains, J. W. & Ketcham, A. S. A standard model for tensiometric studies. *J. of Surg. Res.* **5**, 265-269 (1965). https://doi.org/10.1016/S0022-4804(65)80016-6

15. Takayama, Y. & Aoki, R. Roles of lactoferrin on skin wound healing. *Biochem. Cell Biol.* **90**, 497-503 (2012). https://doi.org/10.1139/o11-054

16. Coger, V. et al. Tissue Concentrations of Zinc, Iron, Copper, and Magnesium During the Phases of Full Thickness Wound Healing in a Rodent Model. *Biol. Trace Elem. Res.* **191**, 167-176 (2019). https://doi.org/10.1007/s12011-018-1600-y

17. Nemeth, E. et al. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. *Science.* **306**, 2090-2093 (2004). https://doi.org/10.1126/science.1104742

18. Malerba, M. et al. Epidermal hepcidin is required for neutrophil response to bacterial infection. *J. Clin. Invest.* **130**, 329-334 (2020). https://doi.org/10.1172/jci126645

19. Peyssonnaux, C. et al. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. *Blood.* **107**, 3727-3732 (2006). https://doi.org/10.1182/blood-2005-06-2259

20. Barthe, C., Hocquellet, A. & Garbay, B. Bacteriostatic activity of the proregion of human hepcidin. *Protein Pept. Lett.* **18**, 36-40 (2011). https://doi.org/10.2174/092986611794328627

21. Recalcati, S. et al. Macrophage ferroportin is essential for stromal cell proliferation in wound healing. *Haematologica.* **104**, 47-58 (2019). https://doi.org/10.3324/haematol.2018.197517

22. Wilkinson, H. N. et al. Tissue Iron Promotes Wound Repair via M2 Macrophage Polarization and the Chemokine (C-C Motif) Ligands 17 and 22. *Am. J. Path.* **189**, 2196-2208 (2019). https://doi.org/10.1016/j.ajpath.2019.07.015

23. Ward, P. P., Mendoza-Meneses, M., Park, P. W. & Conneely, O. M. Stimulus-Dependent Impairment of the Neutrophil Oxidative Burst Response in Lactoferrin-Deficient Mice. *Am. J. Path.* **172**, 1019-1029 (2008). https://doi.org/10.2353/ajpath.2008.061145

24. Mobarra, N. et al. A Review on Iron Chelators in Treatment of Iron Overload Syndromes. *Int. J. Hematol. Oncol. Stem Cell Res.* **10**, 239-247 (2016).

25. Richter, K. et al. A Topical Hydrogel with Deferiprone and Gallium-Protoporphyrin Targets Bacterial Iron Metabolism and Has Antibiofilm Activity. *Antimicrob. Agents Chemother.* **61**, e00481-00417 (2017). https://doi.org/10.1128/AAC.00481-17

26. Mohammadpour, M., Behjati, M., Sadeghi, A. & Fassihi, A. Wound healing by topical application of antioxidant iron chelators: kojic acid and deferiprone. *Int. Wound. J.* **10**, 260-264 (2013). https://doi.org/10.1111/j.1742-481X.2012.00971.x

27. Parquet, M. d. C., Savage, K. A., Allan, D. S., Davidson, R. J. & Holbein, B. E. Novel Iron-Chelator DIBI Inhibits Staphylococcus aureus Growth, Suppresses Experimental MRSA Infection in Mice and Enhances the Activities of Diverse Antibiotics in vitro. *Front. Microbiol.* **9** (2018). https://doi.org/10.3389/fmicb.2018.01811

28. Rund, D. Intravenous iron: do we adequately understand the short- and long-term risks in clinical practice? *Br. J. Haematol.* **193**, 466-480 (2021). https://doi.org/10.1111/bjh.17202