World J. Surg. 28, 301–306, 2004
DOI: 10.1007/s00268-003-7396-7

WORLD
Journal of
SURGERY

Nitric Oxide and Wound Healing

Majida Rizk, M.D.,[1] Maria B. Witte, M.D.,[2] Adrian Barbul, M.D.[1]

[1]Department of Surgery, Sinai Hospital of Baltimore, Johns Hopkins Medical Institutions, 2401 West Belvedere Avenue, Baltimore, Maryland 21215, USA
[2]Eberhard-Karls-Universitaet, Tuebingen, Germany

Published Online: February 17, 2004

Abstract. Nitric oxide is a short-lived free radical that acts at the molecular, cellular, and physiologic level. Since its discovery almost 20 years ago it has proven itself as an important element in wound healing. This review highlights many of the important aspects of nitric oxide in wound healing, including a review of the basic biology of nitric oxide, its role as part of the cytokine cascade and as a promoter of angiogenesis, as well as its more recently elucidated role in apoptosis.

It has been known for over 20 years that arginine enhances wound collagen synthesis and wound breaking strength during normal and impaired healing. The exact mechanism of action is not known, but it is under intense study. One avenue that our laboratory has pursued is to study the expression and activity of the various metabolic pathways of arginine within the wound (Fig. 1). We know that wound arginine is metabolized via both the nitric oxide (NO) synthesis pathway and the urea synthesis pathway. The temporal expression and regulation of these degradative pathways may well explain the biological and pharmacological effect of arginine on wound healing. This article examines the current status of knowledge concerning the role of wound nitric oxide synthesis.

Nitric Oxide is a short-lived free radical formed from the terminal guanidino nitrogen atom of arginine. The guanidino nitrogen accepts five electrons in an oxidation process requiring molecular oxygen, resulting in formation of NO and citrulline. Nitric oxide synthases, the enzymes responsible for this conversion, are homodimeric flavoprotein enzymes (130–150 kDA subunits). Tetrahydrobiopterin, flavine mononucleotide (FMN), flavine adenine dinucleotide (FAD), nicotinamide-adenine-dinucleotide phosphate (NADPH), and oxygen are required as co-factors for full activity [1]. The NO synthases exist in three distinct isoforms, two constitutive (endothelial and neuronal) isoforms and one inducible isoform. The constitutive isoforms are permanently active, generating low concentrations of NO, and they are regulated by intracellular calcium fluxes or exogenous calmodulin. The expression, transcription, and function of the inducible isoform (iNOS) is induced by a variety of cytokines, growth factors, and inflammatory stimuli on target cells; their action leads to release of high levels of NO over and above the amounts generated by the constitutive isoforms.

The high amounts of NO formed by the inducible isoform account for some of its detrimental effects in inflammatory situations such as sepsis [2]. The inducible isoform of NO is also expressed during wound healing, burn injury, endotoxin exposure, arthritis, and inflammatory bowel diseases.

NO Expression in Healing Wounds

Levels of arginine, a semi-essential amino acid [3], become critically low after wounding. Arginase levels are high in wound fluid, and they increase with the age of the wound. Thus arginine competes with iNOS for substrate and may downregulate NO synthesis during wound healing. Indeed, NO synthesis in macrophages can be impaired by arginine consumption by arginase [4]. To date there are no data, however, on the interaction and competition of these two alternate pathways and their affects on wound healing. It should be noted that L-hydroxy-arginine and nitrite, the intermediate and end products, respectively, of NO synthesis, are both strong arginase inhibitors [5, 6]. Furthermore, urea, the end product of arginase activity, inhibits NO formation [7]. Distinct cytokines favor the various degradative pathways. For example, transforming growth factor-beta (TGF-β) and interleukin-4 (IL-4) increase arginase and inhibit iNOS activity, whereas gamma-interferon (IFN-γ), IL-1, and lipopolysaccharide(LPS) work inversely [8–10].

Because NO is difficult to measure directly, stable metabolites are used as surrogates for NO formation. Nitrite and nitrate, two widely used stable end products, can be measured in wound fluid [11]. However, these measurements should not be translated as equimolar formation of NO because non-enzymatic NO formation can occur [12]. Several other direct or indirect detection methods can be performed, such as immunohistochemistry, direct measurement of enzyme content and/or activity, peroxynitrite formation in tissue, gene expression and others. However, no study to date has investigated arginine kinetics after wounding taking into account local and systemic arginine metabolism [13, 14].

Before NO was known, Albina et al. investigated arginine metabolism in wounds and demonstrated increased citrulline forma-

Correspondence to: Adrian Barbul, M.D., e-mail: abarbul@jhmi.edu

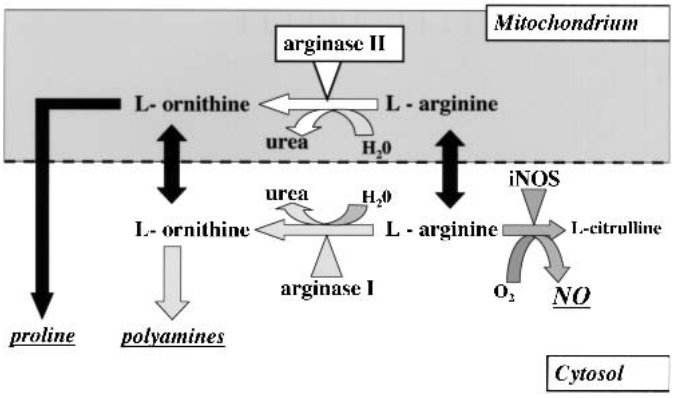

Fig. 1. Arginine metabolism in wounds.

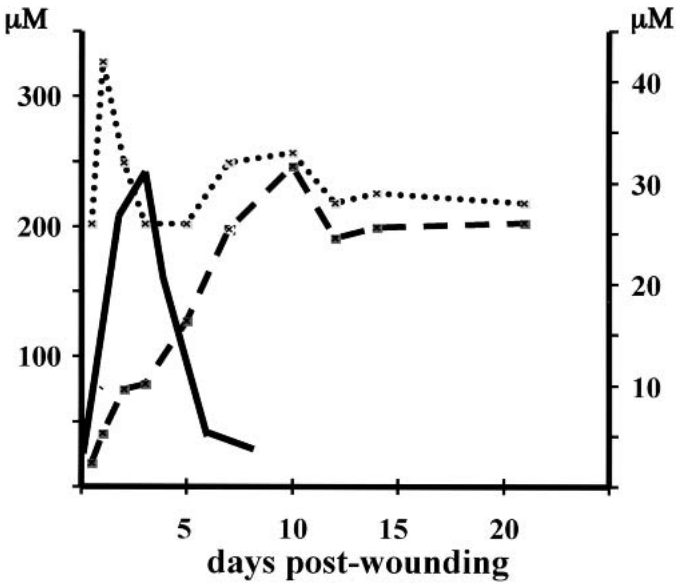

Fig. 2. Time course of NO byproducts (citrulline, ornithine, and NO_2) in wound fluid. Ornithine: dashed line; NO_2: solid line; citrulline, dotted line.

tion, which was imputed to an arginine deiminase-like activity [15]. Up until that report, this pathway of arginine disposal had been described only in bacteria and fungi. Subsequently, generation of NO during wound healing was deduced by demonstrating increased urinary nitrate secretion after wounding [16]. Thereafter several studies confirmed these data and extended the findings to healing after burn injury [17, 18]. In these models, urinary nitrate levels remained elevated until complete healing had occurred. Later experiments confirmed that the highest NOS activity occurs during the early phases of wound healing [19].

With a polyvinyl alcohol sponge model in rats, a progressive accumulation of nitrate/nitrite in wound fluid can be demonstrated, suggesting sustained NO synthesis [20]. However species-specific differences in the kinetics of NO formation exist [21]. With the development of the inducible NOS isoform–specific antibodies and primers for transcriptional and translational analysis, it was demonstrated that iNOS expression is highest in the early phase after acute inflammation. Reverse-transcriptase polymerase chain reaction (RT-PCR) and Northern blotting detect iNOS during the first 5 days in rat models of healing [22, 23].

It is conceivable that the majority of NO synthesis is due to the inflammatory cells present during the early phase of healing, especially macrophages [24]. However, fibroblasts, keratinocytes, and endothelial cells contribute to ongoing NO synthesis, although to a lesser degree [25, 26]. Therefore the overall time course of iNOS activity and NO generation during wound healing has to be viewed as a decreasing curve over time (Fig. 2).

Although the in vitro signals of iNOS induction are well elucidated, little is known of the in vivo signals during wound healing. Of the numerous cytokines and growth factors secreted and released into the wound environment, interleukin-1, tumor necrosis factor-alpha (TNF-α), and INF- γ are the most likely inducers of iNOS. Wound fluid, as a biological reflection of the wound environment, induces NO synthesis in a variety of cells [27].

Although iNOS expression is high during the early phases of wound healing, little is known about the downregulation of iNOS activity at the wound site during the later phases of healing. Presumably iNOS activity can be downregulated by the resolution of the inflammatory response or by cytokine signaling. It is likely that colonized or infected wounds with continued inflammatory responses would continue to generate large amounts of NO, although this has not been studied directly [28]. TGF-β1 is one of the strongest iNOS inhibitors during wound healing [29]. However even during the inflammatory phase of wound healing there is counter-regulation of NO synthesis, as demonstrated by the presence of an unknown factor that reduces iNOS activity, but not by substrate depletion [25].

Mechanism of Action of NO during Wound Healing

Nitric oxide acts via multiple and different mechanisms. Some of its effects are due to its chemical reaction with oxygen leading to formation of reactive radical species [30]. Others are due to its affinity with heme or metal-containing enzymes such as the iron in guanylcyclase. At the molecular level, NO has been shown to act as a signaling molecule that operates via guanylate cyclase to activate cGMP. In addition, NO acts as a cytostatic/cytotoxic molecule inhibiting cytochromes and aconitase, as well as ribonucleotide reductase. Nitric oxide also regulates gene expression by reacting with the thiol binding site of the transcription factor NF-kappa beta. By nitrosylating NF-kappa beta, NO prevents binding to the iNOS promoter, suggesting a feedback inhibition mechanism. Nitric oxide has been shown to induce apoptosis via p53 mechanisms in neuroblastoma cells [31]. Yet, at the same time, NO inhibits activation of caspases and thereby prevents apoptosis.

In higher concentrations NO is cytostatic to multiple cell types including endothelial cells, smooth muscle cells, hepatocytes, and fibroblasts [32–34]. Depending on the cell type, this effect can be cGMP dependent [35] or independent [36]. Target enzymes include the complex I and II of the respiratory chain [37] and ribonucleotide reductase [38], a rate-limiting enzyme in the DNA synthetic pathway. Conversely, NO can stimulate cell proliferation when added in low concentrations [39, 40]. Recent evidence suggests that NO inhibits ornithine-decarboxylase activity, the rate-limiting enzyme for polyamine formation, thus reducing cell proliferation [41].

Nitric oxide regulates gene expression [42, 43] and cellular differentiation [44, 45]. Regulation of gene expression by NO probably occurs indirectly, through amplification of other regulatory mechanisms [46]. For example, although NO is critical for wound

Table 1. Studies of nitric oxide (NO) and wound healing.

Treatment	NO metabolites	Wound breaking strength	Collagen synthesis	Epithelialization	Wound contraction
iNOS knock-out (excisional model)	Decreased	—	—	Decreased	Decreased
iNOS knock-out (incisional model)	No effect	No effect	No effect	—	—
eNOS knock-out	—	Decreased	—	Decreased	Decreased
iNOS inhibition	Decreased	Decreased	Decreased	Decreased	Decreased
Arginine feeding	Increased	Increased	Increased	—	—
Arginine-free diet	Decreased				—
NO donor	Increased	Increased	Increased		—
iNOS transfection	Increased	Increased	Increased	—	Increased

iNOS: the inducible isoform of nitric oxide; eNOS:

collagen deposition, clear-cut enhancement of collagen synthesis or gene expression has not been found (see below). Collagen metabolism and accumulation are tightly regulated through the activity of collagenases and their inhibitors, tissue inhibitors of metalloproteinases (TIMP). Inhibiting the collagenolytic pathway can enhance collagen accumulation. Addition of the NO donor, SNAP, to rat mesangial cells increases gelatinase A activity [47], whereas rat fibroblasts collagenase activity is unaffected by SNAP [35]. Another potential mechanism of posttranslational collagen regulation by NO is regulation of protein kinase C (PKC) activity [48, 49]. By inhibiting PKC activity, NO could potentially downregulate PKC-related collagen synthesis in fibroblasts.

Platelet deposition and degranulation initiates a cytokine explosion of which NO release acts as both stimulant and inhibitor of the inflammation cascade. For instance, NO activates the promoter of the IL-8 gene in a human melanoma cell line. In turn, IL-8 suppresses the expression of iNOS in neutrophils [50]. Transforming growth factor-β1 is another chemoattractant that has been shown to be integrally related to NO; TGF-β suppresses NO production, and at the same time NO can lead to the activation of latent TGF-β1 [51]. Nitric oxide also acts as an immune modulator by attracting monocytes and neutrophils to the wound. Once neutrophils and monocytes are called to action, they become integral players in the cytokine cascade producing TNF-α [52–54]. Nitric oxide affects the expression of TNF-α directly in human peripheral blood monocytes through a cGMP-independent mechanism [55].

The expression of the monocyte-attracting macrophage chemoattractant protein-1, produced by hyperproliferative keratinocytes at the wound edge, appears to be downregulated by NO at the wound site [56]. This possibility supports a temporal relationship for NO in normal wound healing. Nitric oxide is initially involved in the upregulation of the cytokine cascade, acting as a chemoattractant for immune regulatory cells; thus it is vital to the early stage of wound healing. However, the inflammatory phase of wound healing must move into a proliferative phase for wound healing to progress to completion. Nitric oxide has been shown to be involved in this transition, further supporting a temporal role for NO during normal wound healing.

There is increasing evidence for a functional role of NO in wound healing. Inhibition of iNOS by competitive inhibitors decreases collagen deposition and breaking strength in incisional wounds and impairs the healing of other wound models [57–59]. When rats are fed an arginine-free diet, wound healing is impaired: conversely, when humans and animals are fed an arginine-enriched diet there is improved healing as measured by collagen deposition and breaking strength [60–62]. Arginine-supplemented rats have higher levels of NO metabolites in their wound fluid, strongly suggesting that the supplemental arginine is metabolized, at least in part, via NO synthesis [63]. Finally, use of NO donors also improves incisional and excisional wound healing in rats [64–66]. The data demonstrating that NO has a positive regulatory effect on repair is summarized in Table 1.

The mechanism of action of NO on wound healing remains unclear. However, there are data suggesting that at least some effects of NO on wound healing might be systemically mediated: (1) arginine-free nutrition inhibits LPS-induced NO synthesis in several organs, not only at the wound site [67]; (2) NO mediates inflammation-induced edema formation and inhibits cell infiltration into granulomas [68, 69]; (3) the effect of NO on wound healing is not only iNOS-mediated because eNOS knock-out mice also demonstrate impaired healing [70]; and (4) iNOS inhibitors have a high lethality when present in high concentration [54].

In vitro studies of fibroblasts derived from keloids and hypertrophic scars demonstrate low constitutive NOS expression, thus stimulating higher cell proliferation, which is responsible for the high cellularity characteristic of these disorders. In vivo, keratinocyte proliferation is iNOS-dependent [23] and wound reepithelialisation is also NO-dependent, probably mediated indirectly via vascular endothelial growth factor (VEGF) [71]. Interestingly, induction of iNOS in keratinocytes is paralleled by induction of GTP-cyclohydrolase I, the rate-limiting enzyme for tetrahydrobiopterin formation, which is essential for full iNOS activity [72]. Nitric oxide has been shown to increase angiogenesis in ischemic murine tissues, whereas eNOS inhibitors impair angiogenesis in granulation tissue. Vascular endothelial growth factor, a potent angiogenic growth factor, is closely linked to NO; VEGF increases NO production at the gene expression level [73, 74], and the angiogenic effects of VEGF appears to be dependent on NO [75, 76]. It has also been shown that NOS blockade prevents VEGF production, VEGF-induced endothelial cell proliferation, and VEGF-mediated activation of mitogen-activating protein kinase [77].

Endothelial cell migration, endothelial cell adhesion, and endothelial organization are dependent on NO via VEGF [78–81], which is made primarily by keratinocytes during wound healing [82]. Nitric oxide increases VEGF expression by keratinocytes, and iNOS inhibitors can block this process both in vitro and in vivo [83]. Monocyte-induced angiogenesis is NO dependent [84], as is substance P–mediated angiogenesis [85]. Feedback mechanisms exist and appear to act by downregulating PKC-induced VEGF expression [86].

Collagen synthesis correlates with NO synthesis during wound healing. Matrix synthesis is impaired by iNOS inhibition, whereas NO administration and iNOS transfection enhance matrix synthesis [55, 61, 87]. Furthermore, wound-derived fibroblasts are char-

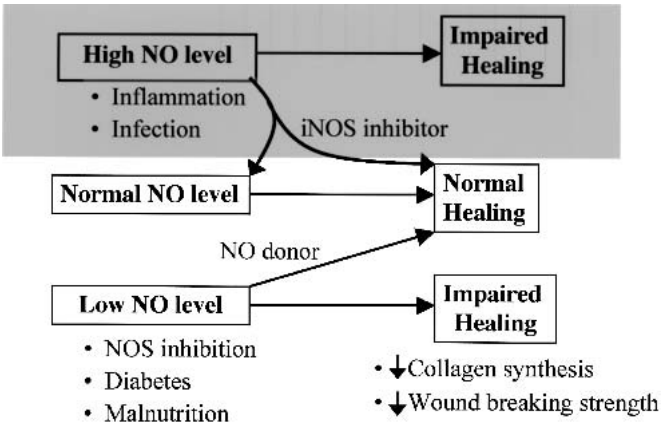

Fig. 3. Nitric oxide and wound healing.

acterized by a distinct phenotype where endogenous iNOS expression correlates with increased collagen synthesis [26].

Wound contraction is a major contributor to closure of open wounds. In excisional wounds closure is delayed by iNOS inhibition [68]. In vitro studies showed that NO induces a locomotor phenotype in keratinocytes [88].

Wound healing is characterized by the organized secretion of growth factors. This represents a potential target for regulating wound healing. Little is known about whether NO can directly affect growth factor or cytokine secretion, activation, or time of action. Arginine is known to downregulate TNF-α after trauma thereby affecting outcome [89]. Both TGF-β and EGF directly and indirectly downregulate NO or the NO-mediated effects [90].

Recently we have examined the possibility that a lack of iNOS gene expression alters wound cytokine expression. Using nonisotopic in situ hybridization quantitative analysis, we studied eNOS, basic fibroblast growth factor (bFGF), TGF-β1, TNF-α, VEGF, and IL-4 expression in incisional wounds and compared expression in wild-type and iNOS-KO mice. It was noted that eNOS and bFGF expression nearly doubled on postoperative day 7 in iNOS-KO incisions and remained two- to threefold elevated thereafter. In additon, TGF-β1 expression was increased approximately 50% to 100% in iNOS-KO wounds on postoperative days 5 and 7, and VEGF and IL-4 expression was 25% to 100% higher in wild-type animals than in iNOS-KO animals at all time points. We hypothesize that the overexpression of TGF-β1 and eNOS may represent mechanisms in iNOS-KO mice that compensate for their loss of functional iNOS, resulting in incisional wound healing equivalent to controls. The impaired expression of VEGF and IL-4, on the other hand, may partially explain the delayed excisional wound healing noted in these animals [91].

Large amounts of NO synthesis at the wound can impair wound healing. Sterile inflammation, as induced by turpentine sterile abscess, results in increased NO synthesis with subsequent impaired collagen production. In this setting iNOS inhibition restores collagen synthesis to normal without affecting the overall inflammatory response (Park et al., unpublished data) (Fig. 3).

Impaired Wound Models

After the discovery that NO is synthesized during wound healing and that inhibition of its production impairs healing, the next step was to investigate whether there is a correlation between NO and the outcome of healing. Several impaired wound models were used to seek such correlation.

In diabetes at least three studies have demonstrated decreased formation of NO metabolites in the wound environment [92–94]. It is not clear whether this decrease is due to the lesser inflammatory response characteristic of diabetes or to a net decrease in NO formation by all wound cells. L-arginine as well as NO donors can partially reverse the impaired healing of diabetes and in parallel restore wound NO levels toward more normal values [61, 87]. More work needs to be done to confirm whether these agents might serve as future treatment options.

Malnutrition and radiation-induced injury are other conditions associated with impaired or delayed healing [95]. Steroids, strong inhibitors of healing, alter arginine metabolism by impairing both the iNOS and the arginase pathways [96].

In one study, iNOS knock-out mice demonstrated delayed closure of excisional wounds, which could be reversed by transfection with iNOS-cDNA [97]. Surprisingly however, there was no effect on collagen deposition or breaking strength in incisional wounds in iNOS knock-out mice [98]. Supplemental L-arginine does not enhance wound healing in iNOS knock-out mice, suggesting that metabolism of arginine via iNOS is an essential pathway in the positive effects of arginine on healing [60].

The eNOS knock-out mice also demonstrated delayed healing in excisional wound models [67]. Wound fluid extracted from these wounds, induces a lesser angiogenic response in the cornea angiogenesis models than in controls, underscoring the importance of eNOS for neoangiogensis during wound healing.

The Future

In summary, L-arginine can be metabolized via distinct pathways during wound healing. Experimental animal models demonstrate a positive effect of NO on wound healing. Human studies are lacking, especially therapeutic studies. Influence of the arginase pathways remains to be elucidated.

Résumé. L'oxyde nitrique est un radical libre de courte durée qui agit au niveau moléculaire, cellulaire et physiologique. Depuis sa découverte il y a plus de 20 ans, son rôle dans la cicatrisation s'est confirmé. Dans cet article on souligne la plupart de ses propriétés dans la cicatrisation des plaies et on fait une revue de la biologie de base de l'oxyde nitrique, son rôle dans la cascade des cytokines, son rôle prometteur dans l'angiogenèse, et son rôle élucidé plus récemment dans l'apoptose.

Resumen. El óxido nítrico es un radical libre de corta vida que actúa a nivel molecular, celular y fisiológico. Desde su descubrimiento hace casi 20 años ha probado ser un elemento importante en el proceso de la cicatrización de heridas. Esta revisión resalta muchos de los más importantes aspectos del óxido nítrico en la cicatrización de heridas, incluyendo una revisión de la biología básica del óxido nítrico, su participación en la cascada de las citoquinas, la promoción de angiogenesis y el recientemente descubierto papel en la apoptosis.

References

1. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem. J. 1994;298:249–258
2. Tsukahara Y, Morisaki T, Horita Y, et al. Expression of inducible nitric oxide synthase in circulating neutrophils of the systemic inflammatory response syndrome and septic patients. World J. Surg. 1998;22:771–777
3. Seifter E, Rettura G, Barbul A, et al. Arginine: an essential amino acid for injured rats. Surgery 1978;84:224–230

4. Chang CI, Liao JC, Kuo L. Arginase modulates nitric oxide production in activated macrophages. Am. J. Physiol. 1998;274:H342–H348
5. Boucher JL, Custot J, Vadon S, et al. N-omega-hydroxyl-L-arginine, an intermediate in the L-arginine to nitric oxide pathway, is a strong inhibitor of liver and macrophage arginase. Biochem. Biophys. Res. Commun. 1994;203:1614–1621
6. Hrabak A, Bajor T, Temesi A, et al. The inhibitory effect of nitrite, a stable product of nitric oxide (NO) formation, on arginase. FEBS Lett. 1996;390:203–206
7. Prahakar SS, Zeballos GA, Montoya ZM, et al. Urea inhibits inducible nitric oxide synthase in macrophage cell line. Am. J. Physiol. 1997;273: C1882–C1888
8. Shearer JD, Richards JR, Mills CD, et al. Differential regulation of macrophage arginine metabolism: a proposed role in wound healing. Am. J. Physiol. 1997;272:E181–E190
9. Corraliza IM, Soler G, Eichmann K, et al. Arginase induction by suppressors of nitric oxide synthesis (IL-4, IL-10 and PGE-2) in murine bone-marrow-derived macrophages. Biochem. Biophys. Res. Commun. 1995;206:667–673
10. Modolell M, Corraliza IM, Link F, et al. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. Eur. J. Immunol. 1995;25: 1101–1104
11. Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate and [15N] nitrate in biological fluids. Anal. Biochem. 1982;126:131–138
12. Zweier JL, Samouilov A, Kuppusamy P, et al. Non-enzymatic nitric oxide synthesis in biological systems. Biochim. Biophys. Acta 1999;1411: 250–262
13. Castillo L, Beaumier L, Ajami AM, et al. Whole body nitric oxide synthesis in healthy men determined from [15N] arginine-to-[15N] citrulline labeling. Proc. Natl. Acad. Sci. U. S. A. 1996;93:11460–11465
14. Beaumier L, Castillo L, Ajami AM, et al. Urea cycle intermediate kinetics and nitrate excretion at normal and ″therapeutic″ intakes of arginine in humans. Am. J. Physiol. 1995;296:E884–E896
15. Albina JE, Mills CD, Barbul A, et al. Arginine metabolism in wounds. Am. J. Physiol. 1988;254:E459–E467
16. Smith DJ, Dunphy MJ, Strang LN, et al. The influence of wound healing on urinary nitrate levels in rats. Wounds 1991;3:50–58
17. Becker WK, Shippee RL, McManus AT, et al. Kinetics of nitrogen oxide production following experimental thermal injury in rats. J. Trauma 1993;34:855–862
18. Carter EA, Derojas WT, Tamir S, et al. Nitric oxide production is intensely and persistently increased in tissue by thermal injury. Biochem. J. 1994;304:201–204
19. Albina JE, Mills CD, Henry WL, et al. Temporal expression of different of l-arginine metabolism in healing wounds. J. Immunol. 1990;144: 3877–3880
20. Schaffer MR, Tantry U, van Wesep RA, et al. Nitric oxide metabolism in wounds. J. Surg. Res. 1997;71:25–31
21. Dull BJ, Gittes RF, Goldman P. Nitrate production and phagocyte activation: differences among Sprague-Dawley, Wistar-Furth and Lewis rats. Carcinogenesis 1998;9:625–627
22. Frank S, Madlener M, Pfeilschifter J, et al. Induction of inducible nitric oxide synthase and its corresponding tetrahydrobiopterin-cofactor-synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound repair. J. Invest. Dermatol. 1998;111:1058–1064
23. Nill MR, Oberyszyn TM, Ross MS, et al. Quantification of inducible nitric oxide gene expression during incisional wound healing. Surg. Forum 1995;XLVI:753–755
24. Reichner JS, Meszaros AJ, Louis CA, et al. Molecular and metabolic evidence for the restricted expression of inducible nitric oxide synthase in healing wounds. Am. J. Pathol. 1999;154:1097–1104
25. Schaffer MR, Efron PA, Thornton FJ, et al. Nitric oxide, and autocrine regulator of wound fibroblast synthetic function. J. Immunol. 1997;158: 2375–2381
26. Paulsen SM, Wurster SH, Nanney LB. Expression of inducible nitric oxide synthase in human burn wounds. Wound Repair Regen. 1998;6: 142–148
27. Witte MB, Efron DT, Kiyama T, et al. Wound fluid regulates nitric oxide expression in fibroblasts. Surg. Forum 1998;XLIX:623–624
28. Mahoney E, Reichner J, Bostom LR, et al. Bacterial colonization and the expression of inducible nitric oxidesynthase in murine wounds. Am. J. Pathol. 2002;161:2143–2152
29. Vodovotz Y, Bogdan C, Paik J, et al. Mechanisms of suppression of macrophage nitric oxide release by transforming growth factor β. J. Exp. Med. 1993;178:605–613
30. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of Nitric Oxide and its Redox-activated forms. Science 1992;258:1898–1902
31. Wang X, Zalcenstein A, Oren M. Nitric oxide promotes p53 nuclear retention and sensitizes neuroblastoma cells to apoptosis by ionizing radiation. Cell Death Differ. 2003;10:468–476
32. Stadler J, Billiar TR, Curran RD, et al. Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. Am. J. Physiol. 1991;260:C910–C916
33. Thomae KR, Nakayama DK, Billiar TR, et al. The effect of nitric oxide on fetal pulmonary artery smooth muscle growth. J. Surg. Res. 1995;59: 337–343
34. Garg UC, Hassid A. Nitric oxide-generating vasodilators inhibit mitogenesis and proliferation of BALB/C 3T3 fibroblasts by cyclic GMP-independent mechanism. Biochem. Biophys. Res. Commun. 1990;171: 474–479
35. Patel MJ, Wypij DM, Rose DA, et al. Secretion of cyclic GMP by cultured epithelial and fibroblast cell lines in response to nitric oxide. J. Pharmacol. Exp. Ther. 1995;273:16–25
36. Witte MB, Thornton FJ, Efron DT, et al. Enhancement of fibroblast collagen synthesis by nitric oxide. Nitric Oxide Biol. Chem. 2000;4:572–582
37. Stadler J, Curran RD, Ochoa JB, et al. Effect of endogenous nitric oxide on mitochondrial respiration of rat hepatocytes in vitro and in vivo. Arch. Surg. 1991;126:186–191
38. Kwon NS, Stuehr DJ, Nathan CF. Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. J. Exp. Med. 1991; 174:761–767
39. Du M, Islam M, Lin L, et al. Promotion of proliferation murine Balb/C3T3 fibroblasts mediated by nitric oxide at lower concentrations. Biochem. Mol. Biol. Int. 1997;41:625–631
40. Efron DT, Kirk SJ, Regan MC, et al. Nitric oxide generation from l-arginine is required for optimal peripheral blood lymphocyte DNA synthesis. Surgery 1991;110:327–334
41. Buga GM, Wei LH, Bauer PM, et al. NG-hydroxy-l-arginine and nitric oxide inhibit Caco-2 tumor cell proliferation by distinct mechanisms. Am. J. Physiol. 1998;275:R1256–R1264
42. Park RW, Kim IS, Jo JS. Bisindolylmaleimide inhibits the PMA-induced down-regulation of collagen synthesis in fibroblasts. Biochem. Mol. Biol. Int. 1996;40:101–109
43. Pilz RB, Suhasini M, Idriss S, et al. Nitric oxide and cGMP analogs activate transcription from AP-1-responsive promoters in mammalian cells. FASEB J. 1995;9:552–558
44. Boss GR. cGMP-induced differentiation of the promyelocytic cell line HL-60. Proc. Natl. Acad. Sci. U. S. A. 1989;86:7174–7178
45. Magrinat G, Mason SN, Shami PJ, et al. Nitric oxide modulation of human leukemia cell differentiation and gene expression. Blood 1992; 80:1880–1884
46. Penova N, Enikolopov G. Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells. Nature 1993;364:450–453
47. Trachtman H, Futterweit S, Garg P, et al. Nitric oxide stimulates the activity of a 72kDa neutral matrix metalloproteinase in cultured rat mesangial cells. Biochem. Biophys. Res. Commun. 1996;218:704–708
48. Clementi E, Sciorati C, Nistico G. Growth factor-induced Ca2+ responses are differentially modulated by nitric oxide via activation of a cyclic GMP-dependent pathway. Mol. Pharmacol. 1995;48:1068–1077
49. Gopalakrishna R, Chen ZH, Gundimeda U. Nitric oxide and nitric oxide-generating agents induce a reversible inactivation of protein kinase C activity and phorbol ester binding. J. Biol. Chem. 1993;268:27180–27185
50. McCall TB, Palmer RMJ, Moncada S, et al. Interleukin-8 inhibits the induction of nitric oxide synthase in rat peritoneal neutrophils. Biochem. Biophys. Res. Commun. 1992;186:680–685
51. Vodovotz Y, Chesler H, Chong SJ, et al. Regulation of transforming growth factor-β1 by nitric oxide. Cancer Res. 1999;59:2142–2149
52. Wahl SM, Wong H, McCartney-Francis. Role of growth factors in inflammation and repair. J. Cell. Biochem. 1989;40:193–199
53. Ansel JC, Armstrong CA, Song I, et al. Interactions of the skin and nervous system. J Investig Dermatol Symp Proc. 1997;2:23–26
54. Imanishi J, Kamiyama K, Iguchi I, et al. Growth factors: importance in

wound healing and maintenance of transparency of the cornea. Prog Retina Eye Res. 2000;19:113–129
55. Lander HM, Sehajpal P, Levine DM, et al. Activation of human peripheral blood mononuclear cells by nitric oxide-generating compounds. J. Immunol. 1993;150:1509–1516
56. Wetzer C, Kampfer H, Pfeilschifter J, et al. Keratinocyte-derived chemotactic cytokines: expressional modulation by nitric oxide in vitro and during cutaneous wound repair in vivo. Biochem. Biophys. Res. Commun. 2000;274:689–696
57. Shaffer MR, Tantry U, Thornton FJ, et al. Inhibition of nitric oxide synthesis in wounds: pharmacology and effect on accumulation of collagen in wounds in mice. Eur. J. Surg. 1999;165:262–267
58. Schaffer M, Tantry U, Gross SS, et al. Nitric oxide regulates wound healing. J. Surg. Res. 1996;63:237–240
59. Murrell GA, Szabo C, Hannafin JA, et al. Modulation of tendon healing by nitric oxide. Inflamm. Res. 1997;46:19–27
60. Bulgrin JP, Shabani M, Smith DJ. Arginine-free diet suppresses nitric oxide production in wounds. J Nutr Biochem. 1993;4:588–593
61. Barbul A, Fishel RS, Shimazu S, et al. Intravenous hyperalimentation with high arginine levels improves wound healing and immune function. J. Surg. Res. 1985;38:328–334
62. Barbul A, Lazarou SA, Efron DT, et al. Arginine enhances wound healing and lymphocyte immune responses in humans. Surgery 1990;108: 331–336
63. Shi HP, Efron DT, Most D, et al. Supplementary dietary arginine enhances wound healing in normal but not inducible nitric oxide synthase knock-out mice. Surgery 2000;128:374–378
64. Witte MB, Thornton FJ, Kiyama T, et al. Nitric oxide enhances wound collagen deposition in diabetic rats. Surg. Forum 1997;XLVIII:665–667
65. Shabani M, Pulfer SK, Bulgrin JP, et al. Enhancement of wound repair with a topically applied nitric oxide-releasing polymer. Wound Rep. Reg. 1996;4:353–362
66. Bauer JA, Rao W, Smith DJ. Evaluation of linear polythyleneimine/nitric oxide adduct on wound repair: therapy versus toxicity. Wound Rep. Reg. 1998;6:569–577
67. Wu G, Flynn NE, Flynn SP, et al. Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. J. Nutr. 1999;129:1347–1354
68. Iuvone T, Carnuccion R, DiRosa M. Modulation of granuloma formation by endogenous nitric oxide. Eur. J. Pharmacol. 1994;265:89–92
69. Iuvone T, Van ON, D'Acquisto F, et al. Differential effect of L-NAME and S-methyl-isothiourea on leukocyte emigration in carrageenin-soaked sponge implants in rat. Br. J. Pharmacol. 1997;121:1637–1644
70. Lee PC, Salyapongse AN, Bragdon GA, et al. Impaired wound healing and angiogenesis in eNOS-deficient mice. Am. J. Physiol. 1999;277: H1600–H1608
71. Stallmeyer B, Kampfer H, Kolb N, et al. The function of nitric oxide in wound repair: inhibition of inducible nitric oxide-synthase severely impairs wound reepithelialization. J. Invest. Dermatol. 1998;113:1090–1098
72. Frank S, Kolb N, Werner ER, et al. Coordinated induction of inducible nitric oxide synthase and GTP-cyclohydrolase I is dependent on inflammatory cytokines and interferon-gamma in HaCaT keratinocytes: implications for the model of cutaneous wound repair. J. Invest. Dermatol. 1998;111:1065–1071
73. Hood JD, Meininger CJ, Ziche M, et al. VEGF upregulates ecNOS message, protein, and NO production in human endothelial cells. Am. J. Physiol. 1998;274:H1054–H1058
74. Vander ZR, Murohara T, Luo Z, et al. Vascular endothelial growth factor/vascular permeability factor augments nitric oxide release from quiescent rabbit and human vascular endothelium. Circulation 1997; 95:1030–1037
75. Ziche M, Morbidelli L, Chouhuri R, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblastic growth factor-induced angiogenesis. J. Clin. Invest. 1997;99:2625–2634
76. Parenti A, Morbidelli L, Cui XL, et al. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-related kinase 1/2 activation in post-capillary endothelium. J. Biol. Chem. 1998;273:4220–4226
77. Morbidelli L, Chang CH, Douglas JG, et al. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am. J. Physiol. 1996;270:H411–H415
78. Papapetropoulos A, Garcia-Cardena G, Madri JA, et al. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. J. Clin. Invest. 1997; 100:3131–3139
79. Shizukuda Y, Tang S, Yokota JA, et al. Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase C delta activity. Circ. Res. 1999;85:247–256
80. Noiri E, Hu Y, Bahou CR, et al. Permissive role of nitric oxide in endothelin-induced migration of endothelial cells. J. Biol. Chem. 1997; 272:1747–1752
81. Noiri E, Lee E, Testa J, et al. Podokinesis in endothelial cell migration: role of nitric oxide. Am. J. Physiol. 1998;274:C236–C244
82. Brown LF, Yeo KT, Berse B, et al. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. J. Exp. Med. 1992;176:1375–1379
83. Frank S, Stallmeyer B, Kamper H, et al. Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes (HaCaT) and during cutaneous wound repair. FASEB J. 1999;13:2002–2014
84. Leibovich SJ, Polverini PJ, Fong TW, et al. Production of angiogenic activity by human monocytes requires an L-arginine/nitric oxide-synthase-dependent effector mechanism. Proc. Natl. Acad. Sci. U. S. A. 1994;91:4190–4194
85. Ziche M, Morbidelli L, Masini E, et al. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth factor and migration in vitro promoted by substance P. J. Clin. Invest. 1994;94:2036–2044
86. Tsurumi Y, Murohara T, Krasinski K, et al. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. Nat. Med. 1997;3:879–886
87. Thornton FJ, Schaffer MR, Witte MB, et al. Enhanced collagen accumulation follows direct transfection of the inducible nitric oxide synthase gene in cutaneous wounds. Biochem. Biophys. Res. Commun. 1998;246:654–659
88. Noiri E, Peresleni T, Srivastava N, et al. Nitric oxide is necessary for a switch from stationary to locomoting phenotype in epithelial cells. Am. J. Physiol. 1996;270:C794–C802
89. Cui XL, Iwasa M, Iwasa Y, et al. Arginine-supplemented diet decreases expression of inflammatory cytokines and improves survival in burned rats. J. Parent. Ent. Nutr. 2000;24:89–96
90. Heck DE, Laskin DL, Gardner CR, et al. Epidermal growth factor suppresses nitric oxide and hydrogen peroxide production by keratinocytes. J. Biol. Chem. 1992;267:21277–21280
91. Most D, Efron DT, Shi HP, et al. Characterization of incisional wound healing in inducible nitric oxide synthase knockout mice. Surgery 2002; 132:866–876
92. Bulgrin JP, Shabani M, Chakravarthy D, et al. Nitric oxide synthesis is suppressed in steroid-impaired and diabetic wound healing. Wounds 1995;7:48–57
93. Schaffer MR, Tantry U, Efron PA, et al. Diabetes-impaired healing and reduced wound nitric oxide synthesis: a possible pathophysiologic correlation. Surgery 1997;121:513–519
94. Witte MB, Thornton FJ, Tantry U, et al. l-Arginine enhances diabetic wound healing. Wound Rep. Reg. 1999;7:A326
95. Schaffer MR, Tantry U, Ahrendt GM, et al. Acute protein-calorie malnutrition impairs wound healing: a possible role of decreased wound nitric oxide synthesis. J. Am. Coll. Surg. 1997;184:37–43
96. Ulland AE, Shearer JD, Coulter C, et al. Altered wound arginine metabolism by corticosterone and retinoic acid. J. Surg. Res. 1997;70:84–88
97. Yamasaki K, Edington HD, McClosky C, et al. Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer. J. Clin. Invest. 1998;101:967–971
98. Efron DT, Witte MB, Tantry U, et al. Incisional wound healing in two different strains of inducible nitric oxide synthase knock-out mice. Wound Rep. Reg. 1999;7:A295