Nutrition in wound healing: investigation of the molecular mechanisms, a narrative review

Abstract: Nutrition can be outlined in terms of epigenetic signals influencing each of the wound healing steps (haemostasis, inflammatory, proliferative and remodelling phase). Specific nutrients, such as amino acids, minerals, vitamins, natural compounds and herbal extracts, target DNA-regulating transcription factors, cytokines, extracellular matrix proteins and glycosaminoglycan, and are specifically involved in the wound healing process. This review focuses on experimental *in vivo* and clinical evidence of dietary supplements administration in pressure ulcers. A good nutritional status is, for example, fundamental to the haemostasis phase of skin wounds. In the inflammatory phase, vitamin A enhances cytokine release, bromelain and amino acids prevent prolonged inflammatory events, while vitamin C enhances neutrophil migration and lymphocyte activation. In the proliferative phase, vitamin C and *Centella asiatica* are required for collagen synthesis. Glucosamine enhances hyaluronic acid production, vitamin A promotes epithelial cell differentiation, zinc is required for DNA and protein synthesis and cell division, and *Aloe vera* supports granulation tissue generation. Finally, in the remodelling phase, amino acids and proteins play a key role in wound scar stabilisation.

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Wound healing is a complex process controlled by soluble mediators, blood cells, and parenchymal cells.[1] In our previous work, we described how epigenetics influences wound healing[2] by targeting DNA, key molecules such as RNA and microRNAs. Epigenetic factors also regulate transcription factors, cytokines, extracellular matrix (ECM) proteins and glycosaminoglycan (GAG). Among these, nutrition is an epigenetic signal that can actively influence every step of the wound healing process.[3]

Wound healing requires dietary amino acids, vitamins and minerals,[4] although some natural compounds, including herbs and extracts, may have a synergistic effect to speed up this process. An adequate nutritional status and health condition is crucial for hard-to-heal wounds, such as pressure ulcers (PU).[5] This raises the question of whether supplementation of selected nutrients, known to be determinants of the wound healing process, might be helpful, either in prevention or therapy, in high risk conditions, such as older age, cancer and in immunosuppressed patients.[6]

The literature describing amino acids, vitamins, minerals, natural compounds, herbs and extracts supplementation, in wound healing is exhaustive, there is a lack of information on genetic background involvement. MacKay et al.[7] schematically reported essential nutrients for each step of wound healing process (Table 1).

Adequate amounts of nutrients are necessary for the synthesis of nucleic acids (DNA and RNA), proteins and other factors involved in functional tissue maturation and differentiation.[8] Malnutrition is largely associated with a delay or failure of the healing process, but nutritional intervention can mitigate malnutrition and improve wound healing, mainly by increasing collagen deposition after trauma.[9] However, the exact molecular mechanisms underlying this effect is not clear.[10] It has been reported that malnutrition involves low levels of wound TGF-β mRNA levels.[1] This unbalanced nutritional condition may be improved by a dietary supplement, promoting wound closure.

Several screening tools, which incorporate historical, haematologic and anthropomorphic data, have been developed and validated for assessing nutritional status with reference to skin ulceration.[11] These include the Subjective Global Assessment (SGA), Malnutrition Universal Screening Tool (MUST) and Mini-Nutritional Assessment (MNA) tool.[12,13] Each has been highly correlated with the risk of malnutrition. In older patients, the MNA is more specific than the SGA.[14]

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Table 1. Essential nutrients in wound healing phases. Modified from MacKay and Miller[6]

Wound healing phase	Effect
Haemostasis	
Drugs, herbs, vitamins, amino acids, minerals	Blood clotting
Inflammatory phase	
Vitamin A	Enhances early inflammatory phase
Bromelain and adequate protein intake	Prevents prolonging inflammatory phase
Vitamin C	Enhances neutrophil migration and lymphocyte transformation
Proliferative Phase	
Vitamin C, *Centella asiatica*	Necessary for collagen synthesis
Glucosamine	Enhances hyaluronic acid production
Vitamin A	Promotes epithelial cell differentiation
Zinc	Required for DNA synthesis, cell division, and protein synthesis
Aloe vera	Support formation of granulation tissue
Remodelling phase	
Amino acids and proteins	Wound remodelling

In this review, we describe an experimental *in vivo* and clinical study with dietary supplement administration in pressure ulcers (PU) and hard-to-heal wounds, with particular focus on gene regulation and molecular pathway.

Dietary supplementation of amino acids

Arginine

Arginine (ARG) is a dietary, conditionally essential amino acid. Its supplementation promotes wound healing, increasing breaking strength and scar collagen deposition.[15] About 50% of ingested arginine is released into the portal circulation and the remainder is directly used in the small intestine.[16] Circulating arginine is a substrate for protein and collagen metabolism in extrahepatic tissues.[17] The kidney metabolises citrulline (the major precursor for arginine) into arginine and exports it into the systemic circulation. Arginine stimulates protein synthesis, the function of T-lymphocytes and regulates nitric oxide (NO) activity.[16] It has been suggested that the presence of NO produced from arginine aids the transition of a wound from the acute inflammatory phase to the proliferative phase of wound healing.[18]

Experimental *in vivo* studies

Shi et al.[19] studied whether arginine can enhance cutaneous wound healing in inducible NO synthase-knockout (iNOS-KO) mice. There were 20 iNOS-KO and 20 wild type (WT) mice divided into four groups of 10 genotypes. They were then randomised to receive either normal food and tap water or food and water, each supplemented with 0.5% arginine (w/w). A 2.5cm dorsal skin incision with implantation of four 20mg polyvinyl alcohol sponges into subcutaneous pockets were conducted in all animals. After 14 days the animals were euthanised. The dorsal wound was harvested for breaking strength determination and the wound sponges were assayed for hydroxyproline content and total wound fluid nitrite/nitrate concentration. Results showed that dietary arginine supplementation enhanced both wound breaking strength and collagen deposition in WT but not iNOS-KO mice. Wound fluid nitrite/nitrate levels were higher in WT mice than the iNOS-KO animals but were not significantly influenced by additional arginine. These data demonstrate that supplemental dietary arginine enhances wound healing in normal mice. The loss of a functional iNOS gene inhibits the beneficial effect of arginine in wound healing. This suggests that the metabolism of arginine via the NO pathway is one mechanism by which arginine enhances wound healing. Effects of 1% dietary ARG-HC1 supplements (0.5% added to a regular commercial rat diet containing 1.8% ARG, 0.5% in drinking water) were studied in (a) hypophysectomised (hypox) rats supplemented with ACTH, L-thyroxine, testosterone propionate, (b) such hypox rats additionally supplemented with bovine growth hormone (hypox+bGH), (c) intact rats (Int), and (d) intact rats supplemented with bovine growth hormone (Int+bGH).

Group (a) hypox rats healed their wounds as rapidly as intact rats (measured by dorsal skin incision breaking strength, accumulation of reparative collagen in polyvinyl alcohol sponges). Group (b) hypox+bGH rats showed increased wound breaking strength and accumulation of reparative collagen in the sc polyvinyl alcohols sponges to levels significantly greater than those of intact controls. bGH given to intact controls did not affect these indices of wound healing. Supplemental ARG-HC1 given to intact rats significantly minimised immediate postoperative weight loss, increased wound breaking strength and sponge reparative collagen accumulation and increased thymic weight. None of these effects of supplemental ARG-HCI was observed in group (a) hypox rats or group (b) hypox+bGH rats. These results supposed that an intact hypothalamic-pituitary axis is necessary for these beneficial effects of supplemental ARG-HC1 given to wounded rats.[20] Moreover, these results agree with previous studies, which demonstrated that supplemental arginine not only lessens the loss of thymic weight after injury, but also lessens the decrease in thymic lymphocyte counts and increases the *in vitro* mitogenic reactivity of thymic lymphocytes from healthy and injured rodents.[21] Probably the pituitary of injured animals secretes both pro- and anti-wound healing factors and a supplementation with a fixed dose of hormones ACTH (adrenocorticotropic hormone), levothyroxine, testosterone propionate, and with and

without growth hormone, may somehow 'replace' these pro-wound healing factors. Arginine might have a pivotal role in this balance.

Clinical studies

van Anholt et al.[22] clinically investigated the potential of a high-protein, arginine- and micronutrient-enriched oral nutritional supplement (ONS) to improve wound healing of PUs.[23] This multicentric, randomised, controlled, double-blind study included non-malnourished patients with category III or IV PUs. Patients were treated with 200ml of the specific ONS or a non-caloric control product three times a day, in addition to their regular diet and standard wound care, for a maximum of eight weeks. Collected data were compared with repeated-measures mixed models (RMMM), analysis of variance, or Fisher's exact tests for categorical parameters. Results demonstrated that the supplementation with the specific ONS accelerated PU healing, indicated by a significantly faster decrease in ulcer size compared with the control after eight weeks ($p=0.016$, RMMM). The decrease in severity score (Pressure Ulcer Scale for Healing) in the supplemented group differed significantly ($p=0.033$, RMMM) from the control. At the end of the study, blood vitamin C levels had significantly increased in the ONS group compared with the control. These results suggested that the nutritional supplementation accelerated healing of PUs and reduced wound care intensity in non-malnourished patients.

Similarly, Desneves et al.[24] investigated the efficacy of a nutraceutical supplementation (arginine 9g, vitamin C 500mg, zinc 30mg) in the healing of PUs.[17] patients with a category II, III or IV PU were randomised to receive daily a standard hospital diet, a standard diet plus two high-protein/energy supplements, or a standard diet plus two high-protein/energy supplements containing the additional nutraceutical supplementation. Anthropometric and biochemical measures were collected, and PU size and severity (by PUSH tool; Pressure Ulcer Scale for Healing; 0=completely healed, 17=greatest severity were measured weekly for three weeks. Results showed that only patients receiving the additional nutraceutical supplementation demonstrated a clinically significant improvement in PU healing (9.47 versus 2.67; baseline and week 3, respectively; $p<0.01$). There were no significant changes in biochemical markers, oral dietary intake or weight in any group. These results support the efficacy of the additional arginine, vitamin C and zinc supplementation in improving the rate of PU healing.

Arginine efficacy in wound healing was also investigated by Zhang XJ et al.[25] The authors analysed the effect of L-arginine supplementation on protein metabolism in skin wound and in muscles of anaesthetised rabbits. L-[ring-13C6] phenylalanine was infused as a tracer on day seven after ear injury, and the scalded ear and uninjured hind limb were used as arteriovenous units to reflect protein kinetics in these two tissues. In study one, an amino acid mixture (10% Travasol) was infused either alone at 1.5ml/kg/hour or at 0.75 ml/kg/hour with supplemental L-arginine to deliver a comparable amount of aminoacid nitrogen. In study two, N(omega)-nitro-L-arginine methyl ester was infused to inhibit nitric oxide synthase during the stable isotope infusion. Results of study one showed that arginine supplementation increased ($p<0.05$) net protein balance in the skin wound and muscle from –6.7±6.2 to –0.8±3.8, and from –4.4±2.4 to –1.9±1.5µmol phenylalanine/100g/hour, respectively, indicating an anabolic effect.[25] Results of study two showed that the N(omega)-nitro-L-arginine methyl ester infusion markedly reduced the blood flow rate in the scalded ear and increased ($p<0.05$) net protein balance in the skin wound and muscle from –8.6±3.4 to –1.0±5.7 and from –3.9±1.3 to –2.2±0.5µmol phenylalanine/100g/hour, respectively.[25]

Sipahi et al.[26] investigated the effect of beta-hydroxy-beta-methylbutyrate, arginine and glutamine supplementation for four weeks on the wound healing of 11 patients undergoing diabetic dialysis. After four weeks, in accordance with the Bates-Jensen scoring, healing was observed on the wound depth score of seven (63.6%) patients and on the wound appearance score of eight (72.7%) patients out of 11. While the wound depth score of four (36.4%) patients and wound appearance score of three (27.3%) patients remained the same, no deterioration was observed on any cases throughout the follow-up period. The supplementation used may thus positively contribute to wound healing in patients undergoing diabetic dialysis.

Barbul et al., demonstrated that the beneficial effects of arginine in wound healing are related, in part, to gene-mediated hormone secretagogue actions of arginine[20] (growth hormone, prolactin, insulin and glucagon).[27,28]

Proline

Proline (PRO) is a non-essential amino acid, the largest constituent of the collagen molecule, with its derivative, hydroxyproline, also well represented. Approximately 99.8% of the body's stores of hydroxyproline are found in collagen, which renders assays of this amino acid useful as a marker for the total amount of collagen present. The hydroxylation mechanism of proline happens post-translationally by the enzyme prolyl hydroxylase, which requires oxygen, ascorbate and iron as cofactors.[29] Both the molecules are essential for collagen biosynthesis, structure and strength.[30] Although plasma levels of proline can be quite variable, its importance in human diet is not well understood. It has been reported that patients with proline deficiency, due to the lack of the enzyme prolyl hydroxylase, have different wound-healing deficits.[31] Physiologically, during the first 10 days of healing, wound proline levels are 30–50% higher than plasma levels, suggesting that import of proline into the wound occurs actively or that biosynthesis of proline takes place in the wound

environment. Then it is essential to provide additional proline into the diet, although it is not clear if supplementation of proline and hydroxyproline directly induces efficient collagen synthesis during the wound healing process. Proline biosynthesis is related to both the citric acid cycle and the urea cycle. Consequently, supplying citric cycle precursors, such as glutamine, may be strategic for enhancing wound collagen synthesis. As to the urea cycle, arginine is converted to ornithine through the action of arginase, a key enzyme of this pathway. Ornithine, through the action of ornithine g-aminotransferase, is converted to glutamic g-semialdehyde, the link to proline synthesis. Experimentally and clinically, it has been reported that supplemental arginine is more effective to support wound healing, as well as in collagen deposition, than proline and glutamine supplementation.[29] This effect is also shared by ornithine, which cannot replace arginine for growth requirement but shares many of its biological and pharmacological activities.

Experimental *in vivo* studies

A study of male Sprague Dawley rats (250–300g body weight) fed with 1% dietary supplement of proline, and which had dorsal skin incision and subcutaneous implantation of polyvinyl alcohol sponges, showed no difference in wound breaking strength or in wound collagen deposition after 10 days of supplementation. This effect may be related to metabolic disposal that does not lead to increased availability in the mitochondria where collagen molecule translation and transcription occurs.[29]

N-acetylcysteine

N-acetylcysteine (NAC) is an antioxidant acetylated precursor of both the amino acid L-cysteine and reduced glutathione (GSH).[32] N-acetylcysteine is a naturally occurring food compound (such as, in garlic and onion) and it is also synthesised by the body. N-acetylcysteine is considered as an antioxidant in three proposed mechanisms. First, N-acetylcysteine has been shown to react directly with various reactive oxygen species (ROS).[33] Secondly, N-acetylcysteine is a cysteine pro-drug and may exert its antioxidant effects by enhancing tissue levels of GSH.[34] Finally, N-acetylcysteine treatment in mouse fibroblasts induces superoxide dismutase enzyme expression through a transit increase in superoxide measured by electron spin resonance spectroscopy.[35]

Experimental *in vivo* studies

Lim Y et al.[36] suppressed NF-κB activation in rats and consequently synthesis and releases of pro-inflammatory cytokines. The authors observed that wound healing time was significantly prolonged and this correlated with decreased expression of inhibitory kappa B alpha (InBa), interleukin-1h (IL-1h) and TNF-α expression and reduced neutrophil infiltration. Genetically modified (zinc superoxide dismutase enzyme was overexpressed) rats were supplemented with N-acetylcysteine, improving the wound healing process during a protein deficient diet and also restoring the expression of InBa, IL-1h and TNF-α, and neutrophil infiltration to normal levels, similar to the control animals. It is possible to conclude that alteration in protein malnutrition may influence wound healing closure in a ROS- and NFnB-dependent way.

Glutamine

Glutamine is a five-carbon, non-essential amino acid[37] with the highest levels in plasma cerebrospinal fluid and skeletal muscle. The majority of cells and tissues are able to synthesise glutamine from glutamate and ammonia, in a process catalysed by the enzyme glutamine synthetase (GS). Glutamine is a key component of nitrogen metabolism with a specific role to store glutamate and ammonia.[38] It is also a precursor of glutathione, proline, purines and pyrimidines.[37] The gastrointestinal tract harbours immune cells and fibroblasts, nutritionally supported by glutamine, these play an essential role in the wound healing process.[39]

Clinical studies

Blass et al.[40] investigated if micronutrient antioxidant supplementation and glutamine accelerated wound healing. They enrolled 20 patients with disorders in wound healing in a randomised, double-blind, placebo-controlled trial. Patients received antioxidant micronutrients (ascorbic acid, alpha-tocopherol, beta-carotene, zinc, selenium) and glutamine (verum) or isoenergetic amounts of maltodextrine (placebo) for 14 days. Serum levels of micronutrients, glutamine and VEGF-A were measured before and after supplementation. Moreover, several parameters of microcirculation were assessed. Results showed that serum levels of micronutrients were not modified, except for selenium, which increased in the verum group (1.1±0.2 versus 1.4±0.2 µmol/l; p=0.009). Glutamine levels decreased only in the placebo group (562±68 versus 526±55 µmol/l; p=0.047). The prevalence of hypovitaminoses and the concentration of VEGF-A remained unchanged. Considering microcirculation, only oxygen (O_2) saturation decreased in the placebo group (56.7±23.4 versus 44.0±24.0 [arbitrary units]; p=0.043). Wound closure occurred more rapidly in the verum than in the placebo group (35±22 versus 70±35 d; p=0.01). These results suggest that oral antioxidant supplementation and glutamine reduced wound healing time.

Dietary supplementation of whey proteins

Whey proteins (WP) promote immune-cell activity and obviously conditions some immunological experiments.

Experimental *in vivo* studies.

Badr et al.[41] investigated if camel whey proteins may have some effect on diabetic wound healing closure in a streptozotocin (STZ)-induced type I diabetic mouse

model. An experimental group was supplemented with whey proteins (100mg/kg body weight dissolved in distilled water 250µl/day for one month), while a control group was supplemented with distilled water (250µl/mouse/day for one month by oral gavage). After one month, diabetic mice showed reduced wound closure related to a significant reduction of tissue anti-inflammatory cytokines IL-10 and to a significant increment of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6). However, an abnormal expression of chemokines involved in leukocyte accumulation at wound sites, such as macrophage inflammatory proteins-1α and -2 (MIP-1α, MIP-2), keratinocyte-derived chemokine (KC), CX3CL1 (a soluble chemokine and a membrane-bound form on the surface of inflamed endothelial cells, epithelial cells, macrophages and vascular smooth muscle cells), and TGF-β, were observed in diabetic mice, compared with non-diabetic mice. The supplementation with camel whey proteins significantly accelerated the closure of diabetic wounds restoring normal levels of anti-inflammatory and pro-inflammatory cytokines. Notably, the supplementation with camel whey proteins also restored normal expression of MIP-1α, MIP-2, KC, CX3CL1 and TGF-β in diabetic wound tissue, compared with the untreated control group. These data support the evidence that supplementation with camel whey proteins may improve diabetic wound closure by restoring the immune response in diabetic mice.

With a comparable protocol of study, the same author investigated the effects of camel whey protein supplementation on the mRNA and protein expression levels of β-defensin-1 (BD-1), BD-2 and BD-3 and subsequently on the wound healing process in a streptozotocin (STZ)-induced diabetic mouse model.[42] Mice were divided into three groups of 10: group one was non-diabetic mice (control); group two was diabetic mice; and group three was diabetic mice that received a daily supplement of undenatured camel whey proteins (100mg/kg of body weight) via oral gavage for one month. Results showed that, compared with the non-diabetic control mice, the diabetic mice exhibited delayed wound closure, characterised by a reduction in hydroxyproline content (indicator of collagen deposition), a marked increment in free radical levels and a prolonged elevation in the levels of the pro-inflammatory cytokines, IL-6, TGF-β, and TNF-α. Mice supplemented with camel whey proteins had accelerated diabetic wound healing compared with untreated diabetic mice. Furthermore, the camel whey protein supplementation reduced free radicals level and restored hydroxyproline content, pro-inflammatory cytokine levels, and expression of BD-1, BD-2 and BD-3 at normal levels in wound tissue. It is known that, in human tissues, BD-1, BD-2 and BD-3 promote wound healing thanks to their antimicrobial effects on wound repair and immune cells. Consequently, the supplementation of diabetic mice with camel whey proteins significantly restored the mRNA and protein expression levels of BD-1, BD-2 and BD-3. These results support the hypothesis that camel whey protein supplementation may be effective for accelerating diabetic wound closure.

Dietary supplementation of minerals

Zinc

Zinc is an important micronutrient with several physiological roles in the body, for example, it is required for activating body growth,[43] it fights against infections with the strongest role on immune system stimulation compared with all other vitamins and minerals.[44] Its wound healing accelerating action is explained by several mechanisms.[23] These mechanisms include the stimulation of DNA synthesis and immune function, upgrading.[45] Unfortunately, the exact mechanism of action of zinc is not fully understood.

Experimental *in vivo* studies

Animal studies confirm that zinc is most important during the later stages of tissue repair and regeneration, as compared with the first inflammatory stage. In fact, during the initial inflammatory phase of wound healing, zinc concentrations gradually decrease, probably because of the production of IL-6, TNF-α, and calprotectin, a zinc-binding protein. The initial reduction of zinc concentration in serum and in the wound bed promotes early phase wound healing by delaying maximal phagocytic and related immune activity within the wound bed, modifying the Langerhans cells, boosting local immunity and with anti-inflammatory effect on phagocytic cells, suitable in the remodelling phase. Subsequently, the zinc concentration in serum and in the wound bed gradually increases, up to 15–20% higher than those found in intact skin.[46]

Dietary supplementation of vitamins

Vitamin A

Vitamin A is required for epithelial tissue development, and normal wound repair.[47] The relationship between vitamin A, immune system function and wound healing is poorly understood.

Experimental *in vivo* studies

Vitamin A deficiency is known to interfere with neutrophil development, increasing the total number of neutrophils but negatively influencing cell maturation and function. Immature neutrophils demonstrate poor migration from local capillaries into infected skin, or an infected wound bed, as well as diminished phagocytosis and oxidative metabolism. Vitamin A deficiency is also correlated with the production of immature, poorly functioning macrophages that are less efficient at ingesting and killing bacteria than mature cells, enhancing the early inflammatory phase of the healing process. Moreover, vitamin A deficiency is associated with production of immature fibroblasts, leading to impaired collagen synthesis and deposition.[48,49] In fact, supplementation

with topical retinol has been shown to increase fibroblast growth and collagen synthesis and reduce matrix metalloproteinase expression in aged and sun-damaged skin.

Vitamin E

Vitamin E encompasses eight lipophilic compounds with different physiochemical properties, which are further subdivided into two groups of four isomers, tocopherols and tocotrienols, which differ slightly in structure, alpha-tocopherol being the most potent and abundant form *in vivo*.[50] Through its scavenging activity, vitamin E protects the cell membranes and polyunsaturated lipids from ROS attack by inducing the activation of various signal transduction pathways and is thus recognised mostly for its role as an antioxidant.[51] Indeed, studies have focused mainly on its antioxidant properties, although the role of the vitamin itself is broader and varied.[52] Vitamin E also modulates the expression of connective tissue growth factor (CTGF)[53] and regulates gene expression and transcription, thereby facilitating the protection of wounds against infections such as methicillin-resistant *Staphylococcus aureus* (MRSA).[54]

Experimental *in vivo* studies

In animal models, it has been reported that vitamin E shifts macrophage production and function.[55] Although the exact mechanism of action is not well understood, it has been supposed that the beneficial effects of vitamin E on immune function go beyond that of a simple antioxidant,[56] while its role in the management of cutaneous wounds remains largely unknown.

Vitamin C

Being an antioxidant, vitamin C reacts with and deactivates biologically significant radicals and oxidants.[57] In general, vitamin C fortifies collagen biosynthesis (lack of vitamin C causes collagen malformation) and the synthesis of ceramides to form strong barrier lipids in the epidermis.[58]

Clinical studies

High-dose supplementation of vitamin C supports the healing of surgical wounds in healthy subjects and hard-to-heal PUs.[6] In clinical practice, supplementation of vitamin C in doses of about 500mg, combined with at least 17mg zinc, and even in combination with arginine, is helpful for wound healing.[59]

It enhances the collagen synthesis,[60] as demonstrated by vitamin C supplementation in an 8-year-old boy with Ehlers-Danlos syndrome Type VI, an autosomal recessive connective tissue disease.[61] In addition, vitamin C is mandatory for the formation of cross-links between collagen fibres, for fibroblast maturation and for angiogenesis.[62] Some studies showed that patients with ulcers have deficient levels of this vitamin, pooled in the wound area where cell growth and differentiation are obviously increased.[40] Vitamin C is known to induce this enzyme in osteoblasts and chondrocytes cells, suppressing the inflammatory phase and promoting fibronectin and collagen I synthesis.[63]

Dietary herbal extracts and natural compounds

Fermented papaya

Papaya is widely known as a medicinal fruit.[64] Several studies support the hypothesis that treatment with papaya preparations may help facilitate the wound healing process.[64–69] Specifically, topical administration of the papaya-derived enzyme papain, may promote enzymatic wound debridement.[70]

Experimental *in vivo* studies

The aqueous extract of *Carica papaya* fruit induces wound healing activity in streptozotocin-induced diabetic rats,[68] although the mechanism of action is not well understood. Collard and Roy[71] investigated if a standardised fermented papaya preparation (FPP) may be effective for promoting wound healing in adult obese diabetic mice. FPP blunted the gain in blood glucose and improved the lipid profile after eight weeks of oral supplementation. However, FPP did not modify the weight of mice during the supplementation period. Results showed that FPP (0.2g/kg body weight) supplementation for eight weeks before wounding was effective in correcting wound closure. Specifically, histological investigations on viable macrophages isolated from the wound site demonstrated that FPP supplementation improved respiratory-burst function as well as inducible NO production. In fact, it is well known that ROS production is a key element of wound healing, and NO availability in diabetic wounds is known to be compromised. Diabetic mice supplemented with FPP showed a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved proangiogenic response. These results support the evidence that diabetic wound restoration may benefit from FPP supplementation by specifically influencing the response of wound-site macrophages and the consequent angiogenic response. Moreover, papain digests necrotic tissue by liquefying eschar, thus facilitating the migration of viable cells from the wound edge into the wound cavity. Papain is also useful in reducing the bacterial burden, decreasing exudates and increasing granulation tissue formation.[70]

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is in the class of essential fatty acid linoleic acid positional and geometric isomers, marked by a conjugated double bond.[72] Conjugated linoleic acid is found naturally in meat and dairy products from cows and sheep due to the process of bacterial bio-hydrogenation of linoleic acid (LA) in the rumen.[73] Although there is evidence for the efficacy of conjugated linoleic acid in different diseases due to its

anti-inflammatory and antioxidant activity, we have few studies about wound healing and skin metabolism.

Experimental *in vivo* studies

Park et al.[74] used a full-thickness excisional wound model after 2-week treatments with control, 0.5%, or 1% CLA-supplemented diet. Results showed that mice fed dietary CLA supplementation had reduced levels of oxidative stress and inflammatory markers. Moreover, the wound closure rate was improved significantly in mice fed a 1% CLA-supplemented diet during early stage of wound healing (inflammatory stage). These results support the hypothesis that dietary CLA supplementation enhances the early stage of cutaneous wound healing because of modulating oxidative stress and inflammatory responses

Collagen peptides

Collagen hydrolysate oral supplement, adsorbed from the intestine in its high molecular weight peptide form, provides beneficial effects on cutaneous wound healing, and skin recovery, although more studies are necessary to verify its mechanism of action.[75] Collagen has a regularly repeated amino acid sequence of Gly-X-Y and glycine. The most frequent tripeptide unit is Gly-Pro-Hyp, which contributes to the maximal stability of the collagen triple helix and to its bioactivity.[76] The Gly-Pro-Hyp tripeptide is partially hydrolysed on intestinal apical membranes, and the Pro-Hyp dipeptide, which is highly resistant to hydrolysis by intestinal proteases, is then absorbed in the intestine.[77]

Experimental *in vivo* studies

Generally, wound healing promoters increase the deposition of collagen, enhancing the tissue strength and forming cross-linkages between collagen fibres. The healing process depends largely on the regulated biosynthesis and deposition of new collagens and their subsequent maturation. Collagen is produced by fibroblasts and helps the wound gain tensile strength during repair.[78] It is likely that the oral intake of collagen promoters (CPs) might enhance the wound healing process by improving the deposition of collagen in the skin.

Marine collagen peptides from Chum Salmon skin

Bioactive peptides derived from the byproducts of Chum Salmon (*Oncorhynchus keta*), a sea fish, and found immunomodulatory properties from meat-derived peptides, and learning and memory-facilitating functions from the skin-derived peptides. Moreover, high-performance liquid chromatography (HPLC) analysis showed high levels of proline and hydroxyproline in peptide samples from fish skin, which represent a large constituent of collagen molecules. Hence, it may be hypothesised that marine bioactive peptides might become candidates for functional food or pharmaceutical applications to enhance wound healing.

Experimental *in vivo* studies

Zhang et al.[79] investigated the efficacy of the administration of marine collagen peptides (MCP) from Chum Salmon skin by using incision and excision rats' models. They divided 96 animals into wo wound models and then within each model animals were randomly divided into two groups: a vehicle-treated group and a 2g/kg MCP-treated group. Wound closure and tensile strength were calculated. Collagen deposition was assessed by Masson staining and hydroxyproline measurement. Angiogenesis was assessed by immunohistological methods. The biological composition of MCP is as follows: glycine (23.77%), glutamic acid (12.22%), proline (9.79%), hydroxyproline (7.51%), aspartic acid (7.29%), alanine (6.59%), arginine (6.08%), lysine (5.66%), leucine (4.64%), serine (4.23%), valine (2.94%), isoleucine (2.57%), threonine (2.53%), phenylalanine (2.51%), histidine (1.61%), methionine (0.03%) and tyrosine (0.03%). Results showed that MCP-treated rats showed faster wound closure and improved tissue regeneration at the wound site, which was supported by histopathological parameters. MCP treatment also improved angiogenesis and helped form thicker and better-organized collagen fibre deposition compared with the vehicle-treated group. Treatment of rats with MCP resulted in an enhancement of wound healing, as evidenced by increased wound contraction, fibroblasts and the formation of new blood vessels. Since collagen and angiogenesis are essential for normal wound closure, the stimulation of their synthesis is required. In this context, it has been supposed that MCP stimulated the collagen synthesis, since its amount was significantly increased during the supplementation period, as measured by Masson staining, hydroxyproline and wound tensile strength. MCP treatment led to increased expression of VEGF, one of the more potent stimulators of angiogenesis, in the first three days of healing. FGF-2 expression had the strongest up-regulation in the MCP-treated wounds. Therefore, it has been hypothesised that the increased VEGF and FGF-2 expression and then increase blood vessel angiogenesis are one of the mechanisms whereby MCP enhances wound healing.

Bromelain

Orally ingested bromelain proteolytic enzymes from *Ananas comosus* (pineapple plant) have also been shown to improve healing time and wound outcome.[7]

Experimental *in vivo* studies

Specifically, bromelain prevents prolonging of the inflammatory phase and reduces oedema, bruising, pain, and healing time following trauma and surgical procedures when administered at a dosage of 500–1000mg daily.[7] Bromelain anti-inflammatory and analgesic activity is due to reduced bradykinin and prostaglandin E_2 levels by activation of factor XII.[80] Bromelain has been demonstrated to inhibit thrombus formation when administered orally due to reduced

levels of high molecular weight kininogen and weak release of thrombin.[81,82] It is also demonstrated that bromelain increases platelet cyclic adenosine monophosphate (cAMP) levels thus increasing prostaglandin I_2 and prostaglandin E_1 levels. It can be hypothesised that dominant endogenous prostaglandins being produced must be from the group that increases platelet cAMP levels.[83]In addition, bromelain has an anti-inflammatory effect by modulating leukocyte cell surface molecules like CD14, CD44, CD16, CD21, CD128a and CD128b, which are involved in leukocyte homing, cellular adhesion, induction of pro-inflammatory mediators and immunomodulatory effect on T-cells by inhibition of T-cell signal transduction, producing effect on Th1, Th2 and immunosuppressive cytokines.[84,85]

Centella asiatica

Centella asiatica has long been used in traditional medicine because of its ability to heal wounds, improve mental clarity and treat skin conditions, such as leprosy and psoriasis.[86] The therapeutic substances are saponin-containing triterpene acids and their sugar esters, of which asiatic acid, madecassic acid and asiaticosides are considered to be the most important.[87]

Experimental *in vivo* studies

Asiaticoside, a saponine isolated from the plant *Centella asiatica*, has been studied for its wound healing activity in non-diabetic as well as in diabetic animals.[88] This plant has some pharmacological activities, such as promotion of fibroblast proliferation[89] and stimulation of collagen synthesis.[90] In addition, histological studies also evidence that orally ingested asiaticoside promoted angiogenesis in injured rats.[88] It is possible that asiaticoside may have a growth factor-like activity or has the ability to stimulate the expression of growth factors such as the basic fibroblast growth factor (bFGF).[88] bFGF has the broadest range of target cells, including all those involved in wound healing viz. endothelial cells, fibroblasts, myoblasts etc.[90] In addition, madecassoside, the main active constituent of *Centella asiatica*, has a strong antioxidant activity through reducing the generation of ROS and reactive nitrogen species, as well as promoting the production of antioxidants.[91]

Aloe vera

Aloe vera is a perennial succulent species belonging to the lily (*Liliaceae*) family.[92] *Aloe vera* juice has a long standing ethnomedicine history for the treatment of skin disorders and healing of burns and wounds.[93] Specifically, oral *Aloe vera* induces significant qualitative and quantitative changes in the glycosaminoglycans (GAG) content[93,94] and supports formation of the granulation tissue.[7]

Experimental *in vivo* studies

Dunphy and Udupa found in day four granulation tissue, an increase in the uronic acid content of *Aloe vera*-treated wounds, as compared with the control, that represents an enhanced synthesis of GAGs. As the content of collagen in the wound starts increasing, the amount of uronic acid declines.[95] In addition, *Aloe vera* induces a significant increase in hyaluronic acid and dermatan sulfate.[93] This modulation is probably due to the polysaccharide fraction extracted from the *Aloe vera* gel, for example, acemannan, although the exact mechanism of action is not well-understood.[96] *In vitro* studies showed that acemannan induces the production of keratinocyte growth factor-1 (KGF-1), VEGF and type I collagen production.[97] While *in vivo* studies demonstrated that it was β-sitosterol from *Aloe vera* which enhanced angiogenesis caused by the increase of Von Willebrand factors, VEGF, VEGF receptor and blood vessel matrix laminin.[98,99]

Conclusion

Commercially available supplements, rich in amino acids, proteins, vitamins, minerals and specific components are efficient tools for wound healing in hard-to-heal wounds. These formulations should be recommended for critically ill patients, with a rational administration in the four different steps of wound healing, that is, in the haemostasis, inflammatory, proliferative and remodelling phases.

Furthermore, selected indications are required for proper nutrient administration in different wounds (burns, surgical, vascular, traumatic, PU etc.) and in prevention.

However, experimental evidence supports nutritional supplementation for promoting the healing of wounds, although the exact genetic mechanism of action is not well understood and lack of clinical validated studies. To support this hypothesis there is also the evidence that malnutrition negatively interfere with wound closure, suggesting a putative role of specific nutrients wound healing phases.

Future research must concentrate on the genetic, biochemical and physiologic differences of the acute and chronic wounds and the interaction with specific nutritional supplements. **JWC**

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