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Nitric oxide and wound healing: an update

Stefano Bacci*

Because of its gaseous qualities, nitric oxide (NO) may pass across membranes and has a relatively short half-life. The enzyme complex nitric oxide synthase (NOS) in humans is responsible for the production of NO. This complex is composed of three distinct homodimeric isoforms: (1) neuronal isoform (nNOS (or NOS I), (2) inducible isoform (iNOS (or NOS II), and (3) endothelial isoform (eNOS/NOS III). L-arginine serves as the substrate for all NO isoforms, while molecular oxygen and NADPH serve as co-substrates. nNOS isoforms are expressed in specific neurons of the central nervous system and are involved in the regulation of blood pressure and synaptic plasticity. Endothelial cells are the primary source of the eNOS isoform, which plays a role in vasodilation, blood pressure regulation, and anti-atherosclerotic and vasoprotective effects. The inducible iNOS isoform is activated in response to stress; inflammatory cytokines, apoptotic bodies, or bacterial antigens induce an increase in enzyme expression.[1]

Cutaneous wound healing is a dynamic and complex physiological process that occurs in response to tissue damage. This process is divided into four complementary and overlapping stages: hemostasis, inflammation, granulation tissue development (proliferation), and remodeling. During hemostasis, endothelial cells produce von Willebrand factor, promoting platelet adhesion, which in turn releases mediators. The release of these molecules produces a fibrin clot that obstructs the lesion and halts the hemorrhage. The contraction of smooth muscles, prompted by elevated calcium ion levels, causes the capillaries to constrict fast, resulting in diminished flow. These actions result in the synthesis of vasoactive metabolites that facilitate vasodilation and relaxation of arterial arteries. The inflammatory phase starts with the rupture of blood vessels, the extravasation of blood components, and the formation of a temporary extracellular matrix. Platelets secrete a variety of vasoactive mediators and chemotactic factors, including interleukin, vascular endothelial growth factor, epidermal growth factor, platelet-derived growth factor, and transforming growth factor beta, which attract inflammatory cells such as mast cells, neutrophils, and macrophages, as well as fibroblasts and endothelial cells, to the wound site. These triggers start the next phase of granulation tissue development, which is characterized by angiogenesis, myofibroblastic differentiation, re-epithelization. During this phase, fibroblasts differentiate into myofibroblasts, which produce extracellular matrix and provide mechanical force to aid in wound healing. The remodeling phase starts after the re-epithelialization process is complete. This phase is characterized by the replacement of granulation tissue by scar tissue, mostly via the activation of matrix metalloproteinases, which degrade extracellular matrix components.[2]

Distinct functions of NO in wound healing: The inflammatory phase of wound healing begins with the secretion of chemoattractant cytokines by degranulating platelets, followed by the activation of mast cells and a subsequent increase in cells, belonging to to the cellular infiltrate. The principal effects of NO include vasodilation, antibacterial activity, antiplatelet aggregation, and the stimulation of vascular permeability, which are particularly pertinent to this phase. NO controls inflammation-related cytokines, including interleukin-8, transforming growth factor-β1, and about cells as monocytes, and neutrophils, which facilitate wound chemoattraction.

The proliferative phase includes cellular growth and differentiation among various cell types. The iNOS inhibitor L-N6-(1-iminoethyl)lysine reduces proliferation in keratinocytes near the wound margin, but low-dose NO enhances keratinocyte proliferation *in vitro*. Epidermal growth factor may signify the onset of the proliferative phase of wound healing by promoting keratinocyte proliferation and inhibiting keratinocyte-derived nitric oxide generation. NO donors promote keratinocyte differentiation, whereas keratinocytes exhibit increased iNOS production as differentiation occurs, likely functioning to reduce proliferation as wound healing advances. NO also promotes the proliferation of endothelial cells, safeguards them against apoptosis, and facilitates the generation of vascular endothelial growth factor. Therefore, the actions of NO on endothelial cells may also pertain to angiogenesis; besides, NO may also influence fibroblast growth. The data supporting the involvement of NO in wound remodeling and contraction is ambiguous, since both iNOS and eNOS deficient animals show significant delays in wound closure. The use of a topical polymer-based NONOate enhanced the closure of rat wounds, indicating a need for nitric oxide in the wound healing process. Nevertheless, *in vitro* research refutes this, since collagen contraction by wound-derived fibroblasts is augmented after NOS inhibition.[3-6]

Donors and competitive inhibitors of NO during wound healing: Competitive inhibitors of NO diminish collagen deposition and tensile strength in incisional wounds, adversely impacting the healing process across several wound types. The use of NO donors augments angiogenic activity by promoting the proliferation and recruitment of endothelial cells. It significantly enhances wound healing by acting as an antibacterial agent and promoting cell migration to the damaged site. Nonetheless, some limitations have constrained the widespread use of NO donors, one of which is the quick degradation and abrupt release of NO from its donor compounds. To avert the release and extended exposure to NO, the oxygen donor is sometimes included in biomaterial scaffolds, such as hydrogels and nanofiber mats. This encapsulation of nitric oxide donors facilitates a prolonged release of nitric oxide at the wound site, therefore inhibiting bacterial growth by impairing cell adhesion via membrane damage and deoxyribonucleic acid deamination.[7] In several conditions, including diabetes, decreased generation of NO metabolites in the wound milieu has been seen. The source of this decline is unclear, maybe due to the diminished inflammatory response linked to diabetes or a general decrease in nitric oxide generation by all wound cells. L-arginine and nitric oxide donors may enhance the protracted healing process linked to diabetes while concurrently normalizing wound nitric oxide levels. Malnutrition and radiation-induced injury are other factors associated with inadequate or protracted wound healing. Steroids, significant barriers to healing, interfere with arginine metabolism by blocking both the iNOS and arginase pathways.[7-9]

NO in chronic wounds: Chronic cutaneous lesions last for 6 weeks and demonstrate

Table 1 | Phases of wound healing and influence of nitric oxide

Phase of wound healing	Action	Influence of nitric oxide on predominant molecules involved in wound healing
Inflammatory	Vasodilation, antibacterial activity, antiplatelet aggregation, stimulation of vascular permeability, wound chemoattraction	Interleukin-8, transforming growth factor-β1
Proliferative phase	Angiogenesis cellular growth and differentiation.	Epidermal growth factor, vascular endothelial growth factor
Remodeling	Ambigous	Ambigous

an inflammatory response that maintains a balance between productive and degenerative processes. Nevertheless, they do not conform to the traditional, systematic, and prompt healing process, advancing through these stages without restoring the anatomical and functional integrity of the tissue. The procedure is hindered by several reasons, with 140 diseases involved. Approximately 6. 85% of adults aged 65 and older possess at least one chronic ailment, whereas 30% have three or more chronic disorders. The morphometric assessment of iNOS indicates a 50% increase in average surface area in chronic wounds, with iNOS expression seen in neurons, granulocytes, macrophages, endothelial cells, and fibroblasts.[10]

Prospective directions: The management of acute and chronic wound failure remains a significant unsolved objective. Twenty-five percent of the delays in hospital discharge may be ascribed to wound failure. Wound dressings that provide NO have been used in experimental animals. Recent investigations have examined NO-releasing nonsteroidal anti-inflammatory drugs in the context of experimental wound healing. The combination of NO with a nonsteroidal anti-inflammatory drug increased collagen production, which was otherwise diminished when the nonsteroidal anti-inflammatory drug was delivered in isolation. High-dose arginine supplementation to increase wound NO generation and improve wound healing is pending broader clinical use.[7-9]

Stefano Bacci*

Department of Biology, Research Unit of Histology and Embryology, University of Florence, Florence, Italy

***Correspondence to:** Stefano Bacci, PhD, stefano.bacci@unifi.it.
https://orcid.org/0000-0002-3651-229X (Stefano Bacci)

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