Hypothesis

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Restoring Sulfur Homeostasis in Diabetic Wounds via SLC7A11-Mediated Cysteine Uptake: Pathophysiological Mechanisms and Surgical Applications of a Dual-Sulfur Regenerative Topical Solution Using N-Acetylcysteine and Methylsulfonylmethane

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Abstract

Background: Chronic diabetic wounds are characterized by persistent inflammatory arrest driven by redox collapse, mitochondrial dysfunction, and extracellular matrix (ECM) instability. Sulfur deficiency has emerged as a central upstream defect linking these pathological domains. Impaired cystine uptake via SLC7A11/xCT limits intracellular cysteine, suppresses glutathione synthesis, and destabilizes redox buffering. Oxidative stress disrupts cytoskeletal signaling, suppresses angiogenic pathways, impairs mitochondrial bioenergetics, and perpetuates chronic inflammation. Concurrent extracellular sulfur depletion compromises disulfide bonding and crosslinking in collagen, elastin, and keratin, weakening tissue mechanics and impairing cell–matrix communication. **Hypothesis:** A dual-sulfur regenerative approach simultaneously addresses intracellular and extracellular sulfur deficits, restoring redox balance, mitochondrial function, and ECM integrity to convert the wound microenvironment from inflammatory arrest to regenerative competence. **Therapeutic Strategy:** A topical formulation combining N-acetylcysteine (NAC; ~2–3% w/v) and methylsulfonylmethane (MSM; ~5–8% w/v) targets complementary compartments. NAC replenishes cysteine, restores glutathione, stabilizes mitochondria, and reprograms inflammatory pathways. MSM enhances ECM crosslinking, restores biomechanical integrity, reduces proteolytic degradation, and reactivates mechanotransduction. Application involves gentle wound cleansing, thin layer topical delivery under semi-occlusive dressing once to twice daily for 2–4 weeks, with monitoring of granulation, epithelialization, exudate, and local inflammation. **Implications:** Intracellular redox normalization, mitochondrial recovery, macrophage phenotype transition, angiogenic restoration, and ECM stabilization converge to initiate coordinated tissue regeneration. Sulfur homeostasis thus serves as a unifying upstream regulator and therapeutic target. **Conclusion:** Targeting both intracellular and extracellular sulfur deficits provides a rational, mechanism-driven strategy to accelerate wound closure, restore tissue quality, and enhance vascular stability. This dual-sulfur approach redefines the biological potential of diabetic wound therapy.

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1. Introduction

Diabetic wounds represent one of the most severe and disabling chronic complications of diabetes mellitus and remain a leading cause of infection-related hospitalization and non-traumatic lower-limb amputation worldwide [1]. Despite major advances in vascular reconstruction, antimicrobial therapy, pressure off-loading, and bioengineered wound dressings, a significant proportion of diabetic ulcers fail to progress toward closure [2]. This persistent therapeutic failure indicates that prevailing clinical models, which predominantly attribute non-healing to ischemia, neuropathy, or infection, do not sufficiently explain the profound biological resistance to tissue repair observed in diabetic wounds [3].

Emerging molecular evidence supports a more fundamental interpretation in which diabetic wounds are sites of metabolic and proteostatic collapse characterized by redox disequilibrium, mitochondrial dysfunction, extracellular matrix (ECM) disintegration, and unresolved inflammation [4,5]. Chronic hyperglycemia drives excessive production of reactive oxygen species (ROS) through mitochondrial electron transport chain over-reduction, activation of NADPH oxidases, increased polyol pathway flux, and signaling through advanced glycation end-product (AGE) receptors [6–8]. These processes disrupt intracellular homeostasis at multiple levels, resulting in oxidative damage to macromolecules, defective protein folding, impaired cellular energetics, and loss of coordinated intercellular signaling required for regeneration [9,10].

A unifying but underrecognized contributor to these abnormalities is disruption of sulfur metabolism. Sulfur is indispensable for intracellular redox buffering, stabilization of protein structure through disulfide bond formation, enzymatic catalysis, and mitochondrial respiration [11,12]. In peripheral diabetic tissues, cysteine bioavailability is frequently limited due to impaired uptake of extracellular cystine via the cystine–glutamate antiporter system xCT, encoded by SLC7A11 [13,14]. This transporter constitutes the principal mechanism by which most non-hepatic cells acquire cysteine equivalents necessary for glutathione synthesis and structural disulfide bonding [15]. Functional suppression of SLC7A11 in the diabetic wound microenvironment leads to intracellular cysteine depletion, exhaustion of glutathione reserves, and uncontrolled accumulation of ROS [16]. These events propagate oxidative damage to proteins, lipids, and nucleic acids, destabilize ECM architecture, impair mitochondrial ATP generation, and perpetuate inflammatory signaling. From a pathophysiological perspective, diabetic wounds can therefore be conceptualized as localized sulfur-deficient, redox-collapsed tissues that are structurally, metabolically, and immunologically incapable of repair. This framework supports a shift away from exclusively symptomatic wound management toward targeted restoration of fundamental molecular deficits, reframing diabetic wounds as a manifestation of SLC7A11-mediated sulfur dysregulation and opening new avenues for regenerative strategies grounded in redox biology, mitochondrial medicine, and proteostasis restoration [12,16].

1. Sulfur Biology as a Foundation of Tissue Integrity

Sulfur occupies a central role in maintaining tissue integrity by governing antioxidant defense systems, protein conformation, mitochondrial bioenergetics, and immune modulation [17]. During wound repair, sulfur availability determines whether resident and infiltrating cells can withstand oxidative stress, synthesize structurally competent matrix proteins, generate sufficient ATP, and successfully transition through the inflammatory, proliferative, and remodeling phases of healing [18–20]. Disruption of sulfur metabolism therefore exerts system-wide consequences that compromise regenerative capacity at molecular, cellular, and tissue levels [21].

Cysteine is the rate-limiting substrate for the synthesis of glutathione, the dominant intracellular antioxidant responsible for maintaining thiol redox balance and detoxifying reactive oxygen species through the glutathione peroxidase and glutaredoxin systems [22]. In most somatic cells, cysteine is derived primarily from extracellular cystine imported via SLC7A11 and subsequently reduced in the cytosol [23].

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In diabetic wounds, multiple hyperglycemia-driven mechanisms markedly increase ROS production, including mitochondrial electron leakage at complexes I and III, activation of NADPH oxidase isoforms, and AGE–RAGE signaling cascades that amplify inflammatory oxidant generation [24–27].

When cysteine availability declines, glutathione synthesis becomes insufficient, leading to oxidation of critical thiol groups in proteins, lipids, and transcriptional regulators [12,28,29]. A major downstream consequence is disruption of Nrf2 signaling, a master regulator of antioxidant gene expression. Under physiological conditions, redox-sensitive modification of KEAP1 permits Nrf2 stabilization and transcription of cytoprotective genes such as HMOX1, NQO1, GCLC, superoxide dismutase, and catalase [30–32]. In sulfur-depleted diabetic wounds, persistent oxidative stress and thiol imbalance impair proper Nrf2 activation while simultaneously enhancing NF-κB signaling, resulting in sustained transcription of pro-inflammatory mediators including TNF-α, IL-1β, and IL-6. This establishes a self-perpetuating redox-inflammatory cycle in which oxidative stress reinforces inflammation and inflammatory cells further amplify ROS production, effectively preventing the transition toward tissue repair [33–36].

Beyond its antioxidant role, sulfur is essential for structural protein integrity through the formation of disulfide bonds between cysteine residues, which stabilize the tertiary and quaternary structures of numerous secreted and membrane proteins [12]. Proper disulfide bond formation within the endoplasmic reticulum, catalyzed by protein disulfide isomerases, is critical for the maturation of collagen, elastin, fibronectin, laminin, and integrins that collectively form the extracellular matrix scaffold [12,37]. In sulfur-deficient conditions, impaired oxidative protein folding leads to accumulation of misfolded ECM precursors in the endoplasmic reticulum and activation of the unfolded protein response via PERK, IRE1α, and ATF6 pathways [38]. Although initially adaptive, chronic unfolded protein response signaling promotes translational attenuation, CHOP-mediated apoptosis, and reduced fibroblast viability and collagen synthesis. Histologically, this manifests as disorganized collagen fibrils, fragmented elastin networks, and diminished matrix tensile strength [39].

The altered matrix composition disrupts integrin engagement and focal adhesion kinase signaling, impairing mechanotransduction processes required for fibroblast migration, myofibroblast differentiation, and wound contraction. Sulfur deficiency therefore directly undermines the structural and signaling framework upon which tissue regeneration depends [40–42].

Sulfur is also indispensable for mitochondrial respiration through its incorporation into iron–sulfur clusters, which serve as essential cofactors for enzymes within complexes I, II, and III of the electron transport chain as well as tricarboxylic acid cycle enzymes such as aconitase [43,44].

Iron–sulfur cluster assembly depends on cysteine-derived sulfur mobilized by mitochondrial cysteine desulfurase systems [45]. In sulfur-restricted diabetic wound cells, defective iron–sulfur cluster biogenesis impairs electron transport efficiency, increases electron leakage and secondary ROS formation, disrupts mitochondrial membrane potential, and reduces ATP synthesis [46,47].

Energy failure has profound functional implications because keratinocyte migration requires ATP-dependent cytoskeletal remodeling, fibroblast proliferation and collagen secretion demand high biosynthetic energy, and endothelial sprouting during angiogenesis depends on mitochondrial signaling and ATP availability. Sulfur-linked mitochondrial dysfunction thus creates a metabolic bottleneck that prevents coordinated progression from inflammation to tissue formation [48,49].

Effective wound healing additionally requires resolution of inflammation and establishment of a pro-repair immune environment, processes that are strongly influenced by intracellular thiol status [50,51]. Adequate cysteine and glutathione levels promote redox conditions that restrain NF-κB activation and favor polarization of macrophages toward a reparative phenotype characterized by secretion of IL-10, TGF-β, and vascular endothelial growth factor [52,53]. In sulfur-deficient diabetic wounds, persistent oxidative stress maintains macrophages in a pro-inflammatory state marked by high production of TNF-α, IL-1β, and matrix metalloproteinases such as MMP-9, which degrade extracellular matrix components and inhibit granulation tissue formation [54–56]. Impaired clearance

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of neutrophils further prolongs protease and oxidant release, amplifying collateral tissue damage [57]. Failure of sulfur-dependent redox signaling to resolve inflammation therefore prevents the coordinated activation of fibroblasts, endothelial cells, and keratinocytes required for the proliferative phase, locking the wound in a chronic inflammatory state that is biologically incapable of regeneration [58,59].

Table 1. Multisystem Consequences of Sulfur Deficiency in the Diabetic Wound Microenvironment.

Biological System	Sulfur-Dependent Component	Molecular Disruption in Diabetes	Cellular Consequence	Tissue/Clinical Outcome
Redox homeostasis	Cysteine → Glutathione synthesis via SLC7A11	Reduced cystine uptake, GSH depletion, ROS accumulation	Oxidative macromolecular damage, redox signaling failure	Persistent oxidative stress, delayed healing
Antioxidant defense regulation	Nrf2–KEAP1 thiol-sensitive pathway	Impaired Nrf2 activation and antioxidant gene transcription	Decreased HO-1, NQO1, SOD, catalase expression	Loss of cytoprotection
Inflammatory control	Redox modulation of NF-κB	ROS-driven NF-κB hyperactivation	Sustained TNF-α, IL-1β, IL-6 production	Chronic non-resolving inflammation
Proteostasis	Disulfide bond formation and ER folding	Misfolded ECM proteins, chronic UPR activation	Apoptosis, reduced fibroblast function	Poor granulation tissue formation
ECM structural integrity	Disulfide-stabilized collagen and matrix proteins	Defective cross-linking and structural weakness	Impaired adhesion, migration, mechanotransduction	Fragile matrix, poor wound contraction
Mitochondrial bioenergetics	Iron–sulfur cluster biogenesis	ETC dysfunction, ↓ membrane potential, ↑ ROS	Reduced ATP production	Impaired keratinocyte, fibroblast, endothelial function
Immune resolution	GSH-dependent macrophage polarization	Persistence of M1 phenotype, impaired M2 transition	Excess TNF-α, IL-1β, MMP-9; low IL-10, TGF-β	ECM degradation, poor angiogenesis
Protease balance	Redox regulation of MMP/TIMP systems	Oxidative activation of MMPs	Excess ECM breakdown	Chronic ulcer persistence

2. Sulfur Dysregulation as a Core Pathophysiological Driver in Diabetic Wounds

Central to sulfur dysregulation in diabetic wounds is impaired function of the cystine–glutamate antiporter system xCT, encoded by SLC7A11, which governs cellular uptake of extracellular cystine and thereby regulates intracellular cysteine availability [13,14]. In the diabetic microenvironment, persistent hyperglycemia, accumulation of advanced glycation end products, and chronic exposure to inflammatory cytokines converge to suppress SLC7A11 expression and functional activity through transcriptional and post-translational mechanisms linked to oxidative stress and inflammatory signaling [60]. Reduced transporter function limits cystine import, leading to intracellular cysteine depletion and progressive exhaustion of glutathione reserves, which undermines the cell's primary

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redox buffering system [13,14,60]. Loss of glutathione-mediated control over intracellular thiol redox balance amplifies oxidative stress, promoting irreversible oxidation of extracellular matrix proteins and cell-surface receptors [61]. Integrins and associated focal adhesion complexes become structurally and functionally compromised, disrupting focal adhesion kinase signaling and cytoskeletal organization required for directed cell migration [62]. Fibroblast proliferation declines due to redox-sensitive cell cycle arrest, while keratinocyte motility becomes impaired as actin dynamics and adhesion turnover lose coordination [63]. The resulting cellular immobility directly contributes to stalled re-epithelialization and poor granulation tissue formation [64].

Concurrently, oxidative stress interferes with stabilization of hypoxia-inducible factor-1α, a transcription factor essential for adaptive responses to tissue hypoxia [65].

Under sulfur-deficient redox conditions, prolyl hydroxylase activity remains aberrantly elevated due to altered redox cofactor availability, promoting premature HIF-1α degradation and blunting transcription of angiogenic mediators such as vascular endothelial growth factor [66]. Endothelial cells within the wound bed therefore fail to mount adequate angiogenic responses, leading to poor neovascularization and persistent tissue hypoxia [67].

From a pathophysiological standpoint, impaired SLC7A11-mediated cysteine transport represents a nodal molecular defect linking metabolic stress to structural failure and immune dysregulation [13,14]. Targeting this sulfur bottleneck offers a rational strategy for restoring the biochemical conditions necessary for tissue regeneration rather than merely addressing downstream consequences of wound chronicity.

Table 2. Molecular Consequences of SLC7A11 Dysfunction in Diabetic Wound Cells.

Cellular Process	Normal Role of SLC7A11	Effect of Diabetic Suppression	Functional Outcome in Wound
Cystine uptake	Supplies cysteine for GSH synthesis	Intracellular cysteine depletion	Redox collapse, ROS accumulation
Antioxidant defense	Maintains glutathione-dependent detoxification	Impaired ROS neutralization	Oxidative macromolecular damage
Cell migration	Preserves integrin and cytoskeletal thiol integrity	Oxidative modification of adhesion proteins	Reduced keratinocyte and fibroblast motility
Angiogenic signaling	Supports redox balance for HIF-1α stabilization	HIF-1α degradation, ↓ VEGF expression	Poor neovascularization
Mitochondrial function	Provides sulfur for Fe–S cluster assembly	ETC dysfunction, ↓ ATP	Energy deficit in repair cells
Inflammatory regulation	Enables redox-mediated resolution signaling	NF-κB persistence, cytokine excess	Chronic inflammation

3. N-Acetylcysteine as an Intracellular Sulfur Restorative in Diabetic Wounds

N-acetylcysteine (NAC) represents a mechanistically targeted intracellular sulfur donor whose therapeutic relevance in diabetic wound healing derives from its ability to correct cysteine-dependent molecular dysfunction rather than functioning solely as a nonspecific antioxidant [68]. In the diabetic wound microenvironment, impaired cystine uptake through SLC7A11 restricts intracellular cysteine availability and constrains glutathione synthesis, precipitating redox collapse [69]. NAC bypasses this transport limitation by entering cells through alternative uptake pathways and undergoing intracellular deacetylation to release cysteine, thereby directly replenishing intracellular thiol pools [70].

Restoration of cysteine availability permits reconstitution of glutathione synthesis and normalization of intracellular redox buffering capacity. As redox equilibrium is re-established, oxidative stress–mediated inhibition of cytoprotective pathways is relieved, allowing reactivation of

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Nrf2-dependent transcriptional programs and attenuation of NF-κB–driven inflammatory gene expression [71,72].

The resulting decline in oxidative and inflammatory pressure enables redox-sensitive signaling networks to recover, facilitating the transition from chronic inflammation to a reparative cellular phenotype [73]. At the mitochondrial level, cysteine restoration supports iron–sulfur cluster biogenesis and limits oxidative damage to components of the electron transport chain [74]. Recovery of mitochondrial membrane potential and improved oxidative phosphorylation efficiency increase ATP production, which is essential for actin remodeling during keratinocyte migration, biosynthetic activity in fibroblasts, and angiogenic function in endothelial cells. By linking thiol restoration to bioenergetic recovery, NAC directly bridges molecular redox repair with functional cellular regeneration [75].

Stabilization of intracellular redox status also protects structural and signaling proteins from oxidative modification, preserving receptor conformation and downstream responsiveness to growth factors such as EGF, PDGF, and TGF-β [76,77]. Collectively, these effects position NAC as a pathophysiologically rational intervention aimed at correcting intracellular sulfur deficiency as a primary driver of diabetic wound chronicity.

Table 3. Mechanistic Effects of N-Acetylcysteine in Diabetic Wound Cells.

Target Domain	Sulfur-Dependent Mechanism Restored by NAC	Downstream Biological Effect	Relevance to Healing
Redox balance	Replenishes cysteine for GSH synthesis	Decreased intracellular ROS	Protection from oxidative injury
Antioxidant signaling	Enables Nrf2 pathway recovery	Increased cytoprotective gene expression	Cellular stress resistance
Inflammatory control	Redox suppression of NF-κB	Reduced TNF-α, IL-1β, IL-6	Transition toward reparative phase
Mitochondrial bioenergetics	Supports Fe–S cluster formation	Improved ATP production	Enhanced migration and proliferation
Growth factor signaling	Preserves receptor thiol integrity	Restored responsiveness to repair signals	Improved granulation and re-epithelialization

4. Methylsulfonylmethane as a Structural Sulfur Donor for Extracellular Matrix Repair

Effective wound healing requires not only restoration of intracellular metabolic competence but also reconstruction of the extracellular matrix that provides structural and biochemical support for regenerating tissue [78].

Methylsulfonylmethane (MSM) serves as a bioavailable organic sulfur source with particular relevance to extracellular protein synthesis and matrix stability in sulfur-deficient diabetic wounds. Whereas intracellular sulfur donors primarily restore redox and metabolic functions, MSM contributes sulfur for structural integration within newly synthesized extracellular proteins [79].

Structural macromolecules critical to wound repair, including collagen, elastin, laminin, and keratin, depend on sulfur-mediated crosslinking and disulfide bond formation to achieve mechanical strength and resilience [80,81]. In diabetic wounds characterized by sulfur insufficiency, newly synthesized matrix proteins often exhibit defective structural maturation, rendering the extracellular matrix vulnerable to degradation and incapable of sustaining effective cell–matrix interactions [82]. Increased sulfur availability in the extracellular compartment supports proper folding, crosslinking, and stabilization of these proteins, improving tensile strength and resistance to proteolysis [12].

Restoration of matrix structural integrity enhances the biomechanical properties of the wound bed, reinforcing the scaffold required for fibroblast attachment, keratinocyte migration, and mechanotransduction. Improved matrix quality also supports integrin signaling and focal adhesion

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formation, facilitating coordinated cellular movement and wound contraction [83]. In addition to its structural role, MSM modulates inflammatory signaling within the wound milieu by reducing expression of matrix-degrading enzymes and limiting amplification of inflammatory cascades, thereby contributing to stabilization of the extracellular environment necessary for regeneration. From a pathophysiological perspective, MSM acts predominantly at the tissue and matrix level, addressing extracellular sulfur deficits that cannot be corrected by intracellular redox restoration alone. Its function is therefore complementary to intracellular thiol donors, forming part of a multi-compartment sulfur restoration strategy aimed at rebuilding both the metabolic and structural foundations of tissue repair [84].

Table 4. Complementary Roles of Intracellular and Extracellular Sulfur Donors.

Therapeutic Agent	Primary Compartment	Main Molecular Target	Principal Biological Effect	Role in Regenerative Strategy
N-Acetylcysteine	Intracellular	Cysteine → Glutathione → Redox systems	Restores antioxidant defense and mitochondrial function	Reverses metabolic and redox collapse
Methylsulfonylmethane	Extracellular / Matrix	Sulfur for structural protein stabilization	Enhances ECM strength and integrity	Rebuilds structural scaffold for repair

5. The Dual-Sulfur Regenerative Hypothesis

Chronic non-healing diabetic wounds arise from the convergence of intracellular metabolic failure and extracellular structural instability, two pathologies that are mechanistically linked through sulfur dysregulation. Restoration of only one biological compartment is insufficient to overcome this integrated failure because cellular recovery cannot translate into tissue regeneration in the absence of a stable extracellular framework, and conversely, matrix repair cannot proceed when cellular metabolism remains redox-collapsed and energy-deprived. A regenerative strategy must therefore correct sulfur-dependent dysfunction across intracellular and extracellular domains in a coordinated manner.

Within this framework, N-acetylcysteine and methylsulfonylmethane fulfill distinct but complementary biological roles. NAC primarily restores intracellular sulfur availability by replenishing cysteine pools required for glutathione synthesis, redox buffering, and iron–sulfur cluster biogenesis. These corrections normalize intracellular redox signaling, revive mitochondrial bioenergetics, and suppress maladaptive inflammatory transcriptional programs, thereby reprogramming keratinocytes, fibroblasts, endothelial cells, and immune cells toward a pro-regenerative phenotype. However, these cellular improvements remain functionally constrained when the surrounding extracellular matrix is mechanically fragile and biochemically disordered.

In contrast, MSM exerts its dominant effects within the extracellular compartment by supplying bioavailable sulfur necessary for protein crosslinking and stabilization of disulfide bonds in structural macromolecules such as collagen, elastin, and keratin. Restoration of extracellular matrix integrity re-establishes tensile strength, elasticity, and spatial organization of the wound scaffold while simultaneously preserving biochemical signaling niches that regulate cell adhesion, migration, and mechanotransduction. Without this extracellular repair, improvements in cellular redox balance and mitochondrial function cannot be translated into coordinated tissue architecture. The dual-sulfur regenerative hypothesis therefore proposes that simultaneous intracellular and extracellular sulfur restoration is required to reverse the chronic non-healing state of diabetic wounds, directly targeting the upstream molecular bottlenecks that prevent progression from persistent inflammation to active tissue repair (Figure 1).

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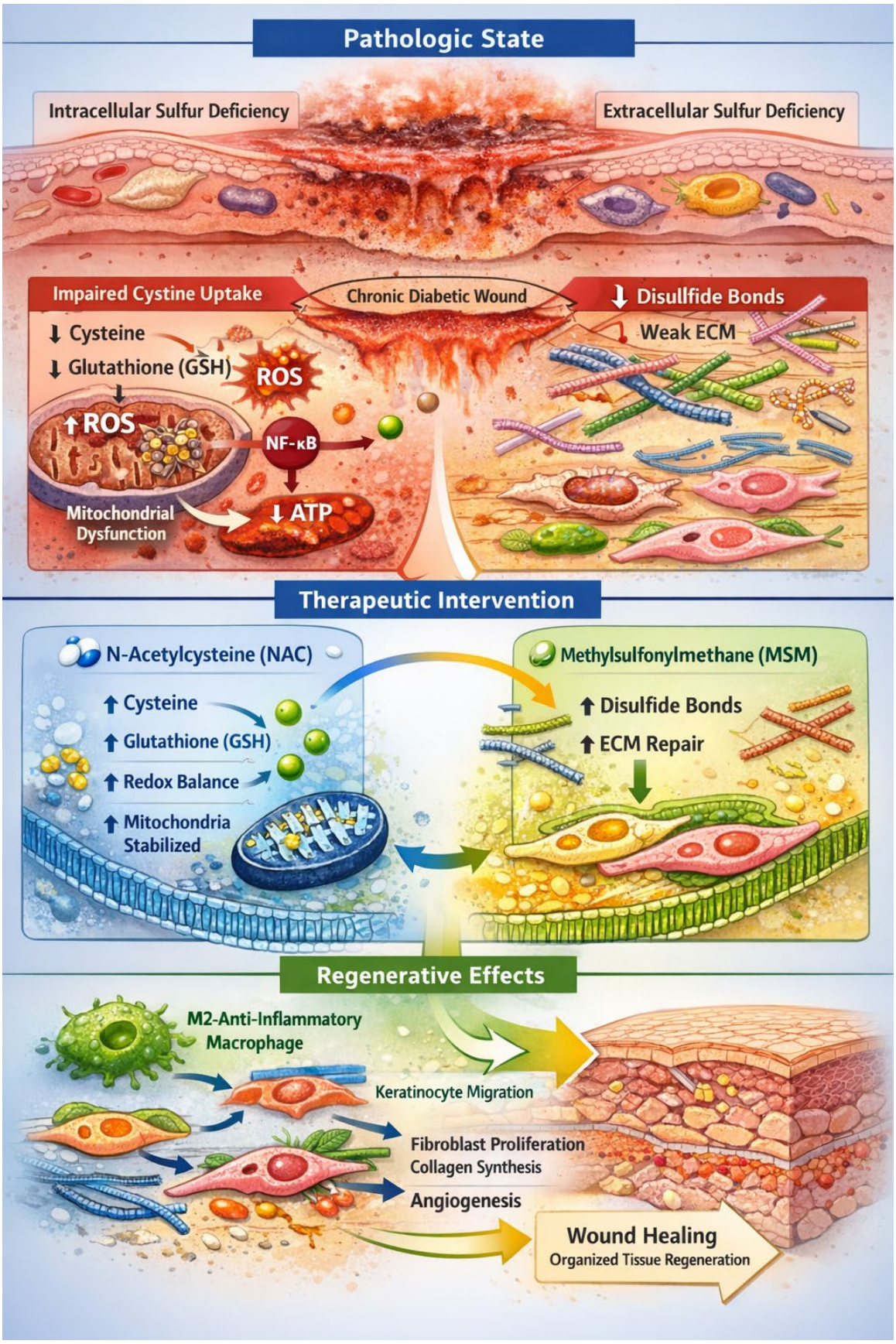

Figure 1. Dual-Sulfur Pathophysiology and Regenerative Hypothesis in Chronic Diabetic Wounds. Schematic representation of the proposed pathophysiological mechanism underlying chronic diabetic wounds and the dual-sulfur regenerative strategy. The diagram illustrates the diabetic wound microenvironment, showing keratinocytes, fibroblasts, endothelial cells, and immune cells (macrophages and neutrophils) in a state of

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persistent inflammation and oxidative stress. Intracellular sulfur deficiency is depicted as impaired cystine uptake via the SLC7A11/xCT transporter, leading to cysteine depletion, reduced glutathione (GSH) synthesis, reactive oxygen species (ROS) accumulation, NF-κB activation, mitochondrial iron–sulfur cluster destabilization, and impaired ATP production. Extracellular sulfur deficiency is shown in the extracellular matrix (ECM), with collagen, elastin, and keratin exhibiting disrupted disulfide bonds, weak crosslinking, impaired mechanotransduction, and reduced cell adhesion and migration. Therapeutic intervention includes topical N-acetylcysteine (NAC) restoring intracellular cysteine, GSH synthesis, redox balance, and mitochondrial function, and methylsulfonylmethane (MSM) reinforcing ECM disulfide bonding and structural integrity. The regenerative cascade highlights macrophage polarization (M1 → M2), keratinocyte migration, fibroblast proliferation, collagen synthesis, endothelial sprouting, and coordinated wound closure. Arrows indicate the sequential flow of molecular and cellular events. Color-coded compartments distinguish intracellular and extracellular domains, emphasizing the integrated sulfur-dependent repair mechanism.

Table 5. Conceptual Framework of the Dual-Sulfur Regenerative Hypothesis.

Pathological Domain	Primary Sulfur Defect	Biological Consequence	Targeted Sulfur Donor	Expected Regenerative Effect
Intracellular redox collapse	Cysteine depletion, low glutathione	Oxidative stress, inflammatory persistence	N-acetylcysteine	Restored redox control and cytoprotection
Mitochondrial dysfunction	Impaired Fe–S cluster biogenesis	Reduced ATP, impaired cell function	N-acetylcysteine	Bioenergetic recovery
ECM structural failure	Defective disulfide bonding in matrix proteins	Weak scaffold, poor mechanotransduction	Methylsulfonylmethane	Matrix stabilization and strength
Impaired cell–matrix signaling	Oxidative damage to adhesion proteins	Reduced migration and organization	Combined NAC + MSM	Coordinated tissue regeneration

6. Integrated Inflammatory Resolution, Mitochondrial Recovery, and Tissue Regeneration: Discussion and Therapeutic Implications

The persistent non-healing phenotype of diabetic wounds can be more accurately understood as the consequence of a unified sulfur-dependent pathophysiological collapse rather than as the additive result of ischemia, neuropathy, or infection. Within the diabetic wound microenvironment, chronic hyperglycemia drives excessive production of reactive oxygen species through mitochondrial electron leakage, activation of NADPH oxidases, and accumulation of advanced glycation end products [85].

This sustained oxidative burden progressively consumes intracellular cysteine reserves, suppresses glutathione synthesis, and disrupts thiol-dependent redox buffering systems that normally constrain inflammatory signaling and preserve protein structure [86]. As redox homeostasis collapses, inflammatory transcriptional programs remain pathologically active, mitochondrial function deteriorates, and extracellular matrix integrity becomes progressively destabilized, creating a self-reinforcing molecular state incompatible with regeneration [87].

From a mechanistic standpoint, sulfur deficiency represents the nodal defect that synchronizes these failures. Impaired cystine uptake and cysteine availability limit glutathione synthesis, allowing oxidative stress to persist and continuously activate redox-sensitive inflammatory pathways. This

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redox imbalance sustains nuclear factor-κB signaling while suppressing cytoprotective transcriptional programs, preventing immune cells from transitioning from tissue-destructive to reparative phenotypes [88]. In parallel, cysteine depletion compromises the biogenesis and stability of mitochondrial iron–sulfur clusters, impairing electron transport chain function and reducing ATP generation [89]. Energy deprivation directly constrains cytoskeletal remodeling, focal adhesion turnover, and directional migration of keratinocytes and fibroblasts, while endothelial cells lose angiogenic competence due to insufficient bioenergetic support and disrupted hypoxia-responsive signaling [90,91].

These intracellular defects are further amplified at the tissue level by sulfur-dependent destabilization of extracellular matrix proteins, where impaired disulfide bond formation and crosslinking weaken collagen and elastin architecture, disrupt mechanotransduction signaling, and prevent coordinated cellular assembly within the wound bed [12].

Within this framework, the dual-sulfur regenerative hypothesis proposes that effective reversal of diabetic wound chronicity requires simultaneous restoration of sulfur availability across both intracellular and extracellular compartments. Replenishment of intracellular cysteine through sulfur donation reconstitutes glutathione synthesis, restores redox buffering capacity, and attenuates oxidative stress-driven inflammatory signaling. As redox equilibrium is progressively re-established, immune cells regain the ability to resolve inflammation, macrophages shift toward reparative phenotypes, and oxidative injury to mitochondrial components is reduced [12]. Recovery of mitochondrial membrane potential and oxidative phosphorylation efficiency restores ATP availability, thereby enabling energy-dependent processes such as keratinocyte migration, fibroblast proliferation, collagen biosynthesis, and endothelial sprouting that are essential for wound closure and neovascularization [92].

Concurrently, restoration of extracellular sulfur availability stabilizes the structural framework required for regeneration. Enhanced sulfur incorporation into matrix proteins facilitates proper disulfide bonding and crosslinking within collagen, elastin, and keratin, improving tensile strength, elasticity, and resistance to proteolytic degradation. A mechanically competent extracellular matrix reactivates integrin-mediated signaling and mechanotransduction pathways, allowing intracellular bioenergetic recovery to be translated into organized tissue architecture rather than disordered cell accumulation. Stabilization of the matrix also preserves growth factor gradients and signaling niches, reinforcing coordinated spatial repair processes.

From a translational perspective, these mechanistic insights support a rational topical therapeutic strategy based on simultaneous intracellular and extracellular sulfur repletion. A dual-sulfur topical formulation combining N-acetylcysteine (NAC) and methylsulfonylmethane (MSM) is proposed to target complementary compartments of wound pathophysiology. Based on existing dermatologic and wound-healing literature, NAC may be incorporated at concentrations of approximately 2–3% (w/v), which have demonstrated efficacy in reducing oxidative stress, restoring glutathione synthesis, and improving cellular migration while maintaining favorable local tolerability [93,94].

MSM may be included at concentrations of approximately 5–8% (w/v), consistent with its documented topical safety profile and its capacity to support structural protein stabilization, reduce matrix degradation, and modulate inflammatory signaling [95].

The proposed method of application involves gentle wound cleansing with sterile saline, followed by application of a thin layer of the dual-sulfur formulation to the wound bed and margins, and coverage with a non-adherent, semi-occlusive dressing to preserve moisture balance while permitting gas exchange. Application may be performed once to twice daily depending on wound severity, exudate levels, and tissue tolerance. A treatment duration of at least two to four weeks is proposed, with periodic clinical assessment of granulation tissue formation, epithelialization, exudate reduction, and local inflammatory signs.

The convergence of intracellular redox normalization, mitochondrial recovery, and extracellular matrix stabilization shifts the diabetic wound microenvironment from a state dominated by

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inflammatory stress and metabolic insufficiency toward one permissive for coordinated regeneration. This sulfur-centered perspective reframes diabetic wounds as locally sulfur-depleted tissues trapped in a redox-collapsed and structurally misassembled state, rather than as lesions limited by perfusion or microbial burden alone. Therapeutic restoration of sulfur homeostasis therefore functions as an upstream molecular reconditioning strategy that enables downstream regenerative pathways to operate effectively. By correcting the fundamental biochemical and bioenergetic constraints that arrest healing, sulfur-targeted interventions hold the potential not only to accelerate wound closure but also to restore tissue quality, vascular stability, and resistance to recurrence, thereby redefining the biological expectations of diabetic wound therapy.

7. Conclusion

Diabetic wound chronicity arises from a convergence of intracellular oxidative failure and extracellular structural instability rooted in sulfur deficiency. Correcting only one compartment leaves the regenerative process incomplete. The proposed dual-sulfur strategy integrates intracellular redox restoration through NAC with extracellular matrix stabilization via MSM, thereby reconnecting metabolic recovery with structural repair. By acting upstream of inflammation, mitochondrial dysfunction, and ECM degradation, sulfur restoration reconditions the wound microenvironment to support coordinated healing rather than merely accelerating closure. This mechanism-driven framework reframes diabetic wound therapy from symptomatic management to metabolic and structural reprogramming, offering a biologically coherent foundation for next-generation regenerative interventions.

Author Contributions: Maher Monir Akl: Conceived and developed the central research hypothesis, designed the immunopathophysiological framework, performed data integration and analysis, and drafted the original manuscript. **Ahmed Ali. El-Nagar:** Contributed to literature review, mechanistic interpretation related to mucosal immunity and epithelial barrier biology, and participated in manuscript revision. **Amr Ahmed:** Provided clinical insight, supervised the translational and public health relevance of the work, and critically revised the manuscript for clinical accuracy. All authors contributed to the conception, design, data collection, analysis, and manuscript preparation equally.

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