PERSPECTIVE ARTICLE

Zinc in wound healing: Theoretical, experimental, and clinical aspects

Alan B. G. Lansdown, PhD, FRC Path[1]; Ursula Mirastschijski, MD, PhD[2]; Nicky Stubbs, RN[3]; Elizabeth Scanlon, RN, MSc[3]; Magnus S. Ågren, DMSci[4]

1. Imperial College Faculty of Medicine, Division of Investigative Sciences, Charing Cross Hospital, London, United Kingdom,
2. Department of Plastic, Reconstructive and Hand Surgery, Otto-von-Guericke University, Magdeburg, Germany,
3. Leeds North West Primary Care Trust, Leeds, United Kingdom, and
4. Department of Surgery K, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark

Reprint requests:
Dr. Magnus S. Ågren, Department of Surgery K, Bispebjerg Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen, Denmark.
Tel: +45-3531 6493;
Fax: +45-3531 3911;
Email: maa02@bbh.hosp.dk

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ABSTRACT

Zinc is an essential trace element in the human body and its importance in health and disease is appreciated. It serves as a cofactor in numerous transcription factors and enzyme systems including zinc-dependent matrix metalloproteinases that augment autodebridement and keratinocyte migration during wound repair. Zinc confers resistance to epithelial apoptosis through cytoprotection against reactive oxygen species and bacterial toxins possibly through antioxidant activity of the cysteine-rich metallothioneins. Zinc deficiency of hereditary or dietary cause can lead to pathological changes and delayed wound healing. Oral zinc supplementation may be beneficial in treating zinc-deficient leg ulcer patients, but its therapeutic place in surgical patients needs further clarification. Topical administration of zinc appears to be superior to oral therapy due to its action in reducing superinfections and necrotic material via enhanced local defense systems and collagenolytic activity, and the sustained release of zinc ions that stimulates epithelialization of wounds in normozincemic individuals. Zinc oxide in paste bandages (Unna boot) protects and soothes inflamed peri-ulcer skin. Zinc is transported through the skin from these formulations, although the systemic effects seem insignificant. We present here the first comprehensive account of zinc in wound management in relation to current concepts of wound bed preparation and the wound-healing cascade. This review article suggests that topical zinc therapy is underappreciated even though clinical evidence emphasizes its importance in autodebridement, anti-infective action, and promotion of epithelialization.

Zinc is a transitional metallic element known from ancient times. It is widely distributed in the human environment, being found in water, air, and virtually all foodstuffs. The medicinal properties of zinc in the form of calamine were documented more than 3,000 years ago in the Ebers Papyrus and in ancient Ayurvedic manuscripts in early Indian medicine,[1,2] but the observation by Raulin in 1869 that the mold *Aspergillus niger* would not grow on a zinc-deficient medium was fundamental in establishing the importance of zinc in biological systems. Subsequent research has shown that zinc is present, albeit in minute concentrations, in all living plant and animal cells, mainly in the form of cofactors or structural components in key enzyme systems in cell replication, protein synthesis, and repair systems following injury. In 1941, Keilin and Mann[3] identified the first metalloenzyme, carbonic anhydrase, with zinc as an essential cofactor, but more recently zinc has been identified in more than 300 different enzymes, of which alcohol dehydrogenase, alkaline phosphatase, angiotensin-converting enzyme, matrix metalloproteinases (MMPs), reverse transcriptase, RNA and DNA polymerases, and superoxide dismutase are well documented.[4,5] In addition to its role in nucleic acid and protein synthesis, carbohydrate metabolism, and oxygen transport, zinc is now known to be instrumental in stabilizing cellular membranes.[6,7] Zinc-finger proteins are a family of more than 2,000 transcription factors that bind specifically to DNA and activate transcription of growth factors,[4,8–10] cytoprotective proteins,[11] and are regulators of adult hematopoietic stem cells.[12] Apart from its importance in protein complexes, the zinc ion is closely involved in intracellular signaling and neurotransmission.[13,14]

Zinc is second only to iron in being the most abundant trace element in the human body,[15] but its nutritional significance came to light only in the 1960s following reports

AE	Acrodermatitis enteropathica
ECM	Extracellular matrix
IL	Interleukin
MICs	Minimum inhibitory concentrations
MMP	Matrix metalloproteinase
MT	Metallothionein
TPN	Total parenteral nutrition

of zinc-responsive growth failure in infants in rural Egypt and Iran.[16,17]

Because of new clinical evidence presented at the World Union of Wound Healing Societies' meeting in Paris 2004, it is timely to re-evaluate the potential benefits offered by zinc therapy in wound management. Although numerous clinical trials claim to show the benefits of using oral or topical zinc therapy in wound management, variations in treatment regimen and zinc formulations used have obscured the true efficacy of the protocols. In keeping with the early studies on supplementary zinc therapy in pilonidal sinus management,[18] evidence is now available to show that not only is zinc beneficial in the healing profile but that it provides an effective level of anti-infective action.[19] Furthermore, a young boy with Hirschsprung's disease with symptoms of zinc deficiency successfully treated with zinc following gastrointestinal surgery provides further irrefutable evidence for the value of zinc in wound healing.[20]

This review summarizes the knowledge on regulation of zinc homeostasis, nutritional role, and metabolism of the trace element, zinc deficiency and diagnosis, and zinc in skin physiology. We also provide an extensive summary of experimental and clinical studies on the physiological and pharmacological roles of zinc in the wound-healing process. The review is concluded by a brief description of commercially available zinc preparations used in wound management and the clinical relevance of zinc absorbed from these products.

CELLULAR ZINC HOMEOSTASIS

Advances in molecular genetics and zinc-specific fluorescent probes have unraveled many of the mechanisms responsible for zinc uptake, intracellular distribution, and elimination.[13] Within cells, 30–40% of the zinc is bound to proteins in the nucleus, 50% is located in the cytoplasm, and the remainder in plasma membranes.[5]

Metallothioneins (MTs) complex up to 20% of intracellular zinc. The implications of MT induction and zinc metabolism in health and disease have been reviewed.[21–23] These ubiquitous, cysteine-rich low-molecular-weight proteins regulate the intracellular supply of zinc to enzymes, gene-regulatory molecules and zinc depots, and protect cells from deleterious effects of exposure to elevated levels of zinc. One MT molecule can bind seven zinc ions.

The ZIP family of membranous transporter proteins is mainly involved in cellular zinc uptake, whereas the ZnT family mediates zinc efflux.[21,24] Energy sources for the zinc channels are elusive, and symport as well as antiport mechanisms have been proposed.[25] Members of the ZIP family consist of 220–650 amino-acyl residues with eight putative membrane-spanning domains. ZIP-1 is the major uptake system in human tissues[26] and is expressed in the small intestine, epidermis, and keratinocytes.[27] The ZnT proteins comprise six putative transmembrane domains with a histidine-rich loop.[24] ZnT-1 is found in plasma membranes and catalyzes efflux from the cytoplasm into the extracellular medium preventing excessive intracellular zinc concentrations.[21,24] ZnT-2 translocates zinc ions to an acidic endosomal compartment, another mechanism for cytoprotection.[21,24] ZnT-4 is constitutively expressed in the mammary gland epithelium where it controls secretion of zinc into breast milk.[28] Mutations of the ZnT-4 gene are responsible for the lethal milk mouse, and pups fed the maternal zinc-deficient milk die before weaning.

Expression of MTs and zinc transporters is transcriptionally regulated by metal-responsive transcription factor-1 that senses zinc levels.[29]

NUTRITIONAL ROLE AND METABOLISM OF ZINC

Zinc was identified as an essential micronutrient by the Wisconsin group of biochemists in 1934. The nutritional value of zinc was widely researched by McCance and Widdowson in the 1940s[30] but the true clinical significance of zinc was not appreciated until much later.[31] The body requirements for zinc in humans are normally satisfied by a well-balanced diet leading to an average daily intake of 10–15 mg per day in concordance with the recommended daily allowance for zinc in healthy adults of 8–15 mg per day.[32,33] Diets rich in protein are usually high in zinc,[32] whereas vegetable diets containing high plant fiber are low in absorbable zinc.[1]

Exogenous zinc is mainly absorbed in the duodenum and proximal jejunum within 3 hours after meal intake.[34,35] Zinc first binds to the apical membrane of the enterocyte, is transported into the cell, and then secreted into the blood or back into the intestine as endogenous zinc.[36] Initial zinc absorption is inversely proportional to mucosal MT concentrations but as MT sequesters zinc, further uptake is suppressed.[37] The quantity of absorbed zinc depends on dietary factors.[35] Intestinal absorption from aqueous zinc solutions may be 80% before food intake but only 5–40% afterward through the action of metal chelators, predominantly phytic acid (inositol hexaphosphate) in seeds, grains, and legumes, which precipitate free zinc ions as insoluble complexes.[38] Other metal ions like iron may compete with zinc for binding sites and inhibit zinc bioavailability.[38] Circulating zinc increases rapidly during the first hour and then declines as the metal is transferred to tissues.

The maintenance of zinc homeostasis relies on reabsorption of endogenous zinc mainly from pancreatic secretion into the distal small bowel, a process that responds to changes in the nutritional zinc status of the individual.[35,39] The liver is a key regulator of zinc homeostasis and controls indirectly excretion of endogenous zinc, which occurs mainly via the gastrointestinal route.[40] Under normal physiological conditions, renal excretion accounts for 10–20% of gastrointestinal excretion of zinc, whereas zinc losses through desquamation and perspiration are normally insignificant.[35]

ZINC DEFICIENCY AND DIAGNOSIS

The total zinc in the human body is estimated to be 0.8–3.0 g.[15] Symptoms and signs of zinc deficiency include ataxia, depression, impaired taste, anorexia, diarrhea, eczematous dermatitis, alopecia, mouth ulcers, and delayed wound healing.[16] True zinc-deficient states are reported in relation to sickle cell anemia, chronic malnutrition, total parenteral nutrition (TPN), malabsorption disorders, chronic alcoholism, chronic liver disease, gut fistulae, Crohn's disease, and ulcerative colitis.[1,41,42] In each case,

zinc supplementation has led to complete remission of the symptoms. Zinc supplements may be effective in preventing or ameliorating types 1 and 2 diabetes.[43]

On a molecular level, ZIP-4 is rapidly induced on microvilli of enterocytes during zinc deprivation to compensate for inadequate zinc intake.[36,44] Furthermore, proinflammatory cytokines induce MTs[45] that suppress gastrointestinal zinc uptake and the zinc transporter protein ZnT-1 that increases hyperzincuria, and thus contribute to a negative zinc balance.

The diagnosis of zinc deficiency in the human body is complicated by the low concentrations present and by controversy and lack of sensitive indices.[46] In serum, about 60% of zinc is bound to albumin, 30% to α2-macroglobulin, and 10% to various other ligands.[47] Normal serum zinc is considered to be in the range 10–18 μmol/L, with higher levels in men than in women[33] but variations in published figures may be due to the accuracy of analytical methods used and circadian rhythms of zinc levels.[48] A serum zinc level below 9 μmol/L is biochemically defined as zinc deficiency[49] but malignancies, hyperactivity,[50] stress, trauma, active tuberculosis, skin diseases, chronic wounds,[51–53] chronic renal insufficiency, uremia,[54] and nephrotic syndrome are predictable causes of hypozincemia. Lower than normal serum zinc in pregnant women is presumably a result of transplacental transfer of zinc from mother to fetus.[55]

According to Halsted and Smith,[56] abnormally low serum zinc merely suggests a state of zinc deficiency, and only clear-cut clinical responses to zinc therapy under controlled conditions constitute definitive evidence. Possibly, the zinc concentration in white blood cells reflects zinc status more accurately than plasma zinc level.[46] Whole-body counting techniques should provide an accurate picture of zinc status.[38,57]

HEREDITARY ZINC DISORDERS

Acrodermatitis enteropathica (AE) occurs as a rare inherited disorder transmitted as an autosomal recessive trait.[58] AE is caused by impaired zinc uptake in the small intestine, manifests early in childhood, and is associated with low serum zinc levels, impaired bowel function, hypogonadism and characteristic skin lesions of erythema, vesiculo-bullous dermatitis, alopecia, stunted growth, decreased resistance to infections, and impaired wound healing.[58,59] Histopathological features of the skin include degenerating keratinocytes, parakeratosis, thick chromatin aggregates, and increased mitosis.[60] AE is frequently fatal unless treated with oral zinc sulfate.[58,59] The etiology has been ascribed to mutations of the ZIP-4 gene, which encodes a zinc transporter protein.[44,61]

The secondary laminopathies are due to mutations in a gene that encodes for a zinc metalloproteinase involved in processing of the intermediate filament prelamin A into mature lamin A and cause mandibuloacral dysplasia and restrictive dermopathy. Skin fibroblasts from these patients show abnormal nuclear morphology.[62]

ZINC IN SKIN PHYSIOLOGY

Zinc is located intracellularly and in extracellular matrix (ECM) in epidermal and dermal tissues in the form of protein complexes where zinc acts as a stabilizer of cell membranes and an essential cofactor, and satisfies a central role in mitosis, migration, and maturation.

The zinc concentration in the epidermis (50–70 μg/g dry weight) is higher than in the dermis (10–5 μg/g dry weight) in human skin, perhaps reflecting the activity of zinc-dependent RNA and DNA polymerases in mitotically active basal cells.[63–66]

Immunohistochemical and in situ hybridization localization studies on normal skin indicate high levels of MTs in the basal epidermis with reduced concentrations in postmitotic keratinocytes, reticuloendothelial cells, and fibroblasts.[22,67–69] MT is associated with increased tissue concentrations of zinc[22] and the zinc content of the skin is significantly lower in the MT-null than in wild-type mice.[67] Furthermore, the epidermis failed to exhibit hyperplasia in MT-null mouse skin challenged with stimulators of cell proliferation comparable to that in animals replete with mRNA for MT genes.[67]

There is a critical balance between zinc and calcium in basal cell mitosis and postmitotic functional maturation involving keratohyalin synthesis and keratinization in normal skin.[70,71] An inverse relationship is seen in the epidermis between the zinc concentration and the state of maturation and keratinization of postmitotic cells. Declining zinc gradients across the epidermis are the reverse of calcium gradients that increase from the basal layer to maximal concentrations in the granular cells.[70,72–74] Calcium-binding proteins like calmodulin hold key roles. Heng et al.[75] demonstrated reciprocity between tissue calmodulin and cAMP levels in epidermal cells, and showed that calmodulin levels decline significantly in the presence of excess zinc. Zinc also modulates the activity of calcium/calmodulin-dependent protein kinase II dose dependently.[76]

The relative importance of zinc and calcium in cell proliferation and maturation is further illustrated in comparative studies of keratinizing epithelia.[77] In thin hairy skin where mitosis is inversely proportional to the hair cover, zinc and calcium levels are appreciably lower than in pressure keratinization on the sole of the foot where the robust epidermis with one to three basal layers is associated with a protracted keratinization and thick compacted stratum corneum. Higher levels of zinc in the sensory epithelia of the nasal mucosa and tongue are not only consistent with high mitotic activity, protracted zones of keratinization, and high levels of protein-bound phospholipids, but reflect the importance of zinc in taste and smell perception.[78]

ZINC IN WOUND-HEALING PHYSIOLOGY

Many of the biochemical and molecular events in wound repair can be expedited by addition of supplementary zinc ion through up-regulation of MTs[22] and zinc metalloenzymes.[79] Furthermore, any defect in the expression of zinc-finger transcription factors in mRNA coding of growth factors is consistent with impaired wound healing.[10,11]

The quantitative and qualitative distribution of zinc in skin wounds is determined by atomic absorption spectrometry and immunohistochemical techniques for demonstrating zinc-binding proteins like MTs.[73] Interleukin-1 (IL-1) is instrumental in modulating zinc metabolism through the differential regulation of MT genes.[45] This mechanism may in part explain the marked increase in zinc

in the early inflammatory phase of experimental wounds.[68,73,80] In the rat wound model, zinc levels in the wound margin increased by 15–20% within 24 hours, increasing to 30% at the time of maximal granulation tissue formation and epidermal proliferation.[73] This early increase was associated with high MT in keratinocytes at the wound margin, macrophages, and dermal fibroblasts, whereas later, MT deposits were associated with proliferating populations of epidermal basal cells. The decline in zinc in the later stages of healing (10–21 days) was consistent with reduced mitotic activity and scar maturation.[73,80,81]

Evidence for the functional role of zinc in repair systems is provided by demonstration of zinc metalloenzymes like alkaline phosphatase, RNA and DNA polymerases, and MMPs.[82] Alkaline phosphatase is a sensitive marker for fine dermal blood vessels and early stages of angiogenesis associated with increased inflammatory activity and connective tissue proliferation.[83] DNA polymerases serve as accurate markers for cell proliferation.[84]

MMPs

MMPs belong to the metzincin clan of metalloendopeptidases.[85] Twenty-five structurally similar human MMPs have been identified,[86] having the following common characteristics:

- an N-terminal hydrophobic domain (signal peptide);
- a propeptide domain; and
- a catalytic zinc-binding domain.

The catalytic domain of MMPs comprises a cleft containing one catalytic tightly bound Zn^{2+}, to which the substrate initially binds before cleavage, and one additional structural Zn^{2+}.[85,87]

As a group, MMPs are capable of degrading essentially all components of the ECM and exhibit diverse proteolytic specificities consistent with their wide range of protein and glycoprotein substrates including cytokines, cytokine receptors, adhesion molecules, and latent MMPs.[86] They are synthesized variously by all cell types in the wound, notably keratinocytes at the wound margin, macrophages, fibroblasts, and endothelial cells under the influence of soluble mediators and cell–ECM contacts.[86] MMPs are synthesized as inactive zymogens with a cysteine residue forming a predomain masking the catalytic site. Disruption of this predomain exposes the zinc ion for catalytic binding to a substrate.[88]

MMPs of particular relevance in wound healing include collagenases (MMP-1, MMP-8, MMP-13), stromelysins (MMP-3, MMP-10), and gelatinase A (MMP-2) and gelatinase B (MMP-9).[79,89–94] The collagenases cleave native triple helical collagen, whereas MMP-2 and MMP-9 degrade fragmented interstitial and denatured collagens, basement membrane type-IV collagens, and gelatin. MMP-3 and MMP-10 have a wide range of substrates in the wound bed.[89,93]

Characteristically, MMPs are up-regulated following injury.[95] Much attention has focused upon the intrinsic mechanisms for up-regulation of these endogenous zinc-dependent enzymes, their proteolytic role in wound debridement and action in modulating cell migration, and reconstitution of the ECM.[79] Current views on wound healing focus upon wound bed preparation as an obligatory first stage in the management of chronic wounds.[96] In pig models, wound repair was shown to be an expression of the debriding efficacy of the collagenases.[97] Administration of synthetic MMP inhibitors that block the early proteolytic and collagenolytic action of MMPs impairs keratinocyte migration and wound contraction during wound healing.[98] Increased MMP expression in scars has been observed at least 1.5 years postwounding[90] but their precise roles in excessive scarring or scarless wound healing need further clarification.[99]

In situ hybridization methods have proved useful in identifying sites of mRNA coding for MMPs and mechanisms of up-regulation.[91,100] Elevated levels of MMP-1 mRNA were demonstrated mainly in 12–24-hour human wound fibroblasts[100] and in migrating epidermal tongues as part of the acute phase in wound healing.[91] Thus, MMP-1 would seem to have a primary role in "initiating" tissue repair[100] and in epithelialization,[94] but then is shut off although keratinocytes up-regulate MMP-1 expression in fibroblasts.[101] Wound healing is severely impaired in collagenase-resistant mice,[102] but MMP-13 knockout animals did not differ in their efficiency of epithelialization, inflammatory response, granulation tissue formation, angiogenesis, and restoration of basement membrane.[103] This may be explained by overlapping functions of the collagenases and supports the concept of redundant activity among the MMPs.[103,104] Intracellular MMP-1 also confers resistance to apoptosis.[105] The membrane-associated MMP-14 appears to be involved in keratinocyte migration and survival.[104] MMP-2 is expressed in uninjured skin including the epidermis and stromal cells of the dermis,[91] and MMP-2 mRNA persists in fibroblasts and endothelial cells throughout the healing cascade, and appears to be involved primarily in the remodeling of scar ECM.[90] In contrast, MMP-9 is expressed in advancing epithelium only in wound sites where it cleaves bonds between basal cells and basement membranes while acting as a local organizer protein.[91,106] The persistence of inflammatory cell-associated MMP-9 is related to poor tissue repair.[91,107] Overexpression of MMP-9 in chronic human wounds[89,91] and in an ischemic rat wound model was associated with high levels of proinflammatory cytokines also being a cause of prolonged proteolysis and delayed healing.[108]

Stromelysins are closely linked to wound contraction as wounds in MMP-3-deficient mice exhibited a normal-looking epithelialization but contraction was impaired.[109] In contrast, MMP-10 expressed in keratinocytes of the epithelial tongue of skin wounds[93,110] appears to act at the cytoplasmic level and recombinant MMP-10 increased migration of cultured human epidermal keratinocytes.[93] On the other hand, mice overexpressing MMP-10 showed normal wound-repair patterns but keratinocytes at the leading edge exhibited reduced ECM deposition with aberrant laminin-5 and β1-integrin expression.[93]

Integrins

Keratinization and keratinocyte migration are modulated by zinc through the expression of integrins α2β1, α3β1, α6β4, and αvβ5.[111] In intact skin, these integrins are expressed mainly in the basal layer and are responsible for

intercellular and cell-basement membrane adhesion, but they become altered in response to inflammation or tissue injury. Each integrin is expressed during epithelialization in skin wounds but their expression in cultured keratinocytes is modulated by zinc. Supplementary zinc promotes induction of α2, α3, αv, and α6 integrin subunits that influence keratinocyte motility in the healing phase.[111]

Experimental studies of zinc in wound healing

Many of the initial studies conducted in the 1950s and 1960s, purporting to show the intrinsic role of zinc and benefits of supplementary zinc in acute wound healing, were inconclusive.[80] Surprisingly, the researchers used galvanized water bottles for their rats and did not allow for this inadvertently added zinc, which advanced wound repair by 30%.

More recent studies have shown unequivocally that topical zinc therapy reduces wound debris and advances epithelialization in surgical wounds in the rat.[112,113] Observations that topical zinc led to reduction of wound debris and necrotic material in wounds of different etiologies[112,114,115] led Ågren to investigate the action of zinc-dependent MMPs in cultured necrotic tissue from porcine wounds.[116] Zinc oxide advanced the enzymatic breakdown of collagen fragments in vitro through MMPs, which, as discussed above, exhibit substrate specificity for most ECM molecules.[86,116] Locally applied zinc oxide also enhanced the repair of ulcerated skin.[117] Conversely, inhibition of MMPs dramatically delays wound healing.[98]

Strong support is given in recent experimental studies to show that added zinc aids surgical wound repair[113,118] and that induced or hereditary zinc deficiency is detrimental. Rats fed a zinc-deficient diet have poor wound healing,[81,118] although skin zinc stores are not depleted unless both zinc and proteins are severely restricted.[119] The mechanisms responsible for impaired biomechanical strength of and collagen metabolism in incisional wounds of zinc-deficient rats are unclear.[120] One study reported a normal rate of collagen synthesis in wounds of zinc-deficient rats.[121] McClain et al.[122] suggested that cross-linking of collagen was compromised. Yet another possibility is the involvement of zinc in metalloproteinases that cleave propeptides of procollagen molecules,[85,123] a step that determines the rate of collagen synthesis.[124] Interestingly, when supplemental zinc is given to zinc-deficient rats, wound zinc increases and healing progresses as normal, but no difference is seen in the healing pattern in normozincemic animals given extra systemic zinc.[118,119] When applied topically for 12 days, zinc oxide was beneficial in the treatment of full-thickness excisional wounds, regardless of the nutritional status of the rats.[125]

Experiments have been carried out in nutritionally balanced domestic pigs to substantiate the therapeutic value of zinc oxide supplement in wound healing and its putative mechanisms of action.[126] We could demonstrate 30% promotion of healing by topical zinc oxide in both partial-thickness and full-thickness wounds.[126–128] Whereas supplementary zinc oxide advanced epithelialization significantly in these experiments, topical zinc sulfate offered no benefits on wound healing (Figure 1).[127] On the contrary, high levels of zinc sulfate (>15 mmol/L) severely delayed

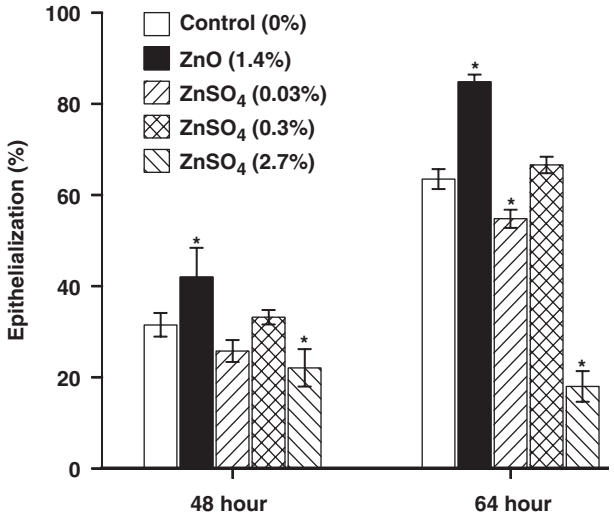

Figure 1. Effect of zinc, in the form of zinc oxide (ZnO) or zinc sulfate heptahydrate ($ZnSO_4$), on epithelialization of partial-thickness porcine wounds 48 and 64 hours postwounding. Zinc concentrations of the treatment groups are given as percentage of elemental zinc per weight vehicle. $*p < 0.05$ compared with control vehicle at the respective time point. Mean ± SEM. Data extracted from Ågren et al.[127]

epithelialization and increased dermal inflammatory cell infiltration. Zinc oxide and the lower zinc sulfate concentrations possessed a mild anti-inflammatory effect.[127] These results indicate that when zinc is added as zinc oxide to wounds, it may exert a pharmacological action on wound healing at the stimulatory level of recombinant growth factors.[129,130] Zinc oxide is advantageous over readily water-soluble zinc compounds e.g., zinc sulfate because it provides sustained release of bioavailable zinc to the wound at noncytotoxic levels.[131–133] Zinc oxide and zinc ion solutions of 500 μmol/L did not elicit cytotoxicity in cultured human dermal fibroblasts,[132] although increased intracellular zinc levels were accompanied by elevated intracellular copper levels.[134]

There are several possible modes of action to explain promotion of epithelialization with supplementary zinc treatment. Increased nuclear MT in wound marginal keratinocytes and in mitotically active cells of the basal epidermis is a positive indication of the involvement of zinc in DNA polymerases in the burst of mitosis that precedes epithelialization.[67,68] Zinc accumulates in proliferating as opposed to stationary epidermal keratinocytes[68] and topical zinc oxide increased keratinocyte proliferation by about 30% in adult murine wounds.[135] Zinc ions mimic the action of growth factors by enhancing intracellular mitogenic signaling pathways,[136,137] and zinc oxide is capable of up-regulating endogenous growth factors, notably insulin-like growth factor-I,[128,138] which may increase epithelialization.[126] In support of these mechanisms is the finding of Mertz et al.,[139] who reported faster epithelialization with a 0.003% zinc/iron solution compared with platelet-derived growth factor-BB in partial-thickness porcine wounds. In addition, an antiapoptotic effect in the epithelium has been ascribed to zinc,[24,140,141] presumably

through its cytoprotective properties against oxidative stress and bacterial toxins.[24,142,143] Modulation of integrin expression[111] and activation of MMPs[98,116] may contribute to enhanced keratinocyte migration with supplementary zinc.

Zinc in gastrointestinal wound healing has been studied in animal models. Vaxman et al.[144] observed a concomitant decreased serum zinc level and increased zinc concentration in the large bowel following laparotomy and construction of colonic anastomoses in rabbits. In dogs, it was found that animals given TPN with a zinc supplement for 2 weeks accumulated almost twice the amount of collagen in 7-day-old colon anastomotic wounds compared with the zinc-free TPN group.[145] Interestingly, serum zinc correlated significantly with collagen deposition in the anastomotic wounds.[145] This indicates a very central physiological role of zinc in collagen accumulation in tissue repair, which can be stimulated by zinc treatment. In another experimental study, zinc given intraperitoneally immediately after operation and daily for 4 days (2 mg/kg/day) increased the bursting pressure of colon anastomoses on the seventh postoperative day in both normal rabbits and rabbits treated with a chemotherapeutic agent.[146] Increased fibroblast infiltration and enhanced epithelialization were observed in the zinc-treated rabbits.[146] We were unable to reproduce these beneficial effects of intraperitoneal zinc sulfate on colon anastomosis repair in a rat model either on postoperative day 3 or 7.

Gastric ulcers in zinc-deficient rats heal slower than in normal controls[147] and zinc compounds can improve mucosal regeneration in zinc-sufficient normal and diabetic rats.[148,149]

CLINICAL VALIDATION OF ZINC IN WOUND MANAGEMENT

Influence of surgery on zinc metabolism and systemic use of zinc in surgical patients

Surgical trauma and infection are associated with redistribution of zinc from the circulation to the liver and possibly other tissues.[150,151] This acute-phase response as a protection against host-accumulated damage or infection is most likely due to induction of MTs because MT-null mice do not react with hypozincemia or hepatic zinc sequestration following lipopolysaccharide administration.[152]

Zorrilla et al.[153] found that impaired wound healing, defined by clinical signs of infection and dehiscence, was significantly related to low levels of zinc in serum obtained from 80 (36 men) patients before they had total hip replacement. We were unable to correlate serum zinc with time to complete healing of open pilonidal wounds in 64 patients (53 men).[19] Zorilla's patients were 66 years old[153] compared with our 25-year-old patients.[19] In a double-blind trial, Faure et al.[154] infused zinc (30 mg/day) pre- and postoperatively for 3 days in 30 patients subjected to major vascular reconstructive surgery. This zinc supplement was sufficient to prevent the postoperative serum zinc decline. Furthermore, significantly fewer wound-healing complications occurred in zinc-treated patients compared with placebo-treated patients.[154] Surgical patients on TPN without trace element supplements are prone to developing symptomatic zinc deficiency,[155] which is relieved by zinc administration.[156] An increased febrile response in patients with pancreatitis and catheter sepsis on TPN with 30 mg elemental zinc supplementation for 3 days has been reported.[157]

Effect of systemic zinc supplementation and chronic wound healing

The majority of studies designed to determine the efficacy of systemic zinc in wound healing have been conducted in patients with chronic wounds. Chronic leg ulcer patients often have abnormal zinc metabolism[57] and low serum zinc levels.[52,53] A recent appraisal concluded that no trial has shown a statistically significant benefit for zinc sulfate in leg ulcer therapy[158] unless there is evidence of low serum zinc.[158] The adverse effects of oral zinc sulfate, usually given as capsules or tablets containing 220 mg thrice daily, include abdominal pain, dyspepsia, nausea, vomiting, and diarrhea.[159]

No correlation was observed between the concentrations of zinc in serum and skin in patients with leg ulcers,[160] suggesting that zinc deficiency condition might exist in spite of normal serum zinc values. In addition to serum zinc measurements, complementary assessment of a patient's dietary intake may provide a useful guide to their zinc status and need for supplementation. Wissing et al.[161] and Raffoul et al.[52] followed cohorts of chronic wound patients and concluded that the patients' zinc intake was in general poor.

Effect of topical zinc on normal and impaired wound healing

Zinc is more commonly used topically, although it is unclear when zinc was first used in the management of skin wounds.[2,162] Pharmacopoeias list zinc sulfate as a local astringent and antiseptic, zinc chloride as an escharotic, and insoluble zinc oxide and calamine as mild antiseptics, astringents, and protective agents, with particular value in treating inflammatory skin conditions and superficial wounds.[163]

The value of topical zinc application in wound care is underpinned by early observations by Henzel et al.[64] They reported that in patients following major surgery, a pronounced decline in blood and tissue zinc, together with increased zincuria and loss of zinc in wound exudates/debris resulted, in up to a 50% reduction in zinc in the granulation tissue and wound margin, creating a local zinc deficit in patients with poor wound healing.

We have investigated the effects of zinc oxide applied topically to chronic and acute wounds in randomized-controlled trials.[19,114,164–166] In a double-blind, placebo-controlled trial, zinc oxide promoted healing of leg ulcers.[166] Furthermore, zinc oxide was as effective as an enzymatic topical debriding agent in the treatment of pressure ulcers.[164] In diabetic foot ulcers, a zinc oxide-medicated occlusive dressing was significantly more effective in debridement compared with autodebridment using a standard hydrocolloid occlusive dressing.[114] Moore[167] strongly advocates topical zinc oxide treatment for diabetic foot ulcers. A debriding effect of zinc oxide has also been

observed in burn wounds.[115] Because hypozincemia[51–53,114] and delayed wound healing are common in these patients, it cannot be concluded from these studies that zinc is also effective in patients with normal zinc stores. In a recently completed double-blind, placebo-controlled trial, topical zinc oxide did not significantly decrease time to closure of open pilonidal wounds.[19] The less obvious effect of zinc in this wound type may be explained by the observations that zinc stimulates epithelialization more than wound contraction in experimental wounds.[126] In support of this, results from smaller clinical trials indicate the beneficial effects of topical zinc on human wounds healing predominantly by epithelialization such as suction-blister wounds,[168] superficial (1 mm deep) small incisions,[169] and split-thickness skin graft donor sites.[170] Taken together, larger-scale trials are urgently required to verify the trend of accelerated wound healing with topical zinc oxide in acute human wounds.

Intriguingly, topical zinc reduced oral antibiotic consumption significantly compared with placebo treatment.[19] Furthermore, *Staphylococcus aureus* was cultured significantly less frequently from zinc oxide-treated than from placebo-treated wounds,[19] substantiating its mild anecdotal antiseptic property.[163]

Zinc is an antimicrobial and anti-inflammatory agent

Zinc is an essential micronutrient also for prokaryotic organisms. Zinc homeostasis in prokaryotes is regulated through a number of specific and nonspecific membrane-bound uptake and efflux pumps.[171] Intracellular free zinc levels are maintained at subtoxic levels through MTs unrelated by evolution to the MT in eukaryotic cells.[172]

At superphysiological levels, zinc inhibits the growth of several bacterial species. Gram-positive organisms appear to be more sensitive to zinc than Gram-negative bacteria. For example, minimum inhibitory concentrations (MICs) of Zn^{2+} on aerobic bacteria isolated from human wound infections were determined in one study.[173] Four susceptibility grades emerged from the study:

1. *Streptococcus* groups A, C, and G (MICs $\leq$0.5–2 mmol/L);
2. *Staphylococcus aureus*, *Streptococcus* group B (MICs 2–4 mmol/L);
3. *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp. (MICs 4–8 mmol/L); and
4. *Proteus* sp., *Pseudomonas aeruginosa*, *Enterococcus* sp. (MICs 8–32 mmol/L).

Similar sensitivity patterns were observed for clinical isolates from burn wounds.[174] Zinc oxide also showed antibacterial activity against aerobic and anaerobic endodontal pathogens.[175,176].

Zinc oxide inhibits attachment[177] and growth of *S. aureus* in vitro.[178,179] In vivo, Sunzel et al.[180] inoculated subcutaneous steel-wire cages implanted in guinea-pigs and rabbits with *S. aureus* (strain 209 P) and demonstrated significantly reduced growth in the presence of zinc oxide.

There is currently a trend in the increased use of antimicrobial agents like silver and iodine.[181,182] The effectiveness of antimicrobial therapies depends on host defense mechanisms and virulence factors.[183] Zinc concentrates naturally in tissues showing high cell turnover, including the bone marrow and thymus, and is considered to be an important regulator for macrophages and polymorphonuclear leukocytes.[184,185] Zinc is also capable of inhibiting nitric oxide formation[186] and prevents sulfhydryl groups from oxidation, other possible mechanisms of the anti-inflammatory activity of zinc.[187] The functionality of zinc in antimicrobial peptides needs further elucidation.[188] Biofilm formation in wounds is an important determinant for the effectiveness of antimicrobial therapy.[183] Although zinc at 1 mmol/L depressed the growth of *E. coli*, the movement of zinc into deeper layers of biofilms was limited.[189] The emergence of methicillin-resistant *S. aureus* (MRSA) is a growing problem in wound care. Ugur et al.[190] reported 13.6% MRSA resistance to zinc. They defined resistance as MRSA strains surviving zinc concentrations above 1 mmol/L. Resistance mechanisms involve the induction of efflux pumps that lowers intracellular Zn^{2+} concentrations.[191]

Thus, as zinc supplements possess mild antimicrobial properties against common wound flora[173] and aid a host's defense system against infections,[178] consideration might usefully be given to zinc products in wound care.

ZINC PRODUCTS

Zinc-containing products available for topical application in wound management include paste bandages, stockings, and occlusive adhesive dressings, alginates, and zinc-saline dressings (Table 1).

Zinc paste bandages or Unna boot composed of open-wove cotton gauze impregnated with zinc oxide paste remain as standard treatments for leg ulcers.[192] Unna boot provides a protective barrier[199] and anti-inflammatory benefit to varicose eczema.[200] Zinc itself may occasionally cause burning, stinging, itching, and tingling when applied to inflamed tissues. Hypersensitivity to topical zinc oxide is absent or rare[201] and most commonly associated with the excipients of the dressing.[165,193] Preservatives, especially parabens but also cetearyl alcohol, can sensitize the skin[193] and have been omitted in newer and sterile bandages.

Zinc paste bandages must be applied loosely and lightly. In dressing leg ulcers, a bandage should be applied from the base of the toes to the tibial tuberosities, ensuring that the heel is completely enclosed. The bandage must be pleated at each turn to accommodate potential edema. Alternatively, the bandage may be cut into strips and overlap each layer by 50%. The bandage must be covered either by a retention bandage such as crêpe or a compression bandage if the arterial circulation is adequate. Paste bandages can be left in place for up to a week for treatment of ulcers and up to 2 weeks if treating the skin alone.

A three-armed randomized clinical trial involving 113 venous leg ulcer patients compared a zinc paste bandage with a zinc oxide-medicated stocking and a calcium alginate dressing.[195] The zinc products and alginate dressing were applied in conjunction with compression bandages. The ulcers healed significantly faster in patients treated with the zinc paste bandage compared with the zinc stocking and the alginate dressing. The authors concluded that the improved healing rates were attributable to improved venous blood return during exercise through the extra compression delivered by the nonelastic paste bandage

Table 1. Nonexhaustive list of commercial zinc-containing wound care products

Product	Manufacturer	Zinc content and other ingredients	Documentation
Zinc paste bandages (Unna boot) [192]			
Calaband®[2, 193]	Mölnlycke Health Care, Göteborg, Sweden	Zinc oxide (9.25%) Calamine (5.75%) Phenosept	
Gelocast®	Smith & Nephew, Hull, UK	Zinc oxide (10%)	
Steripaste®[194]	Mölnlycke	Zinc oxide (15%)	
Varolast®	Hartmann, Heidenheim, Germany	Zinc oxide (15%) Methyl and propyl *p*-hydroxybenzoates	
Viscopaste® PB7[194]	Smith & Nephew	Zinc oxide (10%) Cetearyl alcohol, methyl and propyl *p*-hydroxybenzoates	Venous leg ulcers[195]
Zincaband®[2,193]	Mölnlycke	Zinc oxide (15%) Propyl *p*-hydroxybenzoate	
Zipzoc® (stocking)	Smith & Nephew	Zinc oxide (20%)	Venous leg ulcers[195,196]
Other zinc-supplemented dressings			
Curasorb® Zn	Tyco, Mansfield, MA	Zinc-impregnated (0.18%*) calcium alginate	
Dermagran® Hydrophilic	Dermascience, Princeton, NJ	Zinc ointment (0.05%*) in nonwoven swab Zinc-saline formulation in gauze	Chronic skin ulcers of different etiologies[197]
Mezinc®	Abigo Medical, Askim, Sweden	Zinc oxide (25%) and zinc resinates in an adhesive mass	Diabetic foot ulcers[114] Burns[115] Venous and arterial leg ulcers[165]
Trionic®	Johnson & Johnson Wound Management, Norderstedt, Germany	Zinc (0.03%*), calcium and manganese supplemented alginate	Medium to heavy exudating secondary healing wounds[198]

*Determined by atomic absorption spectrophotometry[129] and given as percentage of elemental zinc per weight dressing material.

and that zinc oxide did not account for the healing differences. This conclusion may be incorrect as the vehicle may have masked any beneficial effect of zinc oxide in the stocking.[132]

Indications for zinc-containing ointments, creams, and lotions in dermatotherapy were recently reviewed by Schwartz et al.[202]

ABSORPTION OF ZINC THROUGH THE SKIN AND FROM WOUNDS

Percutaneous absorption of zinc is greatly influenced by the integrity of the natural skin barrier function afforded by the stratum coreum.[203,204] Regulatory processes are not known but presumably MTs, present in basal epidermal cells and hair papillae,[69] have a role in percutaneous zinc uptake. MTs are induced locally and distally by topical application of zinc.[205–207] Topically applied glucocorticoids also induce MTs.[205] It is unclear presently whether transporter genes of the ZIP or ZnT classes are involved in percutaneous absorption.

Zinc absorption by the skin is influenced by the amphiphilia of the vehicle and physicochemical properties (solubility, pH, molecular weight, partition coefficient) and concentration of the zinc compounds in the topical formulations.[208–210] Although penetration of the zinc ion through intact skin in normozincemic humans is low from topical zinc oxide,[211] dermal absorption was demonstrated with a zinc oxide cream (Triple Care, Smith & Nephew, Hull, UK) applied for 3 hours to the forearm arm of healthy volunteers.[212] Zinc oxide hydrolyzes in the presence of skin surface acidic moisture to release biologically active Zn^{2+}[63]; this readily reacts with sulfhydryl groups in epidermal keratin. Ågren[63] showed that zinc oxide in a rosin-based occlusive adhesive dressing applied to normal forearm human skin led to an initial accumulation of zinc in the stratum corneum of the epidermis and penetrated to deeper levels after longer exposure. Increased zinc levels were measured in interstitial fluid and dermis within 48 hours. No zinc penetration was observed when zinc oxide was incorporated into an occlusive hydrocolloid adhesive dressing presumably due to lack of formation of lipophilic zinc resinates.[213] Gamer et al.[214] also failed to demonstrate zinc penetration from microfine zinc oxide applied to intact porcine skin in vitro. Increased zinc deposition is seen in epidermal keratin with acidic preparations like zinc chloride under occlusive conditions.[141,208,215] The greater acidity favors ionization and the increased state of hydration enhances percutaneous absorption.[216]

The zinc ion bound to epidermal keratin will be lost as superficial keratinocytes are shed naturally, whereas a fraction of zinc penetrating more deeply will be absorbed into the systemic circulation. Maximal systemic absorption of zinc was observed within 1 hour after topical application of ^{65}Zn-labeled zinc chloride or zinc oxide to 6 cm^2 of intact rat skin.[217] A similar distribution of absorbed zinc with the two zinc compounds was observed.[217] Studies in rats have shown that hypozincemias evoked by dietary deprivation can be alleviated by topical application of zinc compounds.[218] Whitehouse et al.[219] noted a therapeutic effect of dry zinc oxide rubbed into a 6 cm^2 area of rat skin. The organic zinc monoglycerolate complex permeated skin more efficiently than zinc oxide and its therapeutic effect was comparable to that of the immunosuppressant cyclosporine in an arthritis model in rats.[219] The clinical relevance of the transdermal route in relieving zinc deficiency symptoms is speculative. Neither Derry et al.[220] nor Morgan et al.[221] were able to monitor an increased serum zinc level after treating extensive skin areas with topical zinc oxide in petrolatum.

Without skin barrier, systemic zinc absorption from topical zinc is substantially increased.[217] In patients with extensive (5–20% of total body surface area) partial-thickness and full-thickness burn wounds, the serum zinc level increased after treatment with a zinc oxide-medicated adhesive dressing.[115,217] In patients with smaller wounds (10 cm^2), no significant difference in serum zinc was observed between the zinc oxide-treated and control-treated patients.[19] An increase in serum zinc can be detected in zinc oxide-treated rats with wounds accounting for more than 5% of the total body surface area.[131,213,217]

CONCLUSIONS

Patients require supplementary oral or topical zinc if normal wound healing is to occur in the face of a pre-existing deficiency state.[64,158,222] Difficulties arise in the diagnosis of hypozincemia,[33] and serum zinc may not be an accurate monitor of subnormal zinc. Evidence is presented here to show that zinc delivered locally provides therapeutic advantages in treatment of not only zinc-deficient chronic wounds but also of surgical wounds, including pilonidal sinus lesions.[19] Topical zinc oxide treatment increases local bioavailable zinc concentrations to fairly constant levels of 1,000–3,000 μmol/L.[19,131,180] These zinc ion levels are atoxic[132] and protective[142,143] to host cells but sufficiently high to augment antibacterial mechanisms in addition to MMP-mediated elimination of necrotic tissue[112,114,116,164] and facilitation of keratinocyte migration.[98,127]

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