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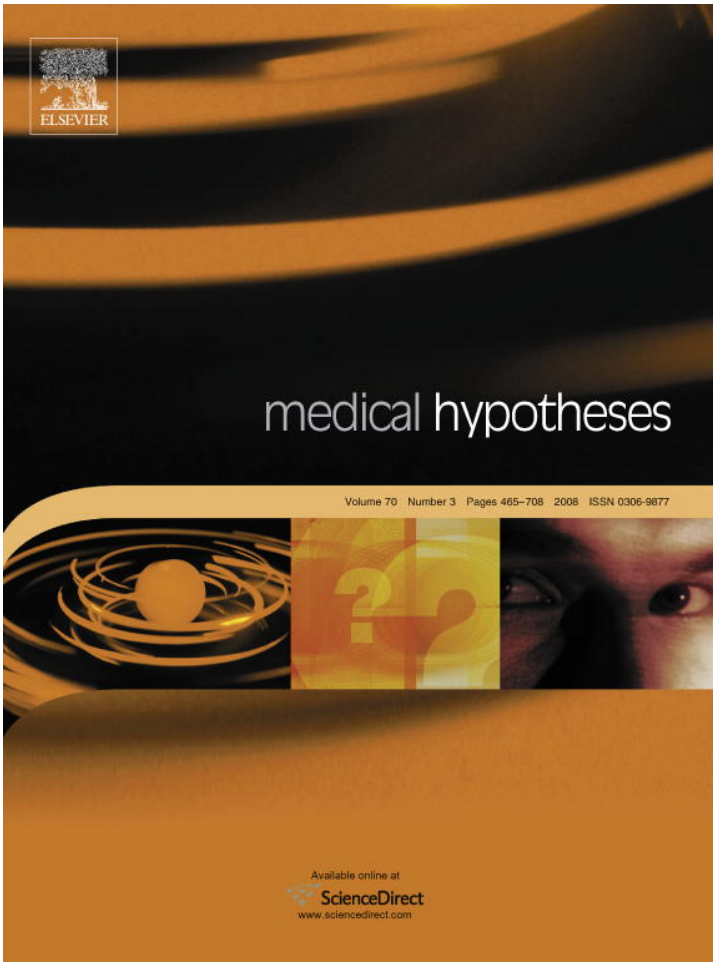

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Could chronic wounds not heal due to too low local copper levels?

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Summary Copper is an essential trace element involved in numerous human physiological and metabolic processes. It plays a key role in many of the processes that together comprise wound healing, including induction of endothelial growth factor, angiogenesis and expression and stabilization of extracellular skin proteins. We hypothesize that in individuals with diabetic ulcers, decubitus, peripheral vascular, or other wounds which might have compromised circulation to the wound site, that part of the incapacity of the wounds to heal is due to low local copper levels. Contamination of wounds is also an important factor causing impaired wound healing. Importantly, copper has potent broad biocidal properties. In contrast, the risk of adverse skin reactions due to exposure to copper is extremely low. We thus hypothesize that introducing copper into wound dressings would not only reduce the risk of wound and dressing contamination, as silver does but, more importantly, would stimulate faster wound repair directly. This would be done by the release of copper from the wound dressings directly into the wound site inducing angiogenesis and skin regeneration.
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Introduction

Wounds normally heal in a very orderly, efficient and highly controlled method of repair distinguished by four distinct, but overlapping, phases: hemostasis, inflammation, proliferation and remodelling [1,2]. These highly complex phases, characterized by high metabolic activity at the wound site, demand a fluent supply of oxygen and nutrients. The altered microenvironment found in wounds, such as low pH, reduced oxygen tension and increased lactate, actually initiate the release of factors by epidermal cells, fibroblasts, macrophages and vascular endothelial cells needed to bring in new blood supply. This process of neovascularisation is stimulated by vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-β. Fibroblasts are responsible for producing the new matrix needed to restore structure and function to the injured tissue. Fibroblasts attach to fibrin and integrin cables and begin to produce collagen, which then becomes cross-linked [3,4].

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Ischemia in chronic wounds

The events of early wound healing reflect a finely balanced environment leading to uncomplicated and rapid wound healing. Chronic wounds or ''complex wounds'' [5] which, for many reasons, have lost this fine balance, are usually associated with systemic pathologies that impair normal healing causing wounds to fail to heal with simple care and requiring special attention. Chronic wounds are characterized by extensive loss of the integument, clear necrosis, or signs of circulation impairment either localized or more extensive, usually in the limbs, leading to extensive loss of substance. A key characteristic common to chronic wounds is ischemia, due to microangiopathic disease in diabetic patients [6], chronic venous insufficiency in chronic venous ulcers [7], vasculitis in patients with autoimmune disease or under immunosuppressive drug therapy [8] or necrosis of the integument in pressure sores [9].

Involvement of systemic copper in wound healing

The function of many factors involved in the efficient and highly controlled repair wound healing mechanism is dependant on their interaction with copper. The following supports this notion:

(i) Inducible VEGF expression is sensitive to copper [10]. Among many known growth factors, VEGF is believed to be the most prevalent, efficacious, and long-term signal that is known to stimulate angiogenesis in wounds. In both *in vitro* and *in vivo* models, $CuSO_4$ clearly promotes angiogenic responses. Topical copper sulphate accelerated the closure of excisional murine dermal wounds. Histological analysis of wound-edge tissue substantiated that $CuSO_4$ treatment not only accelerated wound closure but that the quality of regenerating tissue was distinctly different. $CuSO_4$ treatment was associated with more hyperproliferative epithelial tissue and the density of cells in the granulation layer of copper-treated wounds was clearly higher. Immunohistochemical studies show that wound edges of copper-treated wounds have more prominent VEGF expression [10].

(ii) The export of fibroblast growth factor (FGF)1 and interleukin (IL)-1α, two pro-angiogenic polypeptides, is based on the Cu^{2+}-dependent formation of multiprotein complexes containing the S100A13 protein. The formation of a such a multiprotein aggregate enables the release of FGF1 in response to stress [11].

(iii) Furthermore, copper chelation represses the vascular response to injury [12]. These observations have also led to the development of anti-copper-based, anti-angiogenic strategies for the treatment of cancer [13,14].

(iv) Integrins expressed by basal layer keratinocytes play an essential part in wound healing. Copper modulates integrins expressed by suprabasally differentiated keratinocytes during the final healing phase [15].

(v) The stability of fibronectin mats (Fn-mat), which have potential applications in tissue engineering, is increased by treating them with micromolar concentrations of copper ions [16]. Low concentrations of copper (1 μM) not only caused significant fibronectin stabilisation but the greatest amount of cell ingrowth was observed for copper treated cables [17].

(vi) Expression of the metallothionein gene is upregulated in the skin following topical application of copper, and in wound margins, particularly in regions of high mitotic activity. The action of metallothioneins in these processes may result from the large number of zinc-dependent and copper-dependent enzymes required for cell proliferation and matrix remodelling [18].

(vii) GHK, a tripeptide with high affinity for copper ions that was isolated from human plasma, potently reduces tissue oxidative damage after injury and activates tissue remodelling [19]. Topical application of GHK resulted in accelerated wound healing in ischemic open wounds [20]. Interestingly, the increase in metalloproteinase-2 levels in conditioned media of cultured fibroblasts GHK-Cu(2+), could be reproduced by copper ions alone but not by the tripeptide GHK alone [21], indicating the crucial role of copper in the remodelling of extracellular matrix, a central step in wound repair. Similarly, the copper tripeptide accelerates the growth of normal and irradiated fibroblasts to the point where treated irradiated fibroblasts approximate the population-doubling time of normal controls [22]. Accordingly, it was found that GHK incorporated collagen increases the copper concentration by nine fold at the wound site indicating the wound healing property of GHK can also be linked with copper localization [23].

As delineated above, copper ions play an important role especially, but not only, during

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the proliferation and remodelling phases. It is therefore not surprising that many over-the-counter treatments for wound healing contain copper [24,25]. The importance of copper in wound healing is further demonstrated by the positive effect of its administration in cases of severe burn trauma in children [26] and in the management of phosphorus burns [27].

Copper and wound infections

It is established that infections may delay healing, cause failure of healing, and even cause wound deterioration [28]. Microbial pathogens delay wound healing through several different mechanisms: persistent production of inflammatory mediators; metabolic wastes; toxins; tissue hypoxia; rendering the granulation tissue hemorrhagic and fragile; reducing fibroblast number and collagen production; damaging reepithelization [29,30]; maintaining the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals, and competing with host cells for nutrients and oxygen necessary for wound healing [31]. There is increasing evidence that bacteria within chronic wounds live within biofilm communities, in which the bacteria are protected from host defences and develop resistance to antibiotic treatment [28]. Thus, keeping the wound aseptic or maintaining a low bioburden is an important aim in wound healing, especially when dealing with chronic wounds.

Copper ions, either alone or in copper complexes, have been used for centuries to disinfect liquids, solids and human tissue. Several mechanisms for the potent biocidal activity of copper have been proposed, which include alteration of proteins and inhibition of their biological assembly and activity; plasma membrane permeabilization; and membrane lipid peroxidation [32]. In contrast to the resistant microbes that have evolved to antibiotics in less than 50 years of use, tolerant microbes to copper are extremely rare even though copper has been a part of the earth for millions of years. This lack of resistance to copper may be explained by the capacity of copper to damage in parallel many key factors in micro-organisms [32].

Contrary to the high susceptibility of micro-organisms to copper, human skin is not sensitive to copper and the risk of adverse reactions due to dermal exposure to copper is extremely low [33,34]. Copper is not only considered safe to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices (IUDs) [35,36], but it is an essential metal needed for normal metabolic processes. The National Academy of Sciences Committee established the US recommended Daily Allowance of 0.9 mg of copper for normal adults [37].

The hypothesis

We hypothesize that in diabetic ulcers, decubitus, and other wounds that occur in individuals with potentially compromised blood supply to the wound site, part of the incapacity of the wounds to heal is due to low local copper levels, which occur due to the compromised blood circulation.

Taking together the potent biocidal activities of copper and its roles in the wound healing process, strongly supports the notion that the addition or application of copper or copper containing products, such as Band-Aids and gauze containing copper, to wounds, may significantly enhance the wound healing process. Furthermore, in ischemic patients, with poor local copper supply by the circulatory system, exterior supplement of the copper by elution from copper containing dressings, directly to the wounds, will result in healing where conventional treatments fail.

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