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Review

Revisiting the Regenerative Role of Vitamin C in Skin Ulcer Repair—Mechanistic Insights and Therapeutic Roles

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How To Cite: Stoilov, B.B.; Delaney, C.L.; Truong, V.K.; et al. Revisiting the Regenerative Role of Vitamin C in Skin Ulcer Repair—Mechanistic Insights and Therapeutic Roles. *Regenerative Medicine and Dentistry* **2026**, *3*(3), 10. https://doi.org/10.53941/rmd.2026.100010

Received: 6 February 2026
Revised: 14 April 2026
Accepted: 13 May 2026
Published: 2 July 2026

Abstract: Skin ulcers remain a major cause of morbidity worldwide, affecting millions and burdening healthcare systems with prolonged hospitalisation. Conventional wound therapies rarely achieve full regeneration because the biochemical imbalances that sustain inflammation, oxidative stress and poor collagen deposition remain uncorrected. Among essential nutrients, vitamin C (L-ascorbic acid) has emerged as a decisive molecular regulator of cutaneous repair. Acting as a cofactor for collagen synthesis, vitamin C governs matrix synthesis, promotes angiogenic signalling and maintains immune–redox balance within the ulcer microenvironment. Accumulating evidence demonstrate direct control of cellular metabolism and gene expression by vitamin C through modulation of prolyl hydroxylases, hypoxia-inducible factor-1α, and NF-κB pathways, thus modulating fibroblast proliferation, keratinocyte differentiation, and vascular maturation. It also suppresses pathogen-driven oxidative injury, reinforcing host defence in infected ulcers. This review consolidates biochemical and clinical data to define vitamin C as a clinical modulator of chronic wound healing rather than a supportive micronutrient. The discussion connects mechanistic findings to outcomes from topical and systemic supplementation trials, highlighting how optimised vitamin C delivery can accelerate tissue regeneration and reduce infection risk. Collectively, the evidence establishes vitamin C as a practical, low-cost adjunct capable of bridging nutritional intervention and regenerative medicine for effective ulcer management.

Keywords: Vitamin C; L-ascorbic acid; chronic wound healing; ulcer; collagen; skin regeneration

1. Introduction to Chronic Wounds and Chronic Wounds Treatment

The skin is the largest organ in the human body and possesses many functions in the realms of thermoregulation, sensation, excretion, vitamin D synthesis, storage and absorption, immune response, and prevention of water loss. However, its primary role is to serve as a protective barrier against physical and mechanical harm and damage, as well as a microbial defence by preventing the entry of pathogens. Structurally, the skin comprises of two principal layers: the outer epidermis and the deeper dermis. The epidermis, primarily formed by keratinocytes, provides most of the mechanical barrier properties. The keratinocytes are proliferating away from the basal membrane and are arranged in layers throughout the epidermis thickness. The process of differentiation involves losing all subcellular organelles, including the cytoplasm and the nucleus [1,2]. When reaching their final stage of development keratinocytes are being sealed with moieties rich on lipids, thus forming the water-impermeable barrier that gives this main mechanical protection function of the skin–stratum corneum [3].

In comparison, the dermal layer provides the tensile and elastic properties of the skin. This is owing to the presence of complex extracellular proteins such as collagen, which comprises 70–80% of the dry weight of the dermis [4]. The dermis includes blood and lymph vessels as well as nerve fibres that are part of the peripheral neural system and impart the skin sensory functions. D of being situated in the dermis, some sensory nerve fibres terminate near or within the basal membrane of the epidermis, interacting with keratinocytes and Merkel cells [5,6]. The basal layer by itself consists of proteins (collagen type IV and VII) and glycoproteins (laminin, perlecan, nidogens, heparan sulphate proteoglycans) that provide structure and mediate cell adhesion, dividing the epidermis from the dermis [7]. Compared to the epidermis, the major cell type present in the dermis are the fibroblasts. These cells actively take part in the extracellular matrix (ECM) components synthesis. For the proper skin functioning, cell proliferation and damage repair, a constant and adequate supply of blood is critical to deliver the required micro- and macronutrients. However, due to predispositions and prerequisites (e.g., diabetes, tobacco smoking, aging, pregnancy, hypertonia, hypercholesterolaemia, thrombophilia, obesity, and limited mobility) dysregulated and suppressed blood flow may occur, consequently leading to skin ulceration.

When an injury occurs, four stages of wound healing take place–clot formation, inflammation, proliferation, and tissue remodelling [8,9]. These phases occur in a coordinated and time-dependent manner. However, any disruption in their sequence or timing can lead to abnormal or impaired wound healing. Generally, wounds are classified in two categories–acute and chronic [10]. The first ones are related to mechanical, physical or chemical skin damage and the time for tissue regeneration is relatively short [11]. Unlike acute wounds, the second type are often associated with underlying conditions such as diabetes, vascular insufficiencies, or other systemic disorders. They are characterized by delayed healing, a higher rate of recurrence, and greater persistence. Although, at present, there are no clear evidence that acute wounds to evolve into chronic ones, research of assessing chronic this possibility is still ongoing [12]. In this context, treating chronic wounds requires careful assessment of the underlying conditions. As the prevalence of underlying medical conditions increases with aging populations, so does the incidence of chronic wounds and related complications, such as ulceration. Consequently, the annual costs to healthcare systems for treating chronic wounds increased to tens of billions of dollars in the United States [13]. Amongst the 30 million diabetes patients, about 1 million are expected to develop foot ulcers annually, and further 6–7 million during their lifetime [14]. In addition, venous ulcers associated with varicosis are estimated to affect over 1% of the population [15].

Being chronic wounds, skin ulcers represent a significant challenge for medical practitioners to handle, but also for the patients themselves as ulcers have a severe impact on their quality of life. It has already been extensively demonstrated that the abnormal and slow healing process associated with chronic wound leads to distress, social isolation, anxiety, prolonged hospital stays and even terminal ends for the patients [16,17].

The pathophysiological manifestation of skin ulcers is primarily caused by poor blood circulation, leading to the occurrence of lesions and ruptures that fail to heal. As ulcers progress, they are often characterized by local swelling, erythema (redness), tenderness, pain, discoloration, and changes in skin structure. This leads to an increased risk of infection development. In advanced stages, when antibacterial treatments fail and wounds fail to heal, more invasive interventions may be required. Ultimately, surgical procedures such as skin grafting are often utilized as a last-resort therapeutic strategy to restore tissue integrity and promote healing [18–20].

Appropriate dressing of the wound is of extreme importance, to be kept it clean from contaminations and mechanical irritation [21]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the first line of choice therapeutics to deal with inflammation and pain. Nonetheless, they should be applied in an informed manner, because some would not be suitable for pregnant patients, or those with high blood pressure or diabetes, since these drugs may increase the severity of the conditions, or have severe side effects [22–26]. Non-pharmacological treatments of skin ulcers can be used as adjunct therapies which usually include laying cold compresses, elevating the swollen body parts and simple physical exercises that will increase the blood flow in the peripheral tissues. Although complex to use, negative pressure wound therapy (NPWT) is another method for treatment [27]. Despite its effectiveness, the use of NPWT poses certain challenges which include the need for specialized and trained personnel, requires careful monitoring and precise application to avoid complications.

Even in the absence of an obvious infection, an important consideration is the prophylactic use of antibiotics. Systemic antibiotics may be administered orally to target deep tissue infection, while topical antibiotics are often applied to reduce the risk of surface infections. This is particularly relevant for patients with specific primary medical conditions such as varicosis, atherosclerosis or/and diabetes [28,29]. In combination or individually, these three conditions and prolonged pressure on specific body regions represent the most common underlying causes of skin ulceration [30,31].

This review provides a concise overview of vitamin C in skin biology and wound healing, focusing on its mechanisms of action, delivery strategies, stability, and antimicrobial effects. Vitamin C supports keratinocyte

differentiation and collagen synthesis, stabilizes the ECM through its antioxidant properties, and contributes to local antimicrobial defences, collectively enhancing skin barrier integrity and wound repair.

2. Nutrition Role and Administration Routes of Vitamin C

Nutrients are extremely crucial as vitamin deficiencies alone may have crucial negative impact on the wound healing process, expressed as skin fragility and bleeding, impaired wound healing and increased risk of infections (vitamin C and B). Furthermore, nutrients are related to wound healing impairment (vitamin A, D and K), increased infection susceptibility (vitamin A and E) and increased inflammation due to UV exposure (vitamin D). Although all mentioned vitamins play important and diverse roles in wound healing and prevention, vitamin C is unique in its role of a co-factor in collagen synthesis during tissue repair. The historical role of vitamin C in preventing scurvy during long voyages that are well documented should also be mentioned. The British explorer Captain James Cook famously completed his second Pacific journey in the 18th century without losing any crew members to scurvy, by implementing measures like cleanliness, ventilation, and most prominently a diet rich in citrus fruits and vegetables, key sources of vitamin C.

Ascorbic acid exists in two forms: L-ascorbic and D-ascorbic acid, where the prior is naturally occurring, and the latter is synthetic. It is the left rotating isomer (L-) that is referred to as vitamin C. In fact, D-ascorbic acid has similar physicochemical properties as antioxidant and chelating agent, but it does not act as an enzymatic co-factor, thus considered as an inactive form with negligible biological role [32,33]. Despite that, published research studies have concluded an equal bioavailability of both forms [34]. Nonetheless, vitamin C is still considered essential, meaning that it cannot be synthesized in humans, unlike in other mammal species (due to a mutation in the GULO gene that encodes L-gluconolactone oxidase), and has to be obtained from diet [35].

The route of administration has a major impact on vitamin C bioavailability and ability to exercise its biological role. The oral absorption pathways are influenced by intestinal disorders, systemic diseases or properties of the gastrointestinal tract and metabolization in the liver before reaching the skin. Nevertheless, a recent study has shown that vitamin C-loaded liposomes have resulted in 1.8 times higher bioavailability compared to non-liposomal samples [36]. A study by Łukawski et al. also found enhanced bioavailability after oral administration by vitamin C-loaded liposomes [37]. In another new study, orally applied chitosan particles loaded with vitamin C have been proven to improve immunological parameters and resistance to pathogens in shrimp [38]. There are many other promising developments published in scientific literature that report encapsulation of vitamin C using various polymers and entrapment techniques aimed at protecting the molecule and improving bioefficacy, however, most of these have not been validated by in vivo studies or clinical trials [39–41].

To tackle the issues associated with oral administration, one solution might be an intravenous application (IV). Due to its water-soluble nature, vitamin C is subject to rapid renal clearance, especially when administered in high doses. Once the renal threshold is exceeded, vitamin C is eliminated via urinary excretion, a process termed by the transport maximum [42]. Even when high IV doses are applied, vitamin C concentration is tightly controlled to maintain optimal levels for physiological functions and the excess is excreted through the different pathways [43]. During a healthy physiological process, this fine regulation facilitates vitamin C concentration to be maintained within a specific range without the risk of reaching toxic levels. To date, no advanced intravenous formulations of vitamin C, such as nanoparticles, microemulsions, or controlled-release carriers, have been developed that meaningfully improve IV delivery. This underscores a significant opportunity to design innovative delivery systems that enhance its bioavailability and therapeutic efficacy.

An alternative to address the aforementioned issues is topical administration. This approach results in high enough administration doses locally surpassing the drawbacks of the peroral route and most of the disadvantages of the systemic one associated with intravenous application. Despite that, the overall patient health and current skin conditions like severe acne, melasma, cancer, extensive skin damage and infections should be scrutinized when topical usage is chosen as the therapeutical way of delivering of these nutrients [44–46]. Nonetheless, a key challenge associated with this delivery is the absence of blood vessels in the epidermis and reliance only on passive diffusion via the dermal vascular network [47]. Due to the nature of the epidermis, being composed of lipid-protein complexes, fat-soluble nutrients can penetrate easier. Thus, if not formulated appropriately, drug penetration may still be limited to the upper skin layers. Being water soluble, penetration capacity of ascorbic acid through the upper skin layer and reaching the dermis presents additional challenges. In contrast, B-group vitamins, despite also being water-soluble, can readily penetrate the stratum corneum and reach the dermis via passive diffusion, largely due to their relatively small molecular size [48–50]. Based on this, L-ascorbic acid, which has a lower molecular weight than most essential B vitamins, might be expected to follow a similar transport pathway. However, its highly polar nature and predominant existence as a negatively charged ion (ascorbate) at physiological pH limits

its ability to diffuse across the hydrophobic lipid bilayer of cell membranes. Consequently, vitamin C uptake in skin cells occurs primarily through active transport, mediated by sodium-dependent vitamin C transporters, SVCT1 and SVCT2. Unlike B-group vitamins, which are generally more abundant in circulating blood and tissues, the concentration of vitamin C in the skin's extracellular matrix is relatively limited, necessitating active transport to achieve sufficient intracellular levels. Moreover, whereas most B vitamins primarily function as enzymatic cofactors and do not require substantial tissue accumulation, vitamin C must be actively transported into and retained within skin cells to support its structural and protective roles [51–54].

At present, there are many research studies related to topical formulations for vitamin C delivery, compared to these focused on oral and IV therapies. For instance, a recent study reposted the development of a hydrogel system that can be bioprinted into a scaffold suitable for treatment of full-thickness wounds [55]. Other studies include formulating vitamin C delivery systems based on electrospun wound dressings composed of different polymers [56–59], nanoparticles, liposomes, nanoparticle-in-gel systems [60–62], as well as hydrogels [63–65].

3. Homeostasis-Pathogen Interplay of Vitamin C

3.1. Receptors

Given the transport of vitamin C relays on protein transporters, SVCT1 are mainly expressed in the intestines and kidneys, and in low quantity in the epidermis, while SVCT2 are widely distributed in various tissues, including such that require high metabolic activity, such as brain, muscles, and skin, both in the epidermis and dermis [66–68]. The first type primarily functions to maintain systemic vitamin C homeostasis, whereas the latter serves to protect metabolically active cells from oxidative stress while ensuring a consistent intracellular supply [69]. Although both transporters are membrane-bound proteins that utilize the sodium electrochemical gradient to drive active co-transport, they differ markedly in their uptake kinetics. SVCT1 operates with low affinity but high capacity, an adaptation suited to handling the large quantities of vitamin C absorbed and reabsorbed in the intestines and kidneys. In contrast, SVCT2 exhibits high affinity but low transport capacity, enabling efficient uptake under conditions of low extracellular vitamin C concentrations where cellular demand is high [70,71].

Notable is also the role of Na^+/K^+-ATPase activity as it generates and maintains the sodium electrochemical gradient across the plasma membrane, which is the driving force for SVCT1 and SVCT2. By sustaining this gradient, Na^+/K^+-ATPase indirectly enhances SVCT-mediated vitamin C uptake, thus increasing intracellular accumulation [72,73].

Additionally, a secondary pathway for vitamin C uptake exists via a large family of receptors known as the Sugar Porter (GLUT) family. Although these receptors are primary for hexose transport across different organs and tissues, they also play a crucial role for the gradual reuptake of its oxidised form, dehydroascorbic acid (DHA), following a First order kinetic, which is then metabolized by reduction back to L-ascorbic acid through the glutathione-ascorbate cycle [74–76]. Not all members of this transporter family participate in DHA uptake. The isoforms involved in this transport include GLUT-1 (in most tissues, including cells as the erythrocytes and endothelial cells), GLUT-2 (in the liver, kidneys, and pancreatic β cells), GLUT-3 (in neurons and platelets), GLUT-4 (in adipose tissue and striated muscle) and GLUT-8 (mainly in spermatozoa, testes and brain). Amongst these five, GLUT-8 is the least studied and characterized, mainly because of its occurrence in the brain [77,78], and GLUT-2 was found to have low affinity towards DHA [79]. Furthermore, only GLUT-4 is insulin-sensitive– its activity is regulated by insulin levels, where high levels of insulin can lead to increased GLUT4 uptake of DHA. As with the case of GLUT-2, DHA transport via GLUT-4 was found to be lower compared to GLUT-1 and GLUT-3 [80]. Only GLUT-1 and GLUT- 3 play a main role in transporting DHA, including to the skin cells. [81–84]. GLUT1 receptors function as a key glucose transporter in keratinocytes, fibroblasts, and endothelial cells, whereas GLUT3 are also expressed in keratinocytes and fibroblasts, contributing not only to glucose transport but also to the signalling pathways of M2 macrophages. This noncanonical role of GLUT3 transporters support wound healing and tissue regeneration through immunomodulatory effects. They contribute to M2 macrophage function by regulating intracellular glucose availability, which then fuels metabolic pathways, such as oxidative phosphorylation and the pentose phosphate pathway, necessary for the anti-inflammatory and reparative phenotype. Additionally, GLUT3-mediated glucose uptake can affect signalling cascades (e.g., via AMPK and mTOR pathways) that enhance M2 macrophage polarization, cytokine production (IL-10, TGF-β) and growth factor secretion, thereby promoting tissue regeneration (Figure 1) [85–89].

Collectively, even though DHA directly competes with glucose for uptake, GLUT1 and GLUT3 provide a robust, compensatory pathway for vitamin C uptake in ulcerated tissue, particularly under conditions where SVCT function is compromised. In contrast to SVCT transporters, both GLUT1 and GLUT3 are strongly upregulated by hypoxia and pro-inflammatory signals, largely mediated through hypoxia-inducible factors (e.g., HIF-1α) and

cytokines such as TNF-α, IL-6 and IL-1β [90–92]. This makes them particularly relevant in chronic wounds, where low oxygen tension and persistent inflammation are defining features.

Functionally, these transporters are Na^+-independent and rely on facilitated diffusion, meaning their activity is not directly limited by ATP depletion or disruption of ion gradients, common issues in ischemic or metabolically impaired tissue. As a result, GLUT-mediated uptake remains active even when SVCT-driven transport is reduced. Overall, this interplay establishes a dynamic uptake balance, where both the reduced and oxidized forms of vitamin C contribute to cellular accumulation, ensuring that even under hostile wound conditions, vitamin C can still be delivered through complementary transport mechanisms.

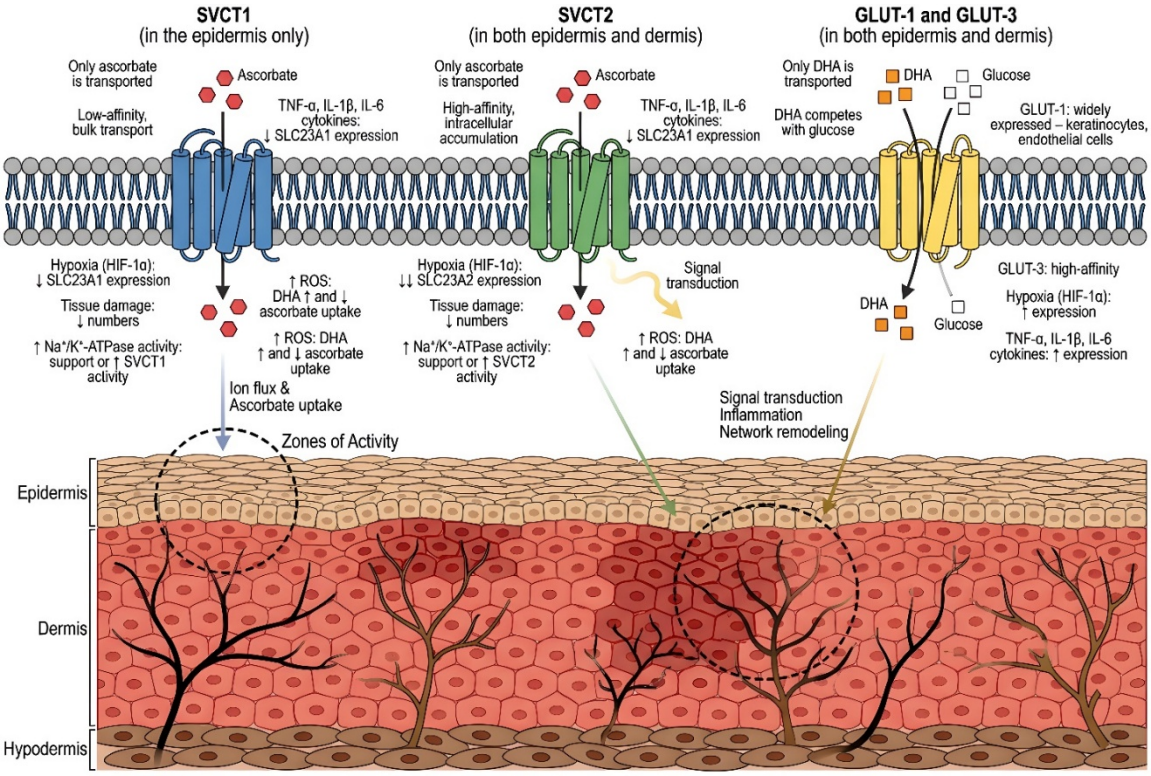

Figure 1. Graphical representation of the distribution and cellular localization of transporters involved in the uptake of vitamin C and DHA, across the epidermal and dermal layers of the skin. Sodium-dependent vitamin C transporter 1 (SVCT1; blue) is predominantly restricted to the epidermis, whereas sodium-dependent vitamin C transporter 2 (SVCT2; green) is expressed in both epidermis and dermis. Facilitative glucose transporters GLUT-1 and GLUT-3 (yellow) are present in both layers and localized to the plasma membrane of keratinocytes and fibroblasts, mediating DHA uptake. Notably, GLUT-3 exhibits prominent intracellular localization within endosomal compartments of keratinocytes and fibroblasts, and is additionally expressed in endosomes of M2 macrophages. The figure also outlines the regulatory effects of vitamin C on these transport systems, emphasizing its role in modulating transporter expression, activity, and intracellular trafficking within the cutaneous microenvironment.

3.2. Bioavailability

Tissue and organ concentrations of vitamin C vary according to their physiological roles and the degree of plasma saturation [68]. The highest levels are observed in the adrenal glands, followed by the liver, with progressively lower concentrations in the brain, kidneys, and cerebrospinal fluid [93–97]. Interestingly, the oral application in animal models of low dose vitamin C (100–1500 mg/kg) led to the highest concentration in different parts of the brain, compared to the other organs and tissues [98]. In vitro studies by Salazar's research team, using primary rat cortical neuron cultures as well as Neuro2a and HSVT-C3 neuronal cell lines, demonstrated that supplementation with 400 µM vitamin C induced SVCT2 overexpression, thereby enhancing ascorbate uptake and promoting cortical neuron differentiation via vitamin C recycling between neurons and astrocytes.[99,100]. Conversely, a small-scale randomized placebo-controlled clinical study demonstrated that high-dose vitamin C supplementation (1000 mg/day) markedly increased skeletal muscle ascorbate levels and SVCT2 expression, without significantly affecting redox status in healthy male participants [101].

Nonetheless, similar to observations in organs such as the brain and skin, tissue and plasma concentrations of vitamin C are tightly regulated and reach a saturation plateau beyond which additional intake does not

meaningfully increase levels. Plasma vitamin C concentrations rise steeply with doses up to 100 mg/day but reach a steady-state saturation plateau between 60 and 80 μmol/L, typically observed at oral doses of 200–400 mg/day in healthy adults. This saturation reflects the limited capacity of tissues to accumulate ascorbate, which is governed by dose-dependent, saturable transport mechanisms and renal reabsorption. Once these systems are engaged to their maximum, excess vitamin C is not further taken up by tissues and is instead excreted in the urine, resulting in only minimal increases in systemic concentrations despite larger doses. Once this saturation is reached, additional intake does not significantly increase plasma or tissue concentrations. Pharmacokinetic studies show that oral doses of 1250 mg produce mean peak plasma levels only about two times higher than those achieved with a daily intake of 200–300 mg. When vitamin C levels exceed the capacity of renal reabsorption, occurring when plasma concentrations reach approximately 60–80 μmol/L, the excess is rapidly eliminated in the urine. [102,103].

It is well established that vitamin C concentrations are considerably higher in the epidermis than in the dermis [104]. Within the epidermis, the lowest levels are typically observed in the outermost layers, likely reflecting continuous exposure to environmental stressors such as UV radiation and oxidative agents [105]. Additionally, considerable variability in reported skin vitamin C levels has been documented, which can be attributed in part to methodological challenges associated with isolating and handling skin samples. Accurate quantification generally requires multiple extraction steps, a process complicated by the inherent instability of vitamin C, which is highly susceptible to degradation and irreversible oxidation under extreme pH, elevated temperatures, or in the presence of strong oxidizing agents. These technical limitations contribute to the observed discrepancies and variability across studies [106]. Another major source of variability in skin vitamin C levels is the anatomical location from which samples are obtained. Generally, higher concentrations are observed in regions of the skin that are actively involved in collagen synthesis, oxidative protection, and tissue repair [107]. Lifestyle and environmental factors, including diet, UV exposure, pollution, and smoking, also exert a significant impact as L-ascorbic acid is particularly susceptible to oxidation under these conditions, where generated free radicals can result in decreased local concentrations [108]. Age is another critical determinant, with older individuals exhibiting markedly lower vitamin C levels in the skin [109,110]. Furthermore, preexisting conditions such as eczema, psoriasis, and diabetes have been associated with reduced cutaneous ascorbate content, underscoring the multifactorial influences on vitamin C distribution in human skin [111–113].

3.3. Pathogens

The pathogens' implications on the various stages of ulceration and the wound healing process can differ significantly [114–117]. For example, during the first stage of ulcer development *S. aureus* and *C. albicans* have capability to colonize the surface area. As the skin breaks open and a shallow wound is formed, the moist environment stimulates the growth of *S. pyogenes* and *P. aeruginosa*. In the third stage, as the ulcer deepens, *E. faecalis* and *K. pneumoniae* become more prominent, as they are able to invade deeper tissues. The fourth stage is often dominated by *P. aeruginosa* and *C. albicans*, both of which are capable of forming biofilms that are difficult to treat (see Table 1). With respect to the wound healing process, during the haemostasis, inflammation and proliferation phases, prominent bacteria species that affect ulcers are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Enterococcus faecalis, Klebsiella pneumoniae*, *Escherichia* coli, *Peptoniphilus asaccharolyticus*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Finegoldia magna*, and *Serratia marcescens* [118].

In general, pathogen infection can significantly impair cellular vitamin C uptake through the dysregulation of vitamin C transporters, particularly SVCT2 [119,120]. During infection, pro-inflammatory cytokines including TNF-α, IL-1β, and IFN-γ activate NF-κB signalling pathways that can suppress SLC23A2 transcription (encoding for SVCT2) and alter transporter localization at the plasma membrane, thereby reducing ascorbate import despite extracellular availability. Concurrently, infection-induced oxidative stress promotes the extracellular oxidation of ascorbate to DHA, shifting cellular uptake toward GLUT-mediated transport rather than SVCT-dependent ascorbate uptake. This redox imbalance not only limits the availability of functional intracellular ascorbate but may also directly damage the transporter proteins. In addition, metabolic reprogramming in activated immune cells, characterized by increased glycolysis (Warburg effect) and upregulation of GLUT-1, further deprioritizes SVCT2-mediated uptake [121–123]. In chronic infection or non-resolving inflammatory microenvironments, sustained cytokine exposure can induce epigenetic modifications that reinforce transporter downregulation, contributing to a persistent "uptake failure" despite local vitamin C presence. Functionally, this results in impaired collagen synthesis, reduced antioxidant defence, and dysregulated immune responses, all of which are critical in chronic wound pathology [124,125].

A research study by Aburawi et al. has shown that 200 mg/mL vitamin C modulated and increased the effect of antibacterial entities like ciprofloxacin at 10 mg/mL concentration against *S. aureus* and *E. coli* [126]. Contrary to these findings, the same study reveals that combination of the nutrient and ciprofloxacin at concentrations of 20 and 40 mg/mL was found to decrease its effectiveness. Similarly, pretreatment with even lower vitamin C concentrations (10 μM) before applying 100 μg/mL ciprofloxacin upon *E. coli* resulted in protecting the bacterial cells and scavenging the antibiotic generated ROS, thereby limiting ciprofloxacin's mechanism of action [127,128].

Another research study has also backed the results where vitamin C successfully reduced *P. aeruginosa*, *S. aureus* and E. *faecalis* bacteria in a dose-dependent manner from 0.16 to 10 mg/mL. Notably, antibacterial inhibition across all three species did not increase substantially at concentrations exceeding 0.625 mg/mL [129]. The positive effect of high-dose vitamin C (12.5–25 mg/mL) was also reported by other study, demonstrating its concentration-dependent inhibitory effect on the growth and biofilm formation of *Streptococcus mutans*, often associated with skin infections, oral cavities and cariogenic effects [130,131].

In different combinations vitamin C has been shown to enhance antibiotic efficacy against *Mycobacterium tuberculosis*, species with the characteristics of both a Gram-positive and a Gram-negative bacterium, which can cause or worsen an existing skin infection. Its co-administration potentiated the bactericidal activity of first-line drugs, particularly isoniazid and rifampin, likely through pro-oxidant, ROS-mediated mechanisms [132]. In the ofloxacin/kanamycin/ ethionamide treatment group, co-administration with vitamin C resulted in a markedly enhanced bactericidal effect, resulting in a more rapid and pronounced reduction in CFU counts compared to antibiotics alone. In another study, vitamin C has been reported to enhance rifampin-mediated killing of *M. tuberculosis*, an effect attributed to its pro-oxidant activity that drives Fenton reaction-dependent ROS generation. The created oxidative stress was further discussed and found to overwhelm bacterial redox defences, thereby improving the efficacy of rifampin, particularly against persistent or latent *M. tuberculosis* populations [133].

Additionally, research has shown that citric and ascorbic acids significantly reduced biofilm formation in *Acinetobacter baumannii*, a Gram-negative bacterium known for causing skin and other connective tissue infections and possessing high drug resistance. This effect was proportionally dose-dependent for vitamin C at the highest tested sub-MIC concentration (1.5 mg/mL), while citric acid had more limited impact [134]. Another recent study demonstrated that even low concentrations of vitamin C (up to 1.25 mg/mL) were effective in inhibiting the growth and biofilm formation of *P. aeruginosa*. Furthermore, a considerable reduction in the minimum inhibitory concentration (MIC) was observed when vitamin C was combined with various antibacterial agents (piperacillin/tazobactam, piperacillin, ceftazidime, gentamicin, ciprofloxacin). In vivo models also showed a reduction in viable bacterial cells and partial improvement of the infection in rats. The study concluded that combining vitamin C with these antibiotics resulted in enhanced efficacy compared to treatment with the antibiotics alone [135].

A research had also shown that vitamin C alone not only reduced an already formed biofilm but can also protect against bacterial cell adhesion to plastic surfaces [136]. In this study two serotypes of *E. coli*, *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Proteus sp.*, *Pseudomonas sp.* were examined, where levofloxacin alone and in the combination with vitamin C were successfully used at 1- and 2-MIC concentrations. The inhibitory effect was even greater in the combination, where almost total elimination of the formed biofilm was achieved. This was attributed to reduction of pH by vitamin C itself and by its ability to inhibit the production of autoinducer-2, the only not species-specific signalling molecule among bacteria that helps them to form biofilms, thus impairing the quorum sensing [137]. In another recent study, the effect of vitamin C was studied for its inhibitory properties against several Gram-positive and Gram-negative species, among which *K. pneumoniae*. Concentration dependency was observed and increased ability to inhibit growth when the doses were higher (20 mg/mL). The substantially larger zones of inhibition at pH 3 compared to pH 8 suggest that, beyond its intrinsic antimicrobial effects, ascorbate may contribute to environmental acidification, thereby enhancing antibacterial activity and offering potential as an adjuvant strategy to improve treatment efficacy [138]. In another recent study, vitamin C at MIC and 0.5-MIC concentrations (MIC_{50} and MIC_{90} were 3.125 mg/mL and 6.25 mg/mL, respectively) was confirmed to positive suppress biofilm formation from *Stenotrophomonas maltophilia*, a multidrug resistant Gram-negative bacteria [139].

In methicillin-resistant *Staphylococcus aureus*, sub-inhibitory concentration of L-ascorbic acid alone and in combination with oxacillin had been found to decrease the expression of *icaA* gene responsible for biofilm formation. In comparison, when oxacillin was used alone, an increase in biofilm formation was observed [140]. Low doses of L-ascorbic acid alone were acknowledged to promote colony spreading and led to increased *agr* gene expression in *S. aureus*, responsible for the bacteria's acute toxicity, whereas when sub-inhibitory doses of L-ascorbic with oxacillin were applied, a minor reduction of *agr* expression was observed with reduced colony

spreading. Regarding this gene, it was previously reported that when *S. aureus* comes into contact with keratinocytes, these epidermal cells detect the toxin phenol-soluble modulin alpha (PSMα) secreted by the bacteria, triggering an immune response which causes a cytokine cascade that first engages T-cells and subsequently promotes neutrophil recruitment [141]. Once *S. aureus* reaches the dermis, downregulation of the agr gene (agr^-) results in reduced toxin production, including PSMα, allowing the bacteria to be contained and digested within the neutrophil's phagosome. In contrast, when agr gene is actively expressed (*agr*+), PSMα facilitates the pathogen's escape from the phagosome, thereby promoting further inflammation [142–144].

In immunocompromised patients, the opportunistic pathogen *Candida albicans* can not only exacerbate existing skin ulcers, but may also be an agent causing their development [145,146]. Although *C. albicans* is typically found in the gastrointestinal tract and the oral cavity, in the context of skin ulcers, it can lead to candidiasis, a mild condition characterized by itching, redness, and discomfort that is generally treatable with antifungal agents. However, if left untreated, candidiasis may progress to infection-induced panniculitis, a more severe condition marked by inflammation of the subcutaneous adipose tissue, resulting in painful nodules and abscesses [147,148]. Furthermore, there are numerous case studies reporting of *C. albicans* being engaged in commensal, cooperative, and synergistic interactions with bacteria commonly found in skin ulcers [149–151]. *C. albicans* may also co-exist in ulcers (commensalism) with *E. coli*, *E. faecalis*, or *K. pneumoniae*, forming mixed-species biofilms that enhance microbial survival and increase resistance to both antifungal and antibacterial therapies. *C. albicans* can synergistically coexist with *S. aureus* leading to an increased secretion of staphylococcal α-toxin and enhanced biofilm production, resulting in elevated resistance to antimicrobial agents [152,153]. In addition, the cooperation between *C. albicans* with *S. aureus* and *P. aeruginosa* is related with the formation of resistant biofilms, regulated through the means of complex cell signalling mechanisms, with *C. albicans* hyphae often serving as a physical scaffold for bacterial attachment [154–157].

The relationship between *C. albicans* and *P. aeruginosa* can also be antagonistic at certain conditions, due to the ability of the bacteria to produce specific antimicrobial compounds, such as phenazines, that inhibit *C. albicans* biofilm formation and growth on nonfermentable carbon sources, as well as suppress their filamentous transition [158,159]. *C. albicans* and *S. mutans* exhibit both cooperative and antagonistic dynamics. While they can mutually reinforce each other's resistance to treatment through metabolite exchange and enhanced colonization [160,161], competition for nutrients and space on the skin surface can lead to antagonism. Notably, *S. mutans* has been shown to secrete tetramic acid (mutanocyclin), which inhibits *C. albicans* filamentation via the cAMP/PKA signalling pathway [162–165].

All the abovementioned findings regarding *C. albicans* are important and should be carefully considered when using vitamin C in the light of its demonstrated antifungal effects [166,167]. In that sense, a recent study have examined honokiol, a natural polyphenol with antifungal activity, extracted from the leaves and bark of Magnolia plants [168,169], alone and separately in combinations with vitamin C and E [170]. The results indicated that even at low concentrations (0.3125 mM) vitamin C had the capacity to drastically enhance its innate antifungal properties through ROS production, whereas vitamin E protected *C. albicans* in a dose-dependent manner. Neither vitamin C nor vitamin E alone induced detectable ROS-positive cells, however, co-treatment of honokiol and L-ascorbic did. In addition to its pro-oxidative action, vitamin C also disrupted the glycolytic metabolism of the fungus, contributing further to its growth inhibition.

In conjunction to its antibacterial and antifungal effects, vitamin C demonstrated antiviral activity against a range of viral serotypes and species, including those implicated in ulcer formation [171]. In that respect, several viral pathogens are known to contribute to skin ulcer development, particularly in immunocompromised individuals. Among the most common are herpes simplex virus (HSV), human papillomavirus (HPV), herpes zoster virus or shingles (HZV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV), all of which can trigger or exacerbate ulcerative skin lesions [172–177].

Published studies have found that oral treatment with acyclovir in combination with vitamin C reduced the recurrence of ocular herpes simplex keratitis, most commonly caused by HSV-1, confirming earlier in vitro findings [178–180], although no research outlined this combination regarding skin ulcers. In contrast, no studies have directly addressed the antiviral effect of ascorbic acid upon herpes zoster virus, but indirectly–intravenous administration of vitamin C has been reported to provide analgesic effects, significantly reducing pain associated with herpetic and postherpetic neuralgia [181,182]. In another recent study, vitamin C was used in combination with famciclovir to bring relief of this medical conditions and was reported to improve the recovery from the disease and to decreased serum levels of the pro-inflammatory cytokines IL-6 and IL-8 [183].

The role of vitamin C in relation to HPV progression remains controversial [184,185]. On one hand, increased serum levels of antioxidant nutrients as vitamin A, B_2, E and B_9 have been associated with a reduced risk of HPV infection [186], whereas, on the other hand, a classical antioxidant as vitamin C was found to be related with the

lowest risk of infection at a serum concentration of 69.5 μmol/L [187]. In the latter case, it was also found that both lower and higher levels of this threshold were associated with an increased infection risk. However, it is important to note that all participants in that study were female patients, with varying baseline vitamin levels (including hypovitaminosis) and differing lifestyle habits such as tobacco use and alcohol consumption. A third study showed that higher daily intake of vitamin C (50 mg) was correlated with a reduced risk of HPV infection progressing through all three stages of cervical intraepithelial neoplasia (CIN) and ultimately to cervical cancer, suggesting a protective effect of the vitamin throughout this progression [188]. Additionally, a separate study reported that vitamin C, when combined with hydrocortisone, stimulated TGF-β1 production and promoted type I collagen deposition by HEK001 cells (HPV-induced keratinocytes), suggesting potential benefits for chronic wound healing [189].

Regarding the effect upon CMV, an analogue of vitamin C with pronounce antiviral properties (L-ascorbic acid-2-phosphate or ASC-2P) has been found to have no effect on the number of cells expressing CMV immediate early antigen (IEA), but significantly inhibited the expression of CMV late antigen (LA) in human foreskin fibroblasts [190]. It has been observed that pretreatment with ASC-2P enhanced its antiviral efficacy, and when combined with ganciclovir and foscarnet resulted in a higher reduction of the virus replication, compared to drugs used alone, where this reduction was found to be insignificant.

Related to HIV, the performed research studies have not identified a specific and strong interconnection between supplementation with nutrients and in that matter with vitamin C, as well [191,192]. Nevertheless, a prior one has found that high concentrations of ascorbic acid were able to inhibit the proliferation and survival of HIV-infected cells in a dose-dependent manner, with marked effects particularly at 1–3 mM [193]. This research also highlighted that the viral infection was found to increase the accumulation of the vitamin in lymphocytic, myeloid, and monocytic cell lines, due to an increased expression of the GLUT receptors. Another prior study observed the inhibition properties of vitamin C (0.57 mM and 0.85 mM) against the HIV-1 production and reduced cell fusion in T-lymphocytes infected by the virus, as well as a significantly reduced extracellular reverse transcriptase activity during prolonged exposure to the vitamin [194].

Table 1. Effect of vitamin C on a broad spectrum of microorganisms, including bacteria, fungi, and viruses, evaluated both as a standalone agent and in combination with selected antimicrobial or antiviral compounds, highlighting its potential modulatory, synergistic, or inhibitory roles across different microbial systems.

Pathogen	In Combination or Alone (-)	Vitamin C Conc.	Effect of Vitamin C Alone	Reference
MRSA	0.25- and 0.125-MIC conc. Oxacillin	1 mg/mL (sub-inhibitory)	Reduced biofilm formation; increased colony spreading	[140]
S. aureus	10 mg/mL Ciprofloxacin	200 mg/mL	Moderate-to-high; dose-dependent	[126]
S. aureus, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *B. subtilis*, *B. licheniformis*	-	20 mg/mL	Effective at high dosage	[138]
E. coli	10 mg/mL Ciprofloxacin	200 mg/mL	Low-to-moderate; dose-dependent	[126]
E. coli	100 μg/mL Ciprofloxacin	10 μM	-	[128,129]
S. aureus, *E. faecalis*, *P. aeruginosa*	-	0.16 to 10 mg/mL	Substantial reduction, but inconsiderable above 0.625 mg/mL	[129]
E. coli, *Proteus sp.*, *Klebsiella sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Pseudomonas sp.*	1- and 2-MIC conc. Levofloxacin	80 and 100 mg/mL	Dose-dependent effect (80 and 100 mg/mL)	[136]
P. aeruginosa	piperacillin/tazobactam, piperacillin, ceftazidime, gentamicin, ciprofloxacin	156.2–1250 μg/mL	-	[135]
S. mutans	-	0.4–25 mg/mL	Effective; dose-dependent	[130]
M. tuberculosis	Rifampin/isoniazid 1.2 μM/7 μM Ofloxacin/kanamycin/ethionamide 14 μM/41 μM/150 μM	1 mM	-	[132]
M. tuberculosis	Rifampin	-	-	[133]
A. baumannii	-	1.5 mg/mL	Anti-biofilm and anti-capsular effects in sub-MIC conc.; dose -dependent	[134]

Table 1. *Cont.*

Pathogen	In Combination or Alone (-)	Vitamin C Conc.	Effect of Vitamin C Alone	Reference
S. maltophilia	-	1- and 0.5-MIC conc.	Effective against biofilm formation	[139]
C. albicans	60 µM Honokiol	10 mM	Low concentrations enhanced its own fungicidal activity	[170]
HSV	800 mg Acyclovir	1000 mg	-	[178]
HZV	-	5–10 g/infusion	Indirect effect towards pain management	[181]
HZV	500 mg/dose Famciclovir	7.5 g/dose	-	[183]
HPV	-	69.5 µM	Reduced risk of infection	[186]
HPV	-	11–114 µM	Reduced risk of infection depending on the dose, but not related to proportional correlation	[187]
HPV	-	50 mg/day	Reduced risk of infection	[188]
HPV	0.6 µg Hydrocortisone	24 µg	Increased MMP-2 activity	[189]
HIV	-	0.1–3 mM	Inhibited proliferation and survival of HIV-infected cells	[194,195]

3.4. Microenvironment Stability

An important affecting factor for vitamin C's stability and effectiveness is the pH of the wound environment. L-ascorbic acid has two pK_a values, where at pH above 4.1 (pK_1) is at its deprotonated form (AH^-)–called ascorbate, while at pH higher than 11.8 (pK_2) results in the other hydroxyl group deprotonating (A^{2-}), forming a dianion of ascorbic acid, referred to as ascorbate ion. As mentioned, due to its strong antioxidant properties, vitamin C can undergo reversible oxidation to DHA not only in response to pH but also in the presence of specific chemical or physical factors, including free radicals, UV light, radiation, body temperature and metal catalysts, primarily Zn^{2+}, Fe^{3+} and Cu. This occurs via a two-step, one-electron oxidation mechanism in which ascorbate is first converted to a transient ascorbate/ascorbyl radical system with different protonation states, followed by a second oxidation or disproportionation step that generates DHA. Furthermore, under physiological conditions, the reaction is effectively limited to this redox cycle, as formation of the fully deprotonated dianion via spontaneous auto-oxidation is not feasible [195]. Similarly, accumulation of irreversible degradation products like 2,3-diketogulonic acid (DKG) is also not possible as it generally requires conditions that promote extensive electron transfer, such as strong oxidizing environments, elevated temperatures, or high concentrations of catalytic metal ions in the absence of protective antioxidants. In physiological settings, including highly inflamed, infected ulcerated skin, pH may raise up to 8.9, temperatures are moderate (~37 °C), and antioxidant systems (glutathione, urate, enzymes) are present. These factors collectively prevent significant accumulation of DHA and its further conversion to DKG. Therefore, while DKG formation is chemically plausible under extreme in vitro conditions, it is highly unlikely to occur in vivo, even in pathological sites with oxidative stress [196].

As DHA is biologically inactive, it can no longer exert its antioxidant or collagen-stabilizing effects. This has important implications for both systemic and topical applications, as the oxidative state of vitamin C directly determines its efficacy. In the context of topical delivery, skin penetration is strongly influenced by the molecule's ionization state. Vitamin C is more lipophilic and able to diffuse across the stratum corneum when in its protonated (non-ionized) form, which predominates at pH values below 4.1 [197]. At higher pH levels, the molecule exists mostly as an anion, which is poorly absorbed through the lipid-rich layers of the skin, reducing its bioavailability. Therefore, formulation strategies often aim to maintain a low pH to preserve vitamin C in its active form while balancing skin tolerability, as excessively acidic preparations may cause irritation. Additionally, the stability of vitamin C in a topical preparation is challenged by environmental factors such as light, oxygen, and trace metal contamination, which can accelerate its conversion to DHA (Figure 2). Proper stabilization techniques, including encapsulation or the use of metal chelators, are often necessary to ensure the compound remains active long enough to penetrate the skin and exert its biological effects [198].

Under physiological conditions, adult skin typically maintains a slightly acidic pH range (pH 4–6), whereas infant skin tends to be neutral to slightly basic [199–201]. This acidic pH is primarily associated with the environment conditions of stratum corneum, while deeper layers such as the viable epidermis exhibit progressively higher pH values, reaching up to pH 7.4 [202]. The formed acidic environment of the upper skin layers has a beneficially protective role by inhibiting bacterial growth and slowing the proliferation of pathogenic species

[203]. In cases of skin injury with bleeding, such as acute wounds, the wound site initially exhibits a neutral pH (approximately 7.4), which gradually shifts toward acidity as the skin regenerates. On the contrary, skin ulcers would impart increased levels of pH, up to 9.25, thus favouring bacterial growth [204,205]. Furthermore, certain bacterial species have ureolytic activity, which by itself is a factor in sustaining these high pH conditions, further creating a medium for bacterial growth. It has long been recognized that elevated pH would also cause another major problem related to the wound healing process relater to reducing oxygen dissociation from oxyhemoglobin in the damaged tissues, thereby leading to reduced healing process [206]. By this way, as it was pointed out earlier, maintaining a lower pH is critical not only to mitigate bacterial growth and enhance healing, but also to stabilize vitamin C when applied topically [207].

To address the above issues, the cosmetic and pharmaceutical industries have developed modified forms of L-ascorbic acid with improved stability. These include the water-soluble magnesium phosphate (MAP) and sodium ascorbyl phosphate (SAP), and the fat-soluble ascorbyl 6-palmitate and tetra-isopalmitoyl ascorbic acid (IPAA) [208,209]. Despite their enhanced stability, a major limitation of these derivatives is their relatively low skin penetration and slow enzymatic conversion to active vitamin C [210–212]. In addition, a variety of vitamin C analogues and derivatives were synthesized over the years, aiming to improve properties such as antiviral, antibacterial, and antioxidant activity. However, some of these compounds were found to exhibit significantly increased cytotoxicity [213].

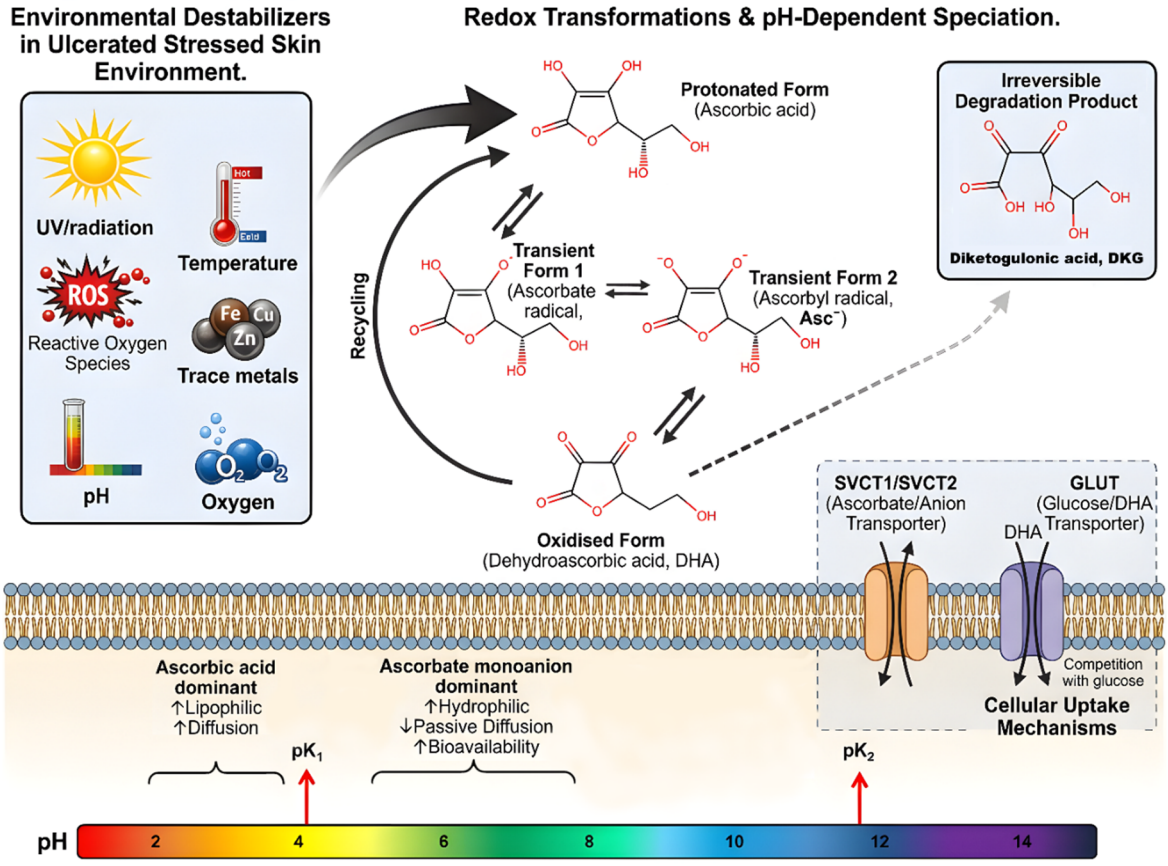

Figure 2. Vitamin C exhibits dynamic redox behaviour that depends on chemical, physical, and physiological conditions, as well as factors influencing its microenvironmental stability at the site of a skin ulcer. The biologically inactive oxidation product is also indicated (DKG), although impossible to form under physiological conditions. While DHA is also an oxidized form, its formation proceeds via a stepwise transformation from the ascorbate monoanion. Initially, oxidation leads to a transient intermediate (Transient form 1), the ascorbate radical, generated by the loss of one electron from ascorbate monoanion. This is followed by further deprotonation to form a second transient species (Transient form 2), the ascorbyl radical, which subsequently converts to DHA, as both transient forms transform simultaneously. The two dissociation constants (pK_1 and pK_2) are also indicated, corresponding to deprotonation events occurring at different pH conditions.

4. Vitamin C in the Context of Ulceration

A randomised, double-blind study has already established that 500 mg/day peroral supplementation of vitamin C has led to 100% foot ulcer healing after 8 weeks in cases of patients having ascorbate deficiency [214]. It should also be noted that some of the patients had preexisting conditions such as neuropathy, vascular disease, diabetes, tabaco smoking and high alcohol consumption. In another study, platelet-rich plasma combined with fibrin glue was evaluated alongside oral supplementation with vitamins C (500 mg/day) and E (400 U/day) as adjunctive therapy to enhance foot ulcer healing in patients [215]. The treatment promoted tissue regeneration, likely through sustained growth factor release, increased angiogenesis, and stimulation of extracellular matrix formation. However, since vitamin C was administered in combination with vitamin E and alongside an active dressing, its independent contribution to the observed effects warrants further targeted investigation.

Collectively, Vitamin C is critical for wound healing due to its roles in collagen synthesis, immune function, and antioxidant defence. Evidence from diabetic foot ulcer studies and broader tissue repair research suggests that maintaining adequate vitamin C status can support healing, but clinical data remain limited, heterogeneous, and often confounded by co-interventions. Further well-designed trials are needed to determine its independent therapeutic efficacy and optimal dosing in tissue regeneration [216,217].

Considering these factors, if the underlying causes of a skin ulcer, such as diabetes, pressure injury, venous insufficiency, trauma, or infection, are not addressed, progressive skin damage and loss of structural integrity are inevitable. In more advanced ulceration (stage 2 and beyond), the lesion extends through the epidermis and potentially the dermis, creating an open wound. This structural disruption will most likely compromise local vitamin C uptake, with sodium-dependent transporters SVCT1 and SVCT2 being particularly affected, whereas some cells may preferentially take up DHA via GLUT transporters [218]. SVCT1, predominantly expressed in the epidermis by keratinocytes, is expected to be the most compromised. However, SVCT2, found in keratinocytes, fibroblasts, and endothelial cells across both skin layers and at the dermal-epidermal junction, would also be adversely impacted [66]. All this can possibly lead to a situation where the damaged skin barrier and altered skin physiology in ulcerated areas can impede the absorption of topically applied vitamin C into the cells, thus reducing the therapy effectiveness. This is despite the fact that the physical penetration into the skin layers itself would be eased because of the ruptured stratum corneum.

The topical application of vitamin C would be expected to result in saturation of the nutrient at the site of the ulceration and take part in all four stages of wound healing (haemostasis, inflammation, proliferation and maturation), summarized in Figures 3 and 4. When an ulcer wound is established as such, each of the four phases of healing would have different implication on the remaining receptors for vitamin C cellular uptake. In fact, the most impacted would be the inflammation and proliferation phases, resonating with the role of GLUT in macrophage glucose and DHA uptake during the former, and to the SVCT-mediated vitamin C uptake for collagen synthesis during the latter.

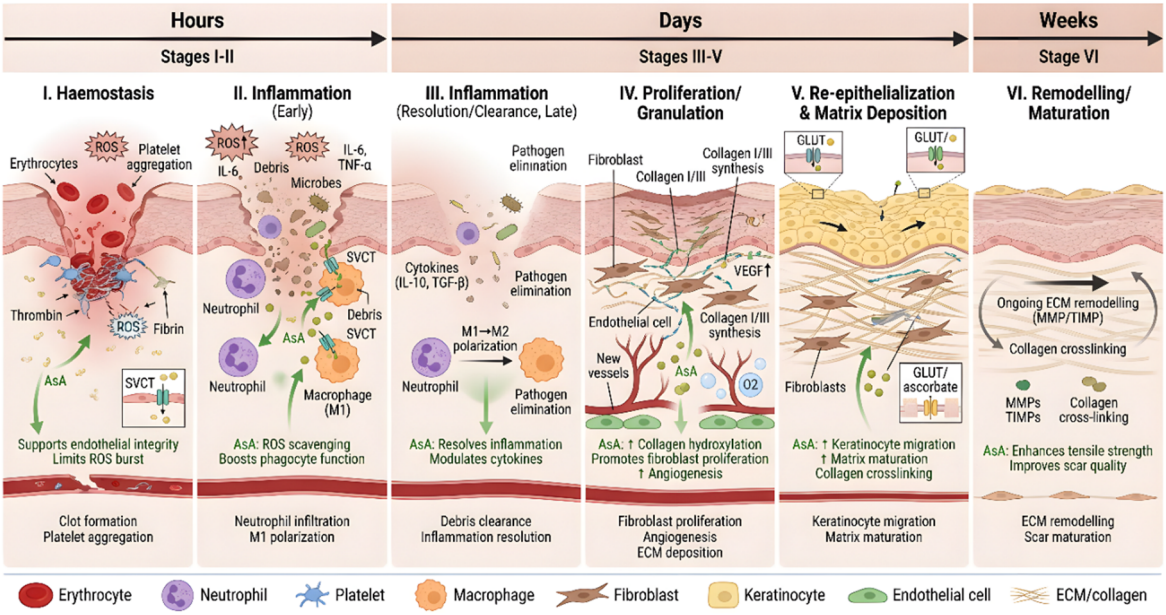

Figure 3. Stage-resolved cutaneous wound healing process and modulation by vitamin C (AsA).

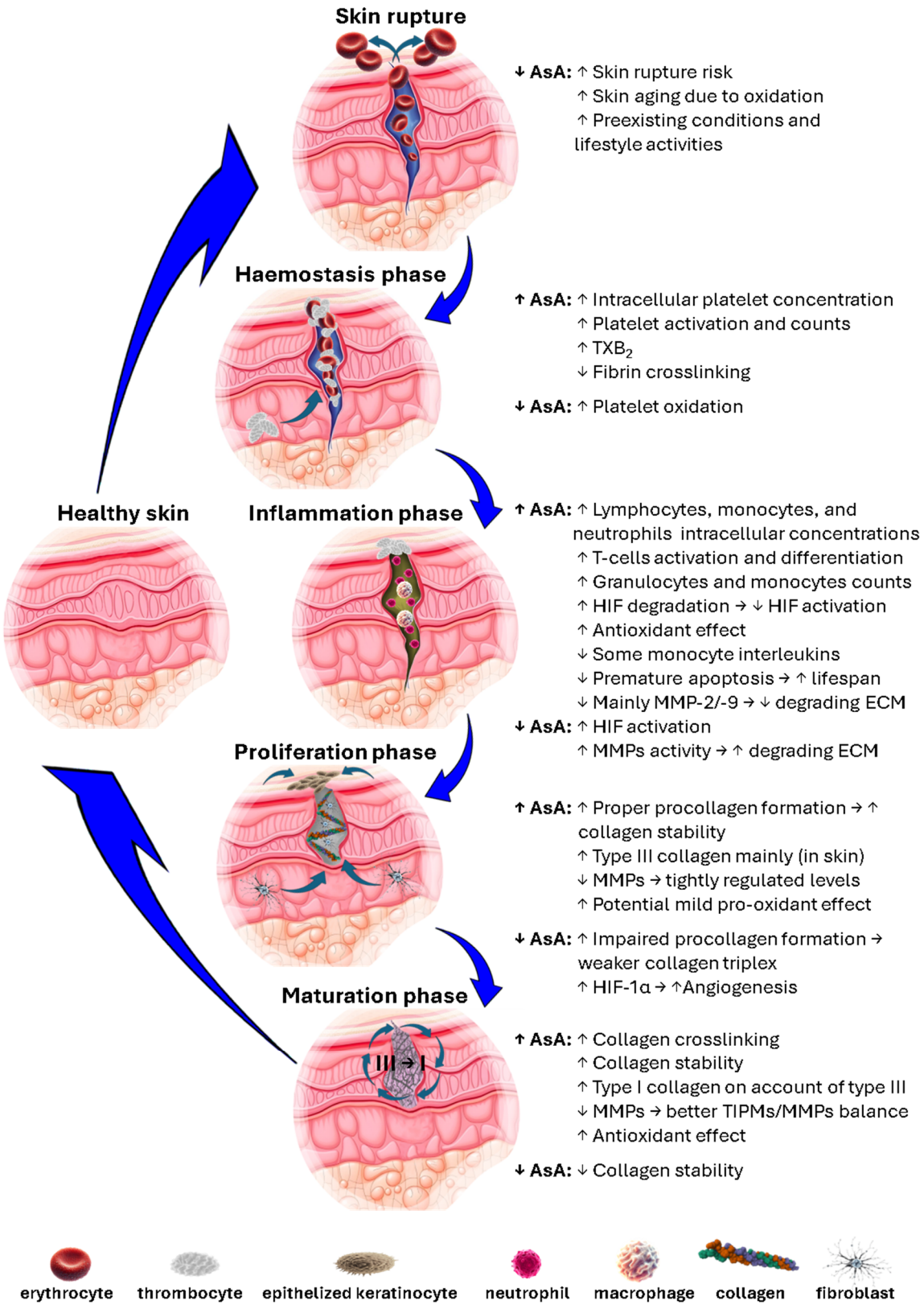

Figure 4. Graphical overview of vitamin C (ascorbic acid, AsA) and its dose-dependent roles throughout the sequential phases of wound healing: haemostasis, inflammation, proliferation, and remodelling. The schematic highlights how varying local concentrations of AsA modulate key biological processes, including coagulation dynamics, oxidative stress balance, immune cell function, fibroblast proliferation, collagen synthesis, angiogenesis, and ECM maturation. Lower to physiological levels are primarily associated with antioxidant protection and support of normal cellular activity, whereas higher concentrations may exert pro-oxidant or regulatory effects that influence tissue architecture and remodelling outcomes. The corresponding cellular components involved at each stage are depicted below and described in the figure legend to provide mechanistic context.

4.1. Haemostasis Phase

To address the initial stage of wound healing, previous research has shown that intravenous (IV) supplementation of vitamin C at low doses, or for short durations, does not significantly affect platelet function, unlike high-dose administration [219]. Following skin rupture and vascular injury, at first, platelets are rapidly recruited to the wound site, initiating a signalling cascade that stimulates megakaryocyte differentiation in the bone marrow to generate new thrombocytes [220]. As also noted, platelet counts may also increase in response to preexisting inflammation, ongoing infections, or chronic diseases such as diabetes [221].

Importantly, platelets express the SVCT2 receptors on their membranes, enabling them to accumulate high intracellular concentrations of ascorbic acid, which has been shown to raise to over 15 mmol/L [219,222]. It was also reported that these elevated doses did not reduce prothrombin time, presumed to be due to the ability of vitamin C to inhibit the expression of CD40L, a transmembrane protein with pro-inflammatory and pro-thrombotic functions [223]. Moreover, high doses of vitamin C increased the levels of pro-inflammatory arachidonic acid metabolites such as PGE_2 and TXB_2 [219]. Specifically, thromboxane B_2 (TXB_2), a stable metabolite of thromboxane A_2 (TXA_2), which is secreted exclusively by platelets, showed more than a threefold increase on the 8th day of high-dose supplementation in in vitro models. In contrast to the results of high ascorbic acid concentration, controlled and low-dose concentration showed comparable TXB_2 levels in the performed in vivo models. This suggested that high-dose vitamin C may substantially enhance platelet activation and proliferation, which could contribute to excessive clot formation and inflammation at wound sites, potentially impairing healing and increasing infection risk.

Nonetheless, other studies suggest a more complex role for vitamin C. For example, an in vitro study reported that high concentrations (1–3 mM) exert modest anticoagulant effects by interfering with fibrin crosslinking during the late stages of coagulation, thereby reducing network density and yielding looser clot structures [224]. Overall, these findings indicate that vitamin C plays a modulatory role in these processes, primarily through its antioxidant properties, as reported previously [225].

During this phase also vitamin C exerts dose-dependent and context-dependent effects on vascular tone during the haemostatic phase of skin ulceration, primarily through its interactions with nitric oxide (NO), endothelial function, and oxidative stress. Nonetheless, this vasoconstrictive effect is most prominent and physiologically relevant during this phase of wound healing, rather than for the later stages. In general, vitamin C plays a role of a vasodilator, but at physiological concentrations, it tends to support vasoconstriction indirectly, which aligns with the goals of the haemostatic phase–limiting blood loss. The nutrient achieves this mainly by reducing oxidative stress, thereby preserving NO bioavailability in a controlled manner rather than allowing excessive or dysregulated NO signalling. Additionally, vitamin C contributes to endothelial stability and supports the integrity of the vascular wall, which facilitates efficient platelet adhesion and clot formation. Its role in maintaining collagen structure is also critical here, as exposed subendothelial collagen is a key trigger for platelet activation [226]. In this context, vitamin C helps maintain a balanced environment where transient vasoconstriction can occur effectively without excessive vascular damage.

In contrast, at higher concentrations, vitamin C may shift toward a pro-oxidant behaviour, particularly in the presence of catalytic metal ions (e.g., Fe^{3+}, Cu^{2+}), which are often elevated in damaged or ulcerated tissue. This can lead to the generation of reactive oxygen species (ROS), which may impair endothelial function and disrupt normal vasoregulatory signalling. Supraphysiological vitamin C doses have also been associated with enhanced NO production or stabilization, promoting vasodilation rather than vasoconstriction, which could counteract haemostatic efficiency if not tightly regulated.

Overall, during the haemostatic phase, low to moderate vitamin C levels support appropriate vasoconstriction and clot formation, whereas high concentrations may dysregulate vascular tone, potentially shifting the balance toward vasodilation or endothelial dysfunction, especially in oxidatively stressed microenvironments such as chronic ulcers. In that sense, there are numerous in vivo studies involving various human populations where different routes of application, regimes and doses ranging from 500 mg to several grams have been employed [227–231]. Thus, defining “low” versus “high” vitamin C doses here is challenging because its vascular effects depend more on local tissue concentration, redox state, and wound microenvironment than on the administered dose, particularly during the haemostatic phase where its role is limited and indirect.

Worth mentioning is also that not only SVCTs, but also GLUTs are expressed in platelets, specifically GLUT-3, which expression is regulated via protein kinase B (PKB/Akt). It has already been demonstrated that the PKB activation and signalling pathway (mainly through phosphoinositide 3-kinase–PI3K) is closely relevant to a cellular response to tissue damage, leading to overexpression of this transducer [232–234]. This pathway is influenced through two distinct mechanisms: PI3K is activated by cytokines and platelet-derived growth factor

(PDGF), while PKB is further activated by thrombin secretion at the wound site [235–237]. Once activated, PKB will facilitate the glucose uptake into platelets, which in turn will lead to an enhanced intracellular accumulation of DHA.

4.2. Inflammation Phase

During the initial stage of wound healing, smooth muscle contraction in damaged blood vessels leads to vasoconstriction, mediated by endothelin, TXA_2 and neural reflexes and followed by platelet aggregation to form a haemostatic plug [238]. As the process progresses, the second stage initiates with vasodilation, triggered by mediators such as histamine, bradykinin, prostaglandins, and NO, which facilitates the migration of immune cells, such as neutrophils and macrophages, to the wound site. Despite their short lifespan, neutrophils are the first leukocytes to infiltrate the injured tissue to initiate phagocytosis upon debris, bacteria, and dead tissue. On the other hand, macrophages are recruited from the bone marrow and blood. At the wound site they differentiate into wound macrophages with highly phagocytic properties. These cells also produce pro-inflammatory cytokines, essential for coordinating the inflammatory response [239,240]. Interestingly, both GLUT-1 and GLUT-3 glucose transporters are abundantly expressed in macrophages. Notably, GLUT-1 expression is upregulated following M1 stimulation by microbial pro-inflammatory signals such as IFN-γ, TNF, and TLR ligands, while GLUT-3 expression increases after M2 stimulation by cytokines including IL-4, IL-6, IL-10, and IL-13 [86]. Importantly, the same study reported that GLUT-3 expression is significantly reduced in wounds of patients with diabetic foot ulcers that exhibit delayed or impaired healing, in contrast to elevated GLUT-3 levels observed in wounds undergoing normal healing. Under normal physiological conditions, similar to thrombocytes, circulating lymphocytes, monocytes, and neutrophils have a substantially higher storage capacity for L-ascorbic acid compared to plasma, maintaining intracellular concentrations of approximately 3.5 mM, 3 mM, and 1.5 mM, respectively [241]. Bone marrow stem cells and hematopoietic progenitor cells were also found to accumulate up to 20-fold more ascorbate than the differentiated ones, largely due to elevated expression of SVCT2 transporters on their membranes [242]. Vitamin C plays a crucial role as a co-factor in the regulation and reducing the impact of the so-called hypoxia-inducible factors (HIFs), a family of transcription factors involved in the cellular response to low oxygen levels and inflammatory processes [243,244]. Reduced plasma levels of the nutrient have already been proven to increase HIF activation, particularly under mild and moderate hypoxia as a stress factor [245]. In this context, sites of inflammation (presumably due to the increased oxidative metabolism of inflammatory cells), and at some point, sites of tumour growth (primarily resulting from outstripping the available nutrient supply and intense metabolic demands), are commonly associated with hypoxia [246–249].

To date, three major isoforms of hypoxia-inducible factors have been identified: HIF-1, involved in the cellular response to hypoxia, promoting angiogenesis and metabolic adaptation; HIF-2, related to erythropoietin production and to oxygen delivery in lungs, heart, and endothelium; and HIF-3 (involved in fine-tuning of the hypoxic response, but so far with limited understanding for its full potential) [250–253]. In neutrocytes, hypoxia has been shown to prolong their lifespan and enhance antimicrobial functions such as phagocytosis and the release of proteolytic enzymes including cathepsin G, proteinase 3, and elastase, where HIF-1 would also be activated, even during normoxia [254–256]. This is so, because hypoxia stabilizes HIF-1α, thus promoting cell survival and adaptation to low oxygen levels. Conversely, through an enzymatic pathway ascorbic acid leads to HIF-1 degradation, thereby reducing its cellular levels [257]. Although this was proven to be more relevant to cancer cells, it was also found that ascorbate deficiency led to impaired capacity of neutrophil cells to provide a proper immune response [258]. To summarise, as the inflammatory phase begins shortly after haemostasis, local hypoxia promotes increased neutrophil activity via HIF-1α stabilisation. As vitamin C becomes available, it facilitates prolyl hydroxylase-mediated HIF-1α degradation, contributing to a gradual reduction in neutrophil activity and modulation of the inflammatory response. However, because ascorbate will likely oxidise rapidly to DHA under physiological conditions, a concurrent severe case of hypoxia will elevate ROS and impair hydroxylase activity, thereby further accelerating vitamin C consumption and disrupting effective HIF-1α regulation. Thus, properly administered doses and optimised delivery routes of vitamin C (e.g., topical or localised delivery), may overcome microenvironment-driven depletion and instability, restoring sufficient bioactive levels to support collagen synthesis, redox balance, and effective tissue repair in skin ulcers.

In that regard also, elevated plasma levels of the nutrient were also found to increase the count of circulating granulocytes and monocytes, suggesting a contribution to more rapid and robust immune response against infections [259]. Furthermore, vitamin C has been shown to inhibit the production of certain interleukins by monocytes stimulated with endotoxins such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), in a dose-dependent manner–higher concentrations leading to greater suppression [260,261]. Owing to its intrinsic

antioxidant properties, ascorbic acid also inhibits apoptosis signalling pathways in T cells and protects monocytes from FAS-induced apoptotic death [262]. Moreover, T cells activation and differentiation were promoted by vitamin C, as well as enhancing their antigen-specific response [263,264].

Skin ulcers have a prolonged inflammatory phase, thus impairing the healing process by preventing it from progressing to the proliferative and maturation phases. If left untreated, this condition may lead to the formation of fibrotic tissue, hypertrophic scars, or even development of skin cancers [265,266]. As mentioned above, high doses of vitamin C have been associated with oxidative stress in tissues, however, these observations were primarily observed in laboratory conditions (especially in the presence of transition metals) and may not translate to in vivo effects, where other factors can mitigate this impact. In fact, vitamin C's role as a potent antioxidant often outweighs its potential to induce oxidative stress [263,267–270]. Nonetheless, when ROS are produced from high vitamin C concentrations, which is more relevant to IV application, this can lead to damage in cellular components, including enzymes involved in glycolysis. This, in turn, may result in reduced production of adenosine triphosphate as the main carrier molecule of energy in cells [271]. In addition, high doses of vitamin C can influence the formation of neutrophil extracellular traps (NETs), extracellular fibres composed of neutrophil DNA that can trap pathogens and release microbicidal proteins [267,268]. During the early stages of sepsis, microbial abundance decreases, although many bacteria trapped by NETs remain viable, primarily due to neutrophil depletion. As mentioned earlier, vitamin C supplementation can reduce neutrophil apoptosis, thereby prolonging their circulation [269,270,272–274].

A major issue, however, is the fact that chronic ulcers fail to transition to the proliferative phase. In this context, even additional vitamin C supplementation often fails to restore SVCT expression because the limitation is driven by microenvironmental dysregulation rather than substrate availability. Persistent inflammation sustains NF-κB activation, suppressing transcription and impairing transporter localization, thereby restricting cellular uptake despite adequate extracellular levels and also shifting uptake toward less efficient GLUT-mediated pathways and thus reducing intracellular ascorbate levels [275]. In parallel, an ongoing and persistent inflammatory signalling induces epigenetic and metabolic reprogramming that stabilizes a low-uptake phenotype, preventing transporter re-expression even under supplementation.

4.3. Proliferation Phase

As previously mentioned, a prolonged inflammatory phase can delay the onset of the proliferative phase, leading to delayed tissue formation, deregulated differentiation (primarily due to persistent collagen degradation), impaired angiogenesis, and ongoing inflammation [276,277]. The proliferative phase typically begins a few days after the ulcer develops and lasts for several weeks, during which time it is characterized by a complex and coordinated interplay of multiple cell types [278,279]. Initially, fibroblast migrate to the wound site and begin producing collagen, a family of proteins comprising a significant part of the ECM in various tissues and a main structural skin component. Among the collagen types, collagen type I and III have most substantial impact on skin structure, with type IV also playing a key role. Type I collagen is the most abundant and provides tensile strength and structural integrity, while type III collagen works in conjunction with type I to contribute elasticity and stability. In addition, Type IV collagen is also having a crucial role, by acting as a component of the basal membrane to support the structural framework of the skin and separating the epidermis from the dermis [280,281]. Together with collagen, new blood vessels form through angiogenesis and ECM components are secreted by the fibroblast (fibronectin, laminin, proteoglycans, glycosaminoglycans, elastin), developing a temporary granulation tissue that fill the wound bed [282]. During this phase also, myofibroblasts cause wound edges to contract, and the wound size to narrow [283]. Concurrently, epithelial cells start to migrate from the wound edges towards the centre to cover the wound surface, thus restoring the barrier function of the skin [284].

Vitamin C plays a central role during the proliferative phase too, where it affects collagen synthesis, angiogenesis and immune cells modulation. Concerning collagen synthesis, L-ascorbic acid acts as a cofactor (together with iron) for the enzymes prolyl and lysyl hydroxylase. During this process, ferrous iron (Fe^{2+}) is converted to ferric iron (Fe^{3+}) in the presence of the co-substrate 2-oxoglutarate, which activates these two enzymes. Ascorbic acid participates by reducing Fe^{3+} back to Fe^{2+} through its own oxidation to monodehydroascorbate (MDHA), thereby facilitating the reactivation of the hydrolases. [285,286]. As hydroxyl groups are added, the peptide chains are further glycolyzed and through a spontaneous chain regrouping and formation, procollagen is formed. Without this prior hydroxylation, the resulting collagen triple helix is weaker, leading to conditions such as scurvy and impaired wound healing [287,288].

Additionally, prolyl hydroxylases require not only ascorbate, but also Fe^{2+}, oxygen, and α-ketoglutarate for catalytic activity. In chronic ulcers, persistent oxidative stress leads to the oxidation of Fe^{2+} to Fe^{3+}, directly

inhibiting enzyme activity even in the presence of vitamin C [289]. Additionally, local hypoxia, an indication for poorly vascularized chronic wounds, limits oxygen availability, further restricting hydroxylase function. Although vitamin C normally acts to maintain iron in its reduced Fe^{2+} state within the enzyme active site, excessive reactive oxygen species can overwhelm this protective effect, rendering the cofactor function ineffective.

Moreover, sustained stabilization of HIF-1α in chronic wounds, driven by hypoxia and inflammatory signalling, creates a feedback loop in which prolyl hydroxylase activity remains suppressed, impairing proper ECM formation and angiogenic balance. This contributes to defective collagen hydroxylation, reduced tensile strength of newly formed tissue, and persistence of a non-resolving wound state. Therefore, the failure of vitamin C supplementation in chronic ulcers is not due to inefficacy of the molecule itself, but rather to a convergence of impaired transport, redox imbalance, cofactor inactivation, and microenvironmental constraints that collectively prevent its proper intracellular utilization and enzymatic function. In addition, because collagen type IV is involved as a component of the basal membrane, a systemic reduction in vitamin C plasma levels would compromise its structural integrity, which in turn, could facilitate the penetration of pathogens deeper into the skin layers [290,291].

Furthermore, Vitamin C also plays a critical role in the regulation of angiogenesis, a process essential for the transition from the inflammatory to the proliferative phase of wound healing, but also not fully understood, as evidenced by the many conflicting reports available in the literature. The nutrient facilitates the hydroxylation and subsequent degradation of HIF-1α, thereby tightly regulating hypoxia-driven angiogenic signalling. Under physiological conditions, this ensures a balanced expression of vascular endothelial growth factor (VEGF) and the formation of functional, mature blood vessels. Nonetheless, in ulcers, where oxidative stress and hypoxia persist, impaired vitamin C availability and function contribute to reduced enzyme activity, resulting in prolonged stabilization of HIF-1α and dysregulated VEGF expression [287,288,292]. This often leads to the formation of immature, leaky, and poorly organized vasculature rather than effective neovascularization. In addition, vitamin C supports angiogenesis through its role in collagen synthesis, providing the structural scaffold necessary for endothelial cell migration and capillary stabilization. It also enhances endothelial cell proliferation and protects against oxidative damage, further promoting vascular integrity. Therefore, insufficient or functionally impaired vitamin C disrupts both the signalling and structural components of angiogenesis, contributing to inadequate perfusion and persistence of a non-healing wound environment [293–298].

Vitamin C exerts a dose-dependent effect on wound healing and angiogenesis. At physiological concentrations in most cells (0.05–0.5 mM), it promotes collagen synthesis, stabilizes the extracellular matrix, and enhances endothelial proliferation and tube formation. However, at higher concentrations (≥1–5 mM), particularly in environments with elevated transition metals or oxidative stress, vitamin C can generate hydrogen peroxide, acting as a pro-oxidant that induces cytotoxicity and impairs angiogenesis [299,300]. This is typically achieved through intravenous infusion, often bypassing the antioxidant role it plays at lower physiological concentrations [301]. In chronic wounds, where oxidative stress is already high, this threshold may be even lower, highlighting the importance of presumably maintaining controlled, sustained delivery rather than exposing cells to excessive local concentrations.

4.4. Maturation Phase

The proliferation phase is followed by the final stage of wound healing known as maturation. This phase may last for several years, depending on the size and severity of the ulcer. The most aspect of maturation is the reorganization of collagen, where collagen type III laid down during the proliferation stage is gradually replaced by type I, which is stronger and more stable. This remodelling of the extracellular matrix results in the formation of scar tissue [302]. During the inflammatory phase, collagenases, or matrix metalloproteinases (MMPs), released from macrophages and neutrophils are also activated. Its concentration increases significantly to aid in the removal of debris and prepare the wound bed for new tissue formation [303–306]. As the healing progresses into the proliferation phase, collagenases (mainly MMP-2 and MMP-9) activity causes a temporary reduction in collagen levels at the wound site. While collagenase levels decrease sharply after the initial phase, it continues to degrade damaged collagen and other ECM components [307]. Throughout the maturation phase its concentration remains considerably lower, but is still crucial for replacing collagen type III with type I [302]. Moreover, during maturation, collagen fibres undergo crosslinking, which involves the formation of covalent bonds between collagen chains. This process is catalysed by a family of enzymes called lysyl oxidases, which are copper- and lysine tyrosyl-quinone-dependent amine oxidases [308,309]. Three lysyl oxidases are particularly important: lysyl oxidase (LOX), being the primary enzyme responsible for initiating the crosslinking of collagen and elastin by oxidizing lysine and hydroxylysine residues; lysyl oxidase-like 1 (LOXL1), supporting collagen crosslinking and

crucial for elastin maturation; and lysyl oxidase-like 2 (LOXL2)–involved in tissue remodelling, fibrosis, and angiogenesis [310–313]. Furthermore, vitamin C plays a vital role as a potent antioxidant by protecting the newly formed and existing tissue from damaging factors such as UV light and ROS (Figure 5) [314,315].

Vitamin C is an essential cofactor for the hydroxylation of lysyl and proline residues in collagen. The hydroxylysine (formed with the help of vitamin C) and lysine residues in collagen, as well as those in tropoelastin (the building block of elastin), serve as active targets for lysyl oxidase (LOX) enzymes, as these enzymes introduce aldehyde groups to form allysine and hydroxyallysine [316]. These moieties then form covalent bonds (Schiff base and aldol condensation products) with other unmodified lysine or hydroxylysine residues on adjacent collagen molecules [317,318]. Further maturation leads to the formation of more stable crosslinks, such as pyridinoline and deoxypyridinoline, which are associated with the maturation of elastin and collagen molecules, thus enhancing their structural integrity [319–321]. Hydroxyproline groups, that form as a product of prolyl hydroxylase's activity with ascorbic acid and iron as cofactors, do not directly participate in this crosslinking process, but are rather critical for maintaining the stability of the collagen triple helix structure with the formation of hydrogen bonds [322,323].

In parallel with collagen crosslinking and matrix stabilization, tissue inhibitors of metalloproteinases (TIMPs) play a critical regulatory role during the remodelling phase of ulcer healing [324]. By inhibiting MMPs, TIMPs help shift the balance from matrix degradation toward matrix preservation, allowing the newly deposited collagen I to accumulate, reorganize, and gain tensile strength. This controlled inhibition is essential to prevent excessive extracellular matrix breakdown, ensuring proper scar formation and structural integrity of the healed tissue. Vitamin C suppresses the expression and activity of MMP-2 and MMP-9, thereby contributing to a favourable proteolytic balance and limiting excessive ECM degradation. Its influence on TIMPs is largely indirect, arising from modulation of oxidative stress and cellular signalling rather than consistent direct upregulation. Consequently, the nutrient primarily regulates the MMP/TIMP relationship through inhibition of MMP activity, with secondary, context-dependent effects on TIMPs [325].

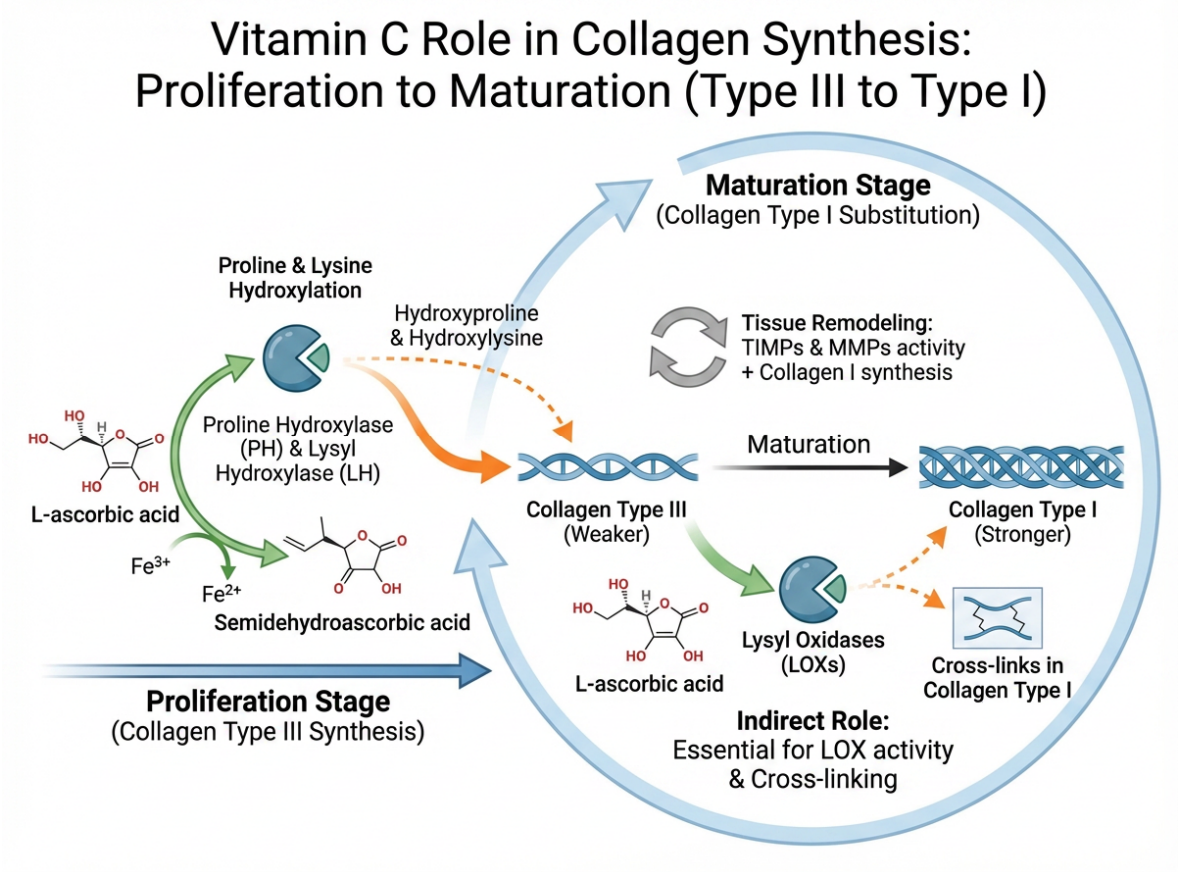

Figure 5. Direct role and mechanism of vitamin C in collagen type III synthesis during the proliferation stage and its involvement (direct and indirect) during the subsequent substitution with the stronger collagen type I during the maturation phase.

5. Vitamin C Extraction, Detection and Quantification

Accurate extraction, detection, and quantification of vitamin C are critical for reliably characterizing its distribution, bioavailability, and stability within biological tissues. This is particularly important in complex environments such as chronic wounds, where spatial heterogeneity, oxidative degradation, and limited transport can significantly influence local ascorbate levels and, consequently, its biological activity.

As mentioned above, there are significant limitations associated with accurate determination of its concentration within skin tissue and the broader understanding of the relationship between plasma levels and cellular saturation [326]. Among these, the most critical issue is related to the highly reactive nature of L-ascorbic acid and its tendency to participate in redox reactions. By this way, while sample collection and homogenization methods are important, the choice of analyte extraction techniques and storage conditions may play an even more pivotal role. This requires serious scrutinization and optimization of downstream processing, as it involves multi-step purification and careful solvent selection. Elevated temperatures during the process, high concentration of polar organic solvents like acetone, methanol (with potential risk of methylation) and trichloroacetic acid may lead to vitamin C oxidation, while cytotoxic solvents like acetonitrile are unsuitable. Instead, ethanol-water mixtures would be considered the most favourable extraction media [322,327,328].

In addition, the common redox dye 2,6-dichlorophenolindophenol (DCPIP), which is often used to quantify ascorbic acid via titration due to its reduction-induced colour change, is not suitable entirely for this purpose [323]. The main reason is the lack of specificity of DCPIP towards the nutrient and its nature to readily react with other molecules that act as reducing agents present in skin cells including glutathione, nicotinamide adenine dinucleotide (NAD), succinate dehydrogenase, cysteine [329–331]. These cause a colour change of DCPIP from blue or pink (in acidic pH) to colourless when reduced. Found in various organs and tissues, the iron-sulphur protein ferredoxin is present in the human body in two forms–Ferredoxin 1 (FDX1) and Ferredoxin 2 (FDX2) [332]. Both are present in the skin and despite their differing biological roles, can also reduce DCPIP [333,334]. It is important to note that other cellular systems involved in electron transport like Cytochrome C and P450 will also react with DCPIP analogically [335,336].

This issue is further compounded by matrix interference from lipids, necessitating extensive and variable sample preprocessing that undermines reproducibility [329]. Analytical performance is further hindered by relatively low sensitivity, susceptibility to optical interference, and semi-quantitative output, particularly in turbid or heterogeneous samples, like tissue and cell cultures. In summary, these drawbacks, along with a lack of methodological standardization, render DCPIP unsuitable for clinical deployment, especially when compared to more advanced techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS), which, although more accurate, remain impractical for rapid or point-of-care use [337]. This is so, because HPLC-based detection typically requires complex and time-sensitive sample preparation, including stabilization, deproteinization, and protection against oxidation, due to the inherent instability of ascorbate during handling and storage. These steps introduce variability and increase the risk of analyte degradation, particularly in real-world clinical workflows where immediate processing is not always feasible. Additionally, the requirement for expensive instrumentation, trained personnel, and controlled laboratory environments limits accessibility and scalability. Collectively, these challenges hinder the broader clinical adoption of HPLC for vitamin C measurement, despite its analytical advantages.

In regard to specificity, ascorbate oxidase (AO) as a plant and fungi synthesized enzyme has the ability to catalyse the oxidation of vitamin C to DHA [338,339]. It has a complex structure of at least 4 Cu atoms that defines its blue colour. When molecular oxygen is present in the system the enzyme catalyses L-ascorbic acid through spontaneous oxidation to DHA, a process that can be visually tracked by the enzyme colour change, from blue to colourless, upon substrate depletion [340,341]. Particularly curious is the case with ferritin–a cytoprotective protein found in the epidermal basal membrane and in other skin appendage as hair follicles, endothelial muscles, and sebaceous glands [342]. It stores iron in the form of ferric iron that needs a reducing agent (such as ascorbate) to transform into ferrous iron, but in order to do so, an additional free or loosely bound iron as a substrate must be available first to interact with ascorbate. In vivo and in vitro models have both shown that in aerobic conditions and neutral pH ascorbate induces iron release from this protein with high affinity [343]. When AO was introduced into that system, it extensively increased the ascorbate related free radical levels, and after an additional iron supplementation, an increased affinity of ferritin to AO was observed [344].

While ascorbate oxidase offers improved specificity compared to redox dyes (e.g., DCPIP) by catalysing the selective oxidation of ascorbate, its performance is highly dependent on enzyme stability and activity, which can be adversely affected by variations in pH, temperature, and the presence of inhibitors or interfering substances, as mentioned above [345]. From a translational perspective, reliance on purified enzymes increases cost, limits shelf-

life and complicates integration into robust platforms. Furthermore, assay standardization across laboratories will also be challenging due to differences in enzyme sources, activity units, and detection formats, thus hindering the reproducibility, scalability, and regulatory acceptance required for clinical deployment. It is unfortunate, however, as a methodology based on AO could have potentially been a powerful and at the same time very accurate and relatively cheap technique for L-ascorbic acid quantitative and qualitative analysis. Moreover, this would be particularly promising given relative low AO toxicity at physiological concentrations and conditions to mammalian cells [346].

Moreover, there are several other enzymes that are tightly related to the red-ox transformation of L-ascorbic acid. One of them is ascorbate peroxidase (APX), a plant and algae enzyme that uses ascorbate to reduce hydrogen peroxide produced by photorespiration and photosynthetic electron transport to water [347]. It is a heme-containing enzyme that also plays a role in scavenging and neutralizing ROS, by the mechanism of oxidizing ascorbic acid in the process [348–352]. Ascorbic acid serves as a substrate for APX, leading to the production of MDHA and then spontaneously oxidising to DHA. From there, it can be reduced back to the protonated form of L-ascorbic acid by other enzymatic systems [353]. In certain plant species, the APX-mediated ROS detoxification process occur in three distinct stages, where the first is characterized by interaction between H_2O_2 and the enzyme with the formation of a transient Compound I (a ferryl heme and a porphyrin pi-cation radical) and water. During the second, this Compound I reacts with ascorbate to form Compound II (only the ferryl heme) and one electron oxidized radical. The third and final stage involves the reduction of Compound II by another ascorbate molecule, regenerating the native form of the enzyme and producing water and an additional radical species [354,355]. Excess or fluctuating H_2O_2 levels can lead to enzyme inactivation or non-specific oxidation reactions, while endogenous peroxides and antioxidant systems present in biological samples may interfere with assay kinetics and accuracy. In addition, APX activity can decrease in the presence of heavy metals, thus leading to increased toxicity [356,357]. Given that the enzyme operates under complex physiological and environmental conditions, and its dependence on sensitive redox interactions, the direct application of APX for the determination of vitamin C would pose significant practical limitation. Similar to other methods, the intrinsic instability of ascorbate during sample handling further contributes to measurement error, and the assay does not readily differentiate between reduced and oxidized vitamin C without additional modifications. Variability in enzyme sources, activity, and assay configurations also hampers standardization and reproducibility across laboratories.

Another enzyme directly involved in the body circulation and recycling of ascorbic acid is monodehydroascorbate reductase (MDHAR), related to ascorbate-glutathione cycle that helps regenerate vitamin C from DHA, as well as neutralizing ROS [358,359]. Thus, this enzyme works together with dehydroascorbate reductase (DHAR) and glutathione reductase (GR) to maintain the balance of ascorbic acid and glutathione in cells [360,361]. However, measuring MDHAR activity would again not be accurate, as its activity is interdependent with other enzymes within the cycle. This dependency introduces variability in signal output and complicates assay calibration, particularly in heterogeneous matrices such as plasma or wound exudate. Furthermore, the transient nature and rapid disproportionation of MDHAR reduce the reliability of intermediate detection, while competing cellular redox systems can further perturb assay kinetics. The enzyme itself is susceptible to degradation, loss of activity under non-ideal pH and temperature conditions, and limited shelf stability. Therefore, a comprehensive evaluation of all key enzymes involved is necessary to accurately reflect the ascorbic acid content and redox status of a biological sample.

2,4-Dinitrophenylhydrazine or DNPH has long been considered a reliable reagent for the quantification of ascorbic acid, offering both direct and indirect detection approaches. The indirect method relies on DNPH conjugation with dehydroascorbic acid (DHA), while the direct pathway involves interaction with L-ascorbic acid itself, both of which are facilitated by the presence of reactive carbonyl groups in these two compounds [362–364]. The method is old, yet, proven to be extremely effective towards plant and animal samples, and is based on first oxidizing ascorbic acid to DHA, then coupling through nucleophilic attack with 2,4-DNPH that leads to the formation of a hydrazone derivative, and finally, prior the spectrophotometric measurement–treatment with concentrated sulfuric acid to produce a specific, red-coloured complex. A major drawback, however, would be the sensitivity of the method and the high probability of DNPH to react with a broad range of carbonyl-containing compounds commonly found in skin samples, which requires additional purification and inactivation of these chemicals [365–368].

Interestingly, currently, there is only one study proposing a direct conjugation of vitamin C with a fluorescent dye for the purpose of determining its distribution in cell lines, while skin derived samples have not been used yet. The central concept involves exploiting the specificity of SVCTs, which recognize and mediate cellular uptake of ascorbic acid via its functional moieties. In this study, sulforhodamine B was conjugated to vitamin C through a linker, and the resulting fluorescent compound was visualized using confocal microscopy using two distinct cell

lines–HepG2 (a human liver carcinoma line) and HEK-293T (human embryonic kidney cells transformed by adenovirus) [369]. This study concluded that the synthesized chromophore had high affinity towards SVCT receptors and exhibited features to be used as a target molecule for the examination of the receptors' expression.

Based on the above, the best way of determining and examining the saturation of ascorbic acid in skin may need to involve methodologies, non-toxic chemicals and specificity towards the targeting systems that usually interact transport proteins and enzymes. As discussed, enzymes have been ruled out as viable options for this task, directing attention to transporters responsible for ascorbic acid uptake in skin cells as a more plausible approach. For that reason, the analogue and radiotracer 6-deoxy-6-[18F] fluoro-L-ascorbic acid (^{18}F-DFA), first introduced in the early 90s, was considered to play this important role [370]. By itself, Fluorine-18 is a positron-emitting radioisotope commonly used in positron emission tomography (PET) imaging with half-life of approximately 110 min and mainly used for oncological radiotracing [371,372]. During its radioactive decay it emits high-speed β+ particles (positrons). A recent study reported that when conjugated with L-ascorbic acid and applied intravenously, the newly formed compound was found to be well tolerated in humans with no serious adverse events. The results showed a typical pattern of distribution and accumulation for L-ascorbic acid in organs and tissues, in accordance with the level of expression of SVCT1 and SVCT2 receptors [373]. Another study demonstrated in rat models that ^{18}F-DFA was highly effective as an imaging agent, specifically targeting SVCT2 receptors in tumour tissues [374].

None of these two papers, nor any other related research study so far had mentioned or examined the distribution of ^{18}F-DFA in skin. Moreover, the difficulties of synthesizing the compound in a multistep process that requires specific equipment and premises were also acknowledged. Furthermore, ^{18}F-DFA is hydrophilic as both of its moieties are Fluorine-18 and L-ascorbic acid, and is primarily designed for systemic distribution [375]. Being so, its topical application and penetration through the skin has to be evaluated practically, because the extent of its absorption is expected to vary depending on the same prerequisites as already mentions for ascorbic acid. Next, if the compound was to be directly injected into the dermis, this would create a depo effect even for a hydrophilic substance. This is because, on one side, the basal membrane will function as a barrier preventing the substance from easily diffusing into the epidermis. On the other side, the subcutaneous fat tissue will be another barrier that will lead to a gradual permeation through the blood vessels and subsequent release into the bloodstream [376,377].

In conclusion, the application of ^{18}F-DFA PET imaging in skin ulcer research presents a promising yet technically constrained approach for probing in vivo vitamin C dynamics and redox-related processes. A key limitation arises from the short half-life (~110 min), which necessitates rapid tracer synthesis, administration, and imaging, thereby restricting experimental flexibility and complicating longitudinal study designs typically required for chronic wound assessment. Additionally, the reliance of PET tracers on systemic delivery poses a significant challenge in the context of chronic ulcers, where impaired vascularization, ischemia, and the presence of necrotic or biofilm-laden tissue can markedly hinder tracer penetration and result in heterogeneous or under-representative uptake.

Interpretation of the PET signal is further complicated by the multifactorial nature of tracer accumulation. Although ^{18}F-DFA is designed to mimic ascorbate transport, its uptake is influenced not only by transporter expression but also by perfusion, inflammatory activity, and systemic pharmacokinetics, limiting specificity. In addition, the relatively low spatial resolution of clinical PET imaging (on the millimetre scale) reduces its ability to resolve superficial and structurally heterogeneous wound environments, where critical biochemical gradients occur at much finer scales [378,379].

From a translational perspective, the suitability of existing preclinical models remains uncertain, as commonly used animal wound models often fail to replicate the impaired healing, vascular deficits, and chronic inflammation characteristic of human ulcers [380–382]. Practical considerations, including high cost, need for specialized radiochemistry infrastructure, and exposure to ionizing radiation, further constrain routine clinical implementation. Collectively, while ^{18}F-DFA PET imaging offers valuable mechanistic insights, particularly in controlled experimental settings, these limitations currently restrict its broader applicability in clinical skin ulcer monitoring and management.

An important matter would also be the assayed skin material–cells, tissues, or organ. In that very regard, a material with abnormal and neoplastic activity would be particularly advantageous, as SVCT receptors (especially SVCT2) are known to be overexpressed in such materials [383]. Given that vitamin C uptake is crucial for cancer cell metabolism and survival, targeting these overexpressed receptors in tumours like malignant melanoma (MM), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) could offer promising therapeutic opportunities. A more thorough consideration reveals that BCC and SCC often remain confined to the epidermis or upper dermis for longer periods before potentially invading deeper tissues. In contrast, MM is known for its ability to penetrate deeper into the skin, reaching the dermis and even beyond, thus allowing the cancer cells to access blood vessels

and lymphatic channels [384–386]. For this reason, YUMM, NRAS and BRAF mouse models would be ideal for this examination [387]. If in vivo models were chosen, then the type of administration would be extremely important, as previous studies on intravenous administration of ^{18}F-DFA showed accumulation in organs other than the skin. Oral administration, while a potential option, may be too slow due to the limited potency of the radiotracer, even if the animals are not fasted prior to intake. Thus, the most logical approach would be intradermal injection, with the possibility of examining epidermal distribution either in conjunction with or separately from topical application.

In vitro studies could involve several distinct cell lines, or, for greater complexity, engineering human skin equivalents using human fibroblasts and keratinocytes, as previously proposed [388]. Although this developed skin model demonstrated significant similarities to native human skin, it was concluded (also elsewhere) that so far cell-based skin systems lacked proper characterization and have shown variations or inability to fully develop a normal skin structure [389–392].

In addition, currently there are a few ex vivo studies regarding biopsy extracted skin samples and referred to as skin-on-a-chip [393–395]. Nevertheless, they all were insightful in terms of providing crucial information about key matters related to skin samples culturing or co-culturing, where two of the these three were even connected to long-term culturing. For this reason, it would be interesting to investigate whether a permeable well insert system would be suitable for culturing biopsy-derived skin samples for tracking the distribution of supplemented ^{18}F-DFA to this system. A major drawback, however, would be the presumed, and to some extent unpredictable, cellular activity in response to the disruption of the organ's integrity [396–398]. These changes could involve mechanically induced stress pathways, hypoxia, inflammatory responses, cell apoptosis, and ECM disruptions, all of which may influence cell migration and signalling. Therefore, rapid and proper processing, along with appropriate storage, would be critical to maintain sample integrity [399].

Taken together, robust analytical approaches are essential not only for ensuring data reliability and reproducibility but also for enabling meaningful interpretation of vitamin C dynamics across different tissue compartments, thereby supporting the development and evaluation of targeted therapeutic strategies (Table 2).

Table 2. Methods for extraction, detection, and quantification of vitamin C in biological samples: principles, advantages, and limitations.

Category	Method	Principle	Advantages	Limitations
Sample Processing	Solvent extraction	Isolation using aqueous/organic solvents	• Fundamental step • Adaptable	• Ascorbate instability • Oxidation risk • Solvent- and temperature-sensitive • Requires optimization
Colorimetric	DCPIP assay	Redox dye reduction with colour change	• Simple • Rapid • Inexpensive	• Low specificity • Interference from cellular reductants • Semi-quantitative • Poor reproducibility
Chromatographic	HPLC/LC-MS	Separation with UV or MS detection	• High sensitivity • Accuracy • Specificity	• Complex preparation • Costly • Requires expertise • Limited point-of-care applicability
Enzymatic	Ascorbate oxidase (AO)	Selective oxidation of ascorbate to DHA	• Higher specificity than dyes	• Enzyme instability • Environmental sensitivity • Poor standardization • Cost
	Ascorbate peroxidase (APX)	Ascorbate-dependent H_2O_2 reduction	• Insight into redox activity	• Complex kinetics • Interference • Not specific for quantification

Table 2. *Cont.*

Category	Method	Principle	Advantages	Limitations
	MDHAR/DHAR/GR system	Enzymatic recycling of ascorbate	• Reflects redox cycling	• Indirect measurement • High variability • Poor reproducibility
Chemical Derivatization	DNPH assay	Hydrazone formation with DHA	• Established • Applicable across matrices	• Non-specific reactions • Requires purification • Moderate sensitivity
Imaging/Tracing	Fluorescent conjugates	SVCT-targeted labelled ascorbate	• Enables cellular localization	• Experimental • Limited validation in skin
	^{18}F-DFA PET	Radiotracer-based imaging	• In vivo tracking • Mechanistic insight	• Short half-life • Low spatial resolution • High cost • Limited skin applicability
Experimental Models	*In vitro*/ex vivo skin	Cell cultures, engineered skin, biopsies	• Controlled conditions • Mechanistic insight	• Poor physiological representation • Variability • Stress artefacts
	In vivo models	Whole-organism studies	• Physiological relevance	• Limited translation to human chronic wounds

6. Summary and Outlook

Wound healing is a dynamic and highly regulated process which involves coordinated processes involving immune activation, cell proliferation, collagen deposition, and tissue remodelling. In chronic wounds such as diabetic ulcers, venous leg ulcers, and pressure ulcers, this process is often disrupted and associated with uncontrolled inflammation, persistent infection, and impaired re-epithelialization. The rising prevalence of these wounds, driven by chronic comorbidities such as diabetes, vascular insufficiency, and systemic lifestyle-related factors including smoking, alcohol use, and exposure to environmental pollutants, places an enormous burden on healthcare systems globally.

Conventional therapies such as nonsteroidal anti-inflammatory drugs (NSAIDs), negative pressure wound therapy (NPWT), surgical interventions, and advanced dressings remain essential components of chronic wound management and regenerative medicine. However, increasing attention is being directed toward bioactive adjuncts that can modulate the wound microenvironment. Among these, vitamin C (L-ascorbic acid), a well-known essential dietary nutrient, has emerged as a biologically relevant and underutilized therapeutic agent that is essential for chronic wound healing outcomes.

Vitamin C exerts multifaceted regulatory effects across all phases of wound healing. During the inflammatory and haemostatic phases, it supports immune modulation by enhancing neutrophil apoptosis, reducing excessive oxidative stress, and attenuating pro-inflammatory cytokine expression. In the proliferative phase, vitamin C acts as a critical enzymatic co-factor in proline and lysine hydroxylation, essential for collagen synthesis, a key structural protein required for development of granulation tissue and dermal matrix integrity. In the final maturation phase, vitamin C promotes collagen remodelling and angiogenesis, supporting vascularization and wound closure. Cellular uptake of vitamin C occurs via the sodium-dependent vitamin C transporters SVCT1 and SVCT2, while the uptake of DHA is facilitated via the GLUTs glucose transporters.

Notably, plasma and tissue levels of vitamin C are frequently depleted in patients with chronic wounds. This is likely due to increased metabolic demand, oxidative degradation, and dietary insufficiencies, leading to a physiological deficit that may impair healing capacity. Furthermore, C continues to exhibit antimicrobial properties in ulcerated tissue, even in the presence of infection and reduced transporter expression, partly through

its pro-oxidant activity under certain conditions and its ability to disrupt bacterial biofilms. The combined antibacterial, antioxidant, and immunomodulatory actions of vitamin C indicate that the nutrient is a promising candidate for incorporation into advanced wound therapeutics.

Several emerging delivery strategies such as liposomes, polymeric nanoparticles, hydrogels, microneedle patches, and plasma-activated systems are being explored to improve vitamin C bioavailability, enhance its retention at the wound site, and overcome its inherent instability. However, the lack of standardized, non-invasive tools to monitor local vitamin C concentrations in skin remains a barrier to optimizing dosing regimens. A deeper understanding of its pharmacokinetics and tissue distribution, particularly under hypoxic and inflamed wound conditions, is essential to guide future therapeutic development.

In addition to its established roles, vitamin C exerts both antioxidant and pro-oxidant effects, it supports immune regulation by enhancing neutrophil function and attenuating excessive cytokine-driven inflammation. Its actions are dose-dependent, with suboptimal concentrations limiting collagen synthesis and immune modulation, while excessively elevated levels may paradoxically reduce efficacy or interact antagonistically with some pro-oxidant–based antibacterial therapies. Even when applied topically, vitamin C's effects can be hindered in chronic or damaged skin, due to impaired transporter/receptor engagement, oxidative inactivation in the wound microenvironment, or local tissue destruction that limits uptake, reducing its overall therapeutic potential. Concurrently, vitamin C drives collagen hydroxylation, extracellular matrix stabilization, and angiogenesis, reinforcing granulation tissue formation and dermal integrity essential for effective wound repair.

Collectively, vitamin C represents a biologically potent, safe, and cost-effective molecule with therapeutic relevance across the entire continuum of wound healing. Its combination of antioxidant, pro-oxidant, immunomodulatory, antimicrobial, and collagen-stabilizing effects uniquely positions it to complement conventional and advanced wound care modalities, particularly in diabetic and other chronic ulcers where impaired perfusion, oxidative stress, and tissue instability hinder healing. The integration of vitamin C into multimodal therapeutic strategies, including pharmacological, physical, and bioresponsive delivery approaches offers the potential to enhance tissue regeneration, prevent infection, and restore functional dermal architecture. Thus, future research should focus on precision dosing, innovative delivery systems capable of maintaining effective tissue concentrations, and clinically validated biomarkers to guide therapy, monitor outcomes, and maximize efficacy. By consolidating these mechanisms and translational approaches, vitamin C has the potential to serve as a central pillar of regenerative wound therapies, bridging basic biological insights with clinically effective, patient-centred interventions.

Author Contributions

Conceptualization and original draft preparation—B.B.S.; validation of information B.B.S., K.A.V., V.K.T., C.L.D.; article visualization as figures—B.B.S.; formal analysis— B.B.S., K.A.V., V.K.T., C.L.D.; review and final version editing—B.B.S., V.K.T., C.L.D., K.A.V. All authors have read and agreed to the final version of the manuscript.

Funding

There is no funding in regard to compiling this manuscript.

Institutional Review Board Statement

Not applicable

Informed Consent Statement

Not applicable

Data Availability Statement

Data sharing does not apply to this Review.

Conflicts of Interest

Given the role as Associate Editor, Prof. Krasimir Vasilev had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper

Abbreviations

ROS, reactive oxygen species; NSAIDs, non-steroidal anti-inflammatory drugs; NPWT, negative pressure wound therapy; UV, ultraviolet; IV, intravenous application; SVCT, sodium-dependent vitamin C transporters; GLUTs, glucose transporters; DHA, dehydroascorbic acid; AsA, L-ascorbic acid; TXB2, Thromboxane B2; PDGF, platelet-derived growth factor; NO, nitric oxide; IFN-γ, interferon-γ; TLR, toll-like receptor; TNF, tumour necrosis factor; IL, interleukin; mM, millimoles/millimolar; μM, micromoles/micromolar; HIF, hypoxia-inducible factors; LPS, lipopolysaccharide; LTA, lipoteichoic acid; NETs, neutrophil extracellular traps; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; LOXL, lysyl oxidase-like; LOX, lysyl oxidase; mg, milligram; mL, millilitre; HSV, herpes simplex virus; HPV, human papillomavirus; HZV, herpes zoster virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; CIN, cervical intraepithelial neoplasia; IEA, immediate early antigen; LA, late antigen; DKG, 2,3-diketogulonic acid; MAP, magnesium ascorbyl phosphate; SAP, sodium ascorbyl phosphate; IPAA, tetra-isopalmitoyl ascorbic acid; DCPIP, 2,6-dichlorophenolindophenol; FDX, ferredoxin; AO, ascorbate oxidase; APX, ascorbate peroxidase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; PET, positron emission tomography; ^{18}F-DFA, radiotracer 6-deoxy-6-[18F] fluoro-L-ascorbic acid; MM, malignant melanoma; SCC, squamous cell carcinoma; BCC, basal cell carcinoma; TIMP, inhibitor of matrix metalloproteinase; MIC, minimum inhibitory concentration; pK_a, the negative $\log_{10}$ of the acid dissociation constant (K_a) of a solution; mg, milligram; μg, microgram; mL, millilitre; MMP, metalloproteinase; NAD, nicotinamide adenine dinucleotide; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectroscopy

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