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Nitric oxide-releasing biomaterials for promoting wound healing in impaired diabetic wounds: State of the art and recent trends

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ABSTRACT

Impaired diabetic wounds are serious pathophysiological complications associated with persistent microbial infections including failure in the closure of wounds, and the cause of a high frequency of lower limb amputations. The healing of diabetic wounds is attenuated due to the lack of secretion of growth factors, prolonged inflammation, and/or inhibition of angiogenic activity. Diabetic wound healing can be enhanced by supplying nitric oxide (NO) endogenously or exogenously. NO produced inside the cells by endothelial nitric oxide synthase (eNOS) naturally aids wound healing through its beneficial vasculogenic effects. However, during hyperglycemia, the activity of eNOS is affected, and thus there becomes an utmost need for the topical supply of NO from exogenous sources. Thus, NO-donors that can release NO are loaded into wound healing patches or wound coverage matrices to treat diabetic wounds. The burst release of NO from its donors is prevented by encapsulating them in polymeric hydrogels or nanoparticles for supplying NO for an extended duration of time to the diabetic wounds. In this article, we review the etiology of diabetic wounds, wound healing strategies, and the role of NO in the wound healing process. We further discuss the challenges faced in translating NO-donors as a clinically viable nanomedicine strategy for the treatment of diabetic wounds with a focus on the use of biomaterials for the encapsulation and in vivo controlled delivery of NO-donors.

1. Introduction

The incidence of diabetes mellitus, a chronic disease characterized by persistent hyperglycemia, is rapidly increasing with currently over 463 million (M) sufferers that will likely reach 700 M by the year 2045 (IDF Diabetes Atlas, 2019) [1]. The long-term complications associated with this disease, including diabetic foot ulcers and non-healing wounds, are a major cause of increased morbidity and mortality [2]. The impact of various kinds of wounds including vascular ulcers, pressure ulcers, and diabetic ulcers is quite high affecting 2.4–4.5 million people in the United States alone and therefore, leading to humanistic and economic burdens even in developed countries [3]. Several factors contribute to the failure of the healing processes such as microbial colonization, lack or insufficient secretion of growth factors resulting in reduced or absence of angiogenic activity, lack of oxygen supply, and diminished release of nitric oxide (NO) [4]. Another major reason for the reduced healing process of wounds in diabetic patients is linked with impaired angiogenesis and vasculogenesis as hyperglycemia usually spoils the functional activity of endothelial nitric oxide synthase (eNOS) which thus release the reduced amount of NO and decreases leading to

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the reduced supply of NO and hence affect angiogenic process [5]. Besides, these factors reduced expression of stromal cell-derived factor-1-alpha (SDF-1α) within peripheral wound tissue due to prolonged inflammation process plays a major contribution in retarding the tissue repair resulting in a stalled closure of wounds [6]. In addition, during diabetes mellitus, the pro-inflammatory phenotype of macrophages is changed/transformed to a pro-reparative phenotype [6]. These changes increase the inflammatory profile of the diabetic wounds and thus result in a decrease in the expression of angiogenesis-related mRNA and consequently a very negligible amount of release of growth factors especially vascular endothelial growth factor (VEGF) that participates in angiogenic and wound healing process [7].

Normally, the healing of acute wounds occurs within thirty days from the event of injury to complete closure while chronic wounds remain stalled and fail to recover indefinitely [7,8]. Although it is obvious that enhanced secretion of growth factors and hormones immensely increase the proliferation of cells to substitute the lost tissue at the injured site [9]. But despite these activities, the timeline of recovery of wounds depends upon the complexity of the wound itself as the acute wound heals faster than chronic wounds. The wound healing process also depends upon the size and depth of the injury. A wound in the epidermis or dermis less than 1 cm area heals faster as the skin possesses enough potential to replace this damaged tissue [10]. However, a deep injury in the dermis and hypodermis area of skin results in delayed recovery of skin and requires a patch of skin to be substituted to speed up the regeneration process [11,12].

Currently, treatment strategies are mostly focused on curing superficial ulcers that are not fully capable of achieving complete control over the microvascular pathology in diabetic patients [13,14]. The prevalent approaches usually rely on the use of antibiotics, compression, debridement, pressure management, and the application of specialized dressings [15]. More recent approaches to treat chronic wounds involve using skin substitutes, vacuum-assisted closure, and electrical stimulation [16]. Various kinds of wound coverage matrices in the form of bioengineered artificial skin substitutes composed of recombinant growth factors, stem cells, and combinations of these entire have huge potential in the treatment of diabetic wounds [17]. But the high cost, inappropriate degradation rates, immune system rejection, as well as slow or poor development of blood vessels, are some major issues that prevent the effective use of these bioengineered constructs [18]. These constructs may also lack suitable biochemical and mechanical properties that are needed for faster and quicker healing of the wounds [19]. Besides these approaches, cell-based therapies that involve the use of stem cells also have gained huge popularity because of the potential of stem cells to transform into any cell type [20,21].

Interestingly, the importance of NO has been recognized for a long time for wide-scale efficacy in diverse biomedical fields [22–25]. NO-donors can release NO upon a stimulus such as exposing them to photothermal shock. On the other hand, the release of NO is governed inside or from various types of cells autocrine and paracrine pathways [26]. As far as chemical aspects of NO are concerned, it is a free radical which is capable of performing numerous biological functions [27]. However, one of the drawbacks of this important molecule is its very short life span (~ 100 ms of its origin) [28]. This disadvantage of a very limited lifetime of NO is resolved by encapsulation of NO-releasing donors into various kinds of wound coverage matrices as shown in Fig. 1A-C. The delivery of endogenous NO-donors may ameliorate wound healing due to their multiple benefits such as antimicrobial potential, vasodilation, and pro- or anti-inflammatory activity [29,30]. Moreover, systemic administration of NO-donor such as S-nitrosoglutathione (GSNO) in rats improves the collagen deposition at wound sites, without disturbing matrix metalloproteinase pathways [31]. Thus, adopting strategies that involve the development of wound healing matrices impregnated with NO-donors could be an alternative option for quicker recovery of impaired diabetic wounds.

Increasing the supply of oxygen, growth factors, and other nutrients in impaired diabetic wounds immensely accelerates the healing process [32]. Growth factor impregnated dressings promote angiogenic activity

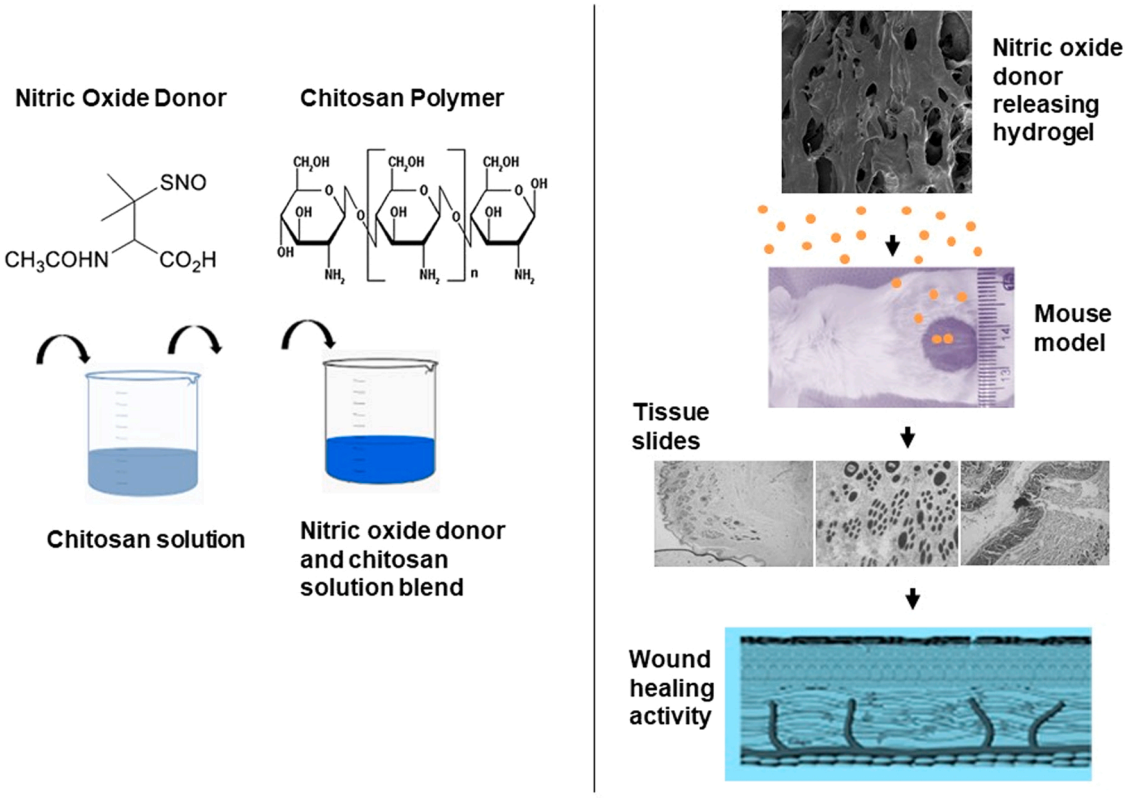


Fig. 1. (A) Schematic showing the encapsulation of NO-donors into wound healing matrices and controlled release of NO for wound healing application.

and thereby healing process as evident from the research work of our group and several other related research groups [33–36]. For example, it has been shown that the migration of HaCat keratinocytes with growth factor-loaded platforms has increased significantly (Fig. 1B) [33–36]. Also, it can be claimed that some proteins involved in mediating the binding of growth factors with their targets can control the migration of cells in the wound healing process [37]. However, wide-scale application of these dressings containing growth factors has been compromised by their high costs, short life due to fast degradation [38]. Another strategy that can be used as an alternative for accelerating wound healing activity could be the use of NO. Evidence shows that NO promotes angiogenic activity like the growth factors by improving the proliferative ability of the endothelial cells that participate in the formation of a thick network of blood vessels in the wound area [39]. NO is naturally involved in promoting angiogenic activity and wound healing closure process [40]. Topical application of NO impregnated wound healing dressings such as hydrogels can produce the same effects as observed naturally inside the body [41].

Keeping in view the role of NO in wound healing, in this review article, we have focused on the challenges faced in diabetic wound management, use of NO-donors in the wound healing process, and mechanism of NO release as well as its effects on the healing process. We have also highlighted the relevance of encapsulation of NO-donors in wound healing matrices for the controlled and extended-release of NO in diabetic wounds.

2. Nitric oxide (NO) and biological system

NO is a very important signaling molecule in the cells involved in various physiological functions [23]. As far as the biomedical significance of NO is concerned, the first evidence was reported by Ascanio Sobrero in 1846 [42]. He showed that the placement of nitroglycerin on the tongue for a short while produces a severe headache that may last for several hours [43]. In 1859, Frederick Guthrie, an English chemist later proved that inhalation of amyl nitrite (a NO-donor) results in the arterial throbbing of the neck and reduces the heart rate [44]. This discovery leads to the application of amyl nitrite in the biomedical field for the treatment of cardiovascular diseases as this chemical agent help to dilate blood vessels and thus, helps to reduce blood pressure [44]. In 1970, Ferid Murad and his colleagues started working to understand the mechanism involved in the vasodilation potential of nitroglycerin and how nitroglycerin affects the enzyme guanylate cyclase (GC) [45]. Murad and his colleagues found that nitrite-containing compounds stimulate GC by enhancing the synthesis of cyclic guanosine monophosphate (cGMP) from guanosine-5'-triphosphate (GTP), which later induce vasodilation [46]. Later, Murad observed that GC activity is improved manifold whenever it is exposed to the NO agent. From this observation, Murad concluded that NO produced by nitrite-containing compounds stimulates GC which in turn results in subsequent vasodilation events and lowering of blood pressure. Robert Furchgott and his colleagues tested the effects of acetylcholine on blood vessel relaxation and found that the presence of endothelial cells is necessary for vasodilation activity as the vessels composed of smooth muscle cells only fail to show vasodilation activity [47]. Later, Furchgott and their colleagues concluded that endothelial cells produce endothelium-derived relaxing factor (EDRF) which is principally involved in producing the vasodilation effect [48]. In 1986, Louis Ignarro while working at the University of California found that the function of both NO and EDRF is identical in vasodilation [49]. After a sequence of studies about the role of NO in biological systems, it was concluded that NO is an endogenous signaling molecule for vasodilation within mammalian tissues. The discovery that NO is a signaling molecule in cells is considered a huge development in the field of Medicine and Physiology and recognition of this discovery, Murad Furchgott and Ignarro were awarded a Nobel Prize in 1998 [50, 51].

2.1. Generation of nitric oxide in the human body

The synthesis of NO is achieved endogenously by a set of three enzymes known as nitric oxide synthases (NOS) [52]. L-arginine is catabolized by NOS in the presence of NADPH and oxygen-producing NO, citrulline, and $NADP^{+}$. NOS enzymes may be neuronal NOS (nNOS), eNOS, or inducible NOS (iNOS) [52]. Among these enzymes, neuronal nitric oxide synthase and eNOS are expressed constitutively while iNOS is expressed inductively during infections or immune responses. Further studies revealed that iNOS is also produced constitutively from keratinocytes, lung, liver, kidney, colon, and neurons [53–56]. The release of eNOS becomes higher in stress conditions especially during hyperthermia, exercise, and shear stress [55,57]. The expression of nNOS increases in the myocardium, myometrium, skeletal muscle, vascular smooth muscle cells, and macula densa during stress situations [58]. It can be concluded that several types of cells are involved in the production of NOS and thus, NO can be generated by the application of stimuli to these cells [59]. Later studies showed that iNOS, eNOS, and nNOS are usually found in the mitochondria so they were named mitochondrial NOS (mNOS) [60]. Further studies revealed that post-translational modification of the nNOS gene produces mitochondrial nitric oxide synthase [61]. Chemically, heme protein in the dimeric form is present in all NOS isoforms and each monomeric unit has a molecular mass of 126–160 kDa. All isoforms are produced separately by different genes and amino acid sequences of these isoforms show 51–57% homology [62]. Additionally, NOS isoforms are produced in different situations. For instance, iNOS generates a higher amount of NO and can produce NO for extended periods compared to nNOS and eNOS. In addition, NOS can mold differently into specific structures and can localize at specific places to perform a specific function [52]. One of the main properties of NOS is that they perform their functions in proximity and thus affect downstream targets [63]. Thus, we can say that NOS formation is regulated at transcriptional and translational levels depending upon substrate availability and their subcellular localizations that allow NO to perform functions by targeting specific proteins [63].

2.2. Biological properties of nitric oxide

NO plays a crucial role in various processes in the body including cardiovascular regulation, neurotransmission, antibacterial activities, and wound healing potential [64]. NO promotes wound healing activity by acting on the inflammatory cells such as macrophages to release NO which initiates complex cascade processes that help in blood vessel formation [65]. The role of NO as a wound healing promoter was proved in a series of experimental reports. In this direction, Albina et al. found that during wound healing activity, the metabolism of L-arginine increases significantly as this compound breaks into NO and citrulline [66]. Keeping in view the study of Albina et al. some researchers used supplementary arginine on wounds and found that this treatment accelerates the wound healing process [67]. In another study, Smith et al. observed that the amount of nitrate in the urine of rats inflicted with injuries increased manifold [65]. They anticipated that this increase in NO was associated with the generation of NO during the wound healing process. Bulgrin et al. during their work reported the same results about the generation of NO during wound healing activity [68]. During their investigation, it was observed that feeding the rats with a low amount of arginine leads to reduced production of nitrate and consequently a low amount of NO [68]. During their work, Bulgrin and colleagues fed the rats by 0 or 3% L-arginine enriched diet and inflicted a deliberate wound on their skin. Initially, there was a continuous increase in the amount of nitrate generation but after a while, the endogenous arginine was not enough for a prolonged time to maintain a higher level of this compound, and nitrate level started returning quickly to a baseline value [68]. However, feeding the rats with a higher concentration of arginine enriched diet (3%) resulted in maintaining higher levels of nitrate throughout wound healing activity. This supported the fact that an

increase in the amount of NO was associated with wounds [69]. The role of NO in the wound healing process was further confirmed by Shi et al. during their work by feeding the diabetic Lewis rats with an L-arginine enriched diet [70]. They observed that feeding the rats with this diet not only accelerates the wound healing activity but also increases the production of nitrate/nitrite levels in wound fluids [70]. NO-donor also manifests the same effects on the wound healing process by producing the NO [71]. Researchers also worked on the role of the iNOS pathway in the generation of NO during the wound healing process to explore the exact mechanism involved in skin regeneration. Reicher et al. found that expression of the iNOS pathway in the first 72 after an injury is tremendously increased and afterward the expression of the pathway slowly subsides [72]. Inhibiting the iNOS pathway during the wound recovery process resulted in a gradual decrease in the synthesis of collagen, epithelization, and production of NO. The role of the iNOS pathway in wound healing activity was further confirmed by providing the supplementary arginine in knocked-out mice by Shi et al. and it was revealed that this supplementary arginine in the iNOS knocked-out mice fails to show any effects on the wound recovery process. Thus, it was concluded that the iNOS pathway helps in the metabolism of arginine to generate NO which acts as a signaling molecule for the wound healing process [73]. Some important biological properties of NO are illustrated in Fig. 2A.

3. Therapeutic applications of nitric oxide

NO produced endogenously in the body performs several functions and can be used in diverse ailments. It is interesting to mention here that varying the concentrations of NO on the same process/disease may produce different effects and it is very important to use an optimum amount of NO for the treatment of a specific disease. Thus, a specific relationship exists between biological processes and NO concentration in a biological activity taking place inside a cell. For instance, a concentration of NO around 1–30 nM leads to improved vasodilation and enhanced angiogenic activity [74]. Further increasing the concentration of NO up to 100 nM produced protective effects by reducing cell apoptosis (anti-inflammatory and wound healing) while concentrations above 400 nM induce cellular apoptosis [74]. Additionally, every cell shows a different dose-response against NO concentration as higher concentrations of NO are mostly lethal to normal cells and may damage the cells and thus cause the death of tissues. However, higher concentrations of NO prove extremely beneficial in cancer chemotherapy and preventing antibacterial infections [67]. Increasing the exposure of NO to the tissues further improves its therapeutic efficacy. The development of scaffolds for clinical applications requires better control over the release of NO to get required effects as naturally displayed by NO produced endogenously in the animal's body. NO released from the surface of non-thrombogenic endothelium ranges between 0.05 and 0.4 $\times$ 10^{-10} mol cm^{-2} min^{-1} and thus scaffolds releasing NO must produce NO close to this range to obtain hemocompatibility ns [75]. Sometimes, localized

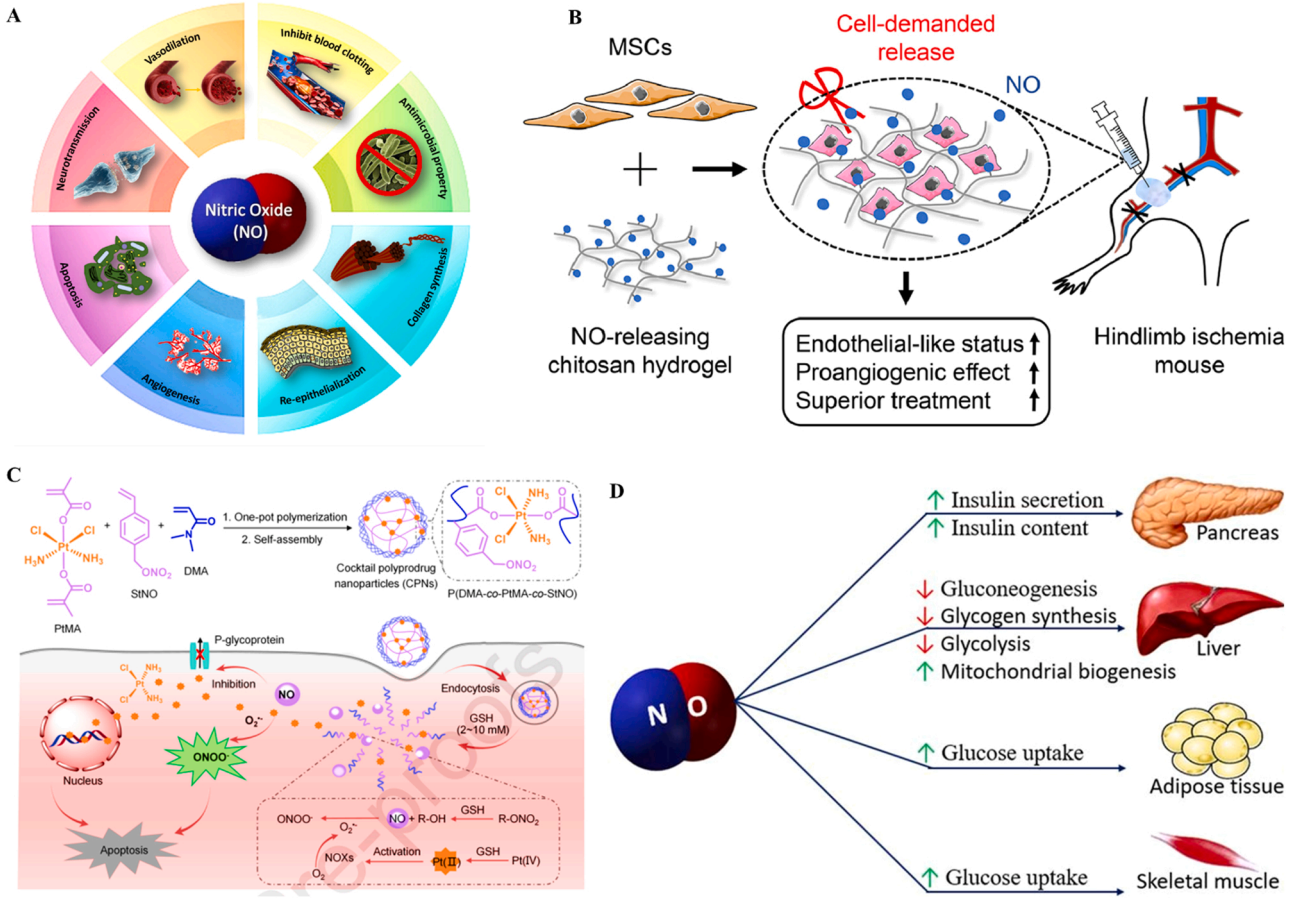


Fig. 2. (A) the biological properties of NO in vasodilation, blood clotting, neurotransmission, apoptosis, angiogenesis, re-epithelization, collagen synthesis, and antimicrobial activities. Among these biological properties, angiogenic activity, epithelization, and collagen synthesis are useful for the wound healing process. (B) NO-releasing hydrogel for hindlimb ischemia therapy. Reproduced from Ref. [76] with permission from Elsevier. (C) Development of cocktail polyprodrug nanoparticles (CPNs) to inhibit the proliferation of cisplatin-resistant cancer cells. Reproduced from Ref. [77]. (D) Regulation of carbohydrate metabolism by NO. Reproduced from Ref. [78] with permission from Elsevier. Abbreviations: Prodrug crosslinker monomer (PtMA), NO prodrug monomer (StNO), N, N-dimethyl acrylamide (DMA), P-glycoprotein (P-gp), peroxynitrite (ONOO-) for collaborative inhibition of cisplatin-resistant cancers.

delivery of NO is required for antithrombotic applications to avoid the systemic implications of this molecule as systemic use of NO may cause severe hemorrhage. It has also been indicated that a NO-releasing hydrogel can be potentially used for improving the therapeutic impacts of mesenchymal stem cell therapy (Fig. 2B) [76] (Fig. 2B). Furthermore, it has been shown that polyprodrug nanoparticles which release cisplatin and NO can be used to combat resistant cancer cells (Fig. 2C)[77]. Recently, it has been also implicated that NO-releasing agents can be developed to regulate carbohydrate metabolism (Fig. 2D) [78].

The therapeutic application of NO gas is largely limited by the high reactivity of this molecule at normal physiological conditions. NO consumption is widely affected by the amount of oxygen and hemoglobin in the body. The rate of NO consumption is much higher in the presence of oxygen and hemoglobin and is equal to the square of NO concentration [79]. In extravascular tissues, the half-life of NO remains in the range of 0.0λ to > 2 s and is mainly associated with the concentration of oxygen [80] while in whole blood half-life of NO in the presence of erythrocytes is 1.8×10^{-3} s [81]. The consumption of NO is tremendously increased in whole blood due to the high affinity and reactivity of NO with the iron of heme molecule or oxyhemoglobin [82]. Because of all these limitations, it is highly recommended to use S-nitrosothiols (RSNOs) materials as donors for NO therapeutic applications.

3.1. Wound healing properties of nitric oxide

Some of the commonly identified biological functions of NO are that it inhibits platelet formation, inactivating the monocytes besides performing anti-inflammatory activities [83–87]. Although NO plays a very important role in wound-associated activities, there is still little recognition of this material in tissue engineering applications [88,89]. NO produced endogenously performs many functions but one of the most important functions of this is that it is a vasodilator. It promotes angiogenic activity by recruiting proliferation and promoting cell proliferation of endothelial cells. It tremendously enhances wound healing activity by acting as an antimicrobial agent and promotes the migration of cells to the injured site [89–92]. NO is produced consistently endogenously which helps naturally in the wound recovery process, however, the application of hydrophobic and hydrophobic NO-donors exogenously can tremendously accelerate the wound closure process as shown in Fig. 2 [93]. But certain limitations have restricted the wide-scale applicability of NO-donors and one of them is faster degradation and burst release of NO from its donor molecules [74,94]. To avoid burst release and a lifetime of NO, this signaling molecule is incorporated into biomaterials scaffolds such as hydrogel or nanofiber mats [94–96]. This encapsulation of NO-donors results in a continuous supply of NO to the wound site and thus prevents the growth of bacteria by inhibiting cell attachment by membrane damage, and deoxyribonucleic acid (DNA) deamination [14,97]. Furthermore, the use of NO over the injured site stops bleeding triggering homeostasis, decreases inflammation, and enhances the wound recovery process [14,88,98].

3.2. Biomaterial matrices for wound healing

Wound healing matrices used in healthcare are normally prepared from biodegradable natural or synthetic materials [99]. The matrices made up of natural materials and those that have low costs, fast recovery rates, and are environment-friendly for users have gained wider acceptance [100]. In the healthcare industry, wound healing matrices that display high swelling properties (that can absorb extra exudates) and that possess high porosity (to allow better diffusion of gases especially oxygen) are preferred [101]. Besides, wound healing dressings impregnated with antibacterial and angiogenic agents are also considered very useful as these can easily inhibit bacterial growth and can promote angiogenic activity [102]. It is seen that wounds that can absorb moisture and can heal dressing maintain wound site moist heals the wounds faster. In contrast, wound healing dressings that fail to stop the loss of water by evaporation leads to a reduced supply of nutrients to the cells and thus decrease wound healing recovery [103]. Among various wound healing biomaterials dressings, hydrogels display hydrophilic matrix properties with the ability to absorb a large amount of water as compared to their size which is used to keep the wound surface moist for improved wound healing [104,105]. Another useful aspect of hydrogels made up of biomaterials is that they facilitate the debridement of necrotic tissues from the wounds which prevent microbial contamination from wounds [106]. Besides this benefit, there are other advantages of hydrogel scaffolds such as their flexible and rubbery nature along with the ability to be removed easily from wounds [107]. One of the unique features of hydrogel is the presence of a large number of pores in their surfaces which allow smooth flow of gases, water, and nutrients through them thus providing these materials to the cells laden inside them. These nutrients also travel to the affected wound healing tissues on which hydrogel dressings are applied [108]. Topical NO release from the PAA:F127/GSNO hydrogels is triggered by exudate absorption and leads to increased angiogenesis and collagen fiber organization, as well as TGF, IGF-1, SDF-1, and IL-10 gene expression in the cortical tissue [109]. The catalysis of -galactosidase controls the release of NO from the PCL/CS-NO dressing under physiological conditions. Application of wound dressing composed of PCL/CS-NO biomaterials in vivo wound healing significantly accelerated wound healing by enhancing re-epithelialization and granulation formation, as well as improving the organization of regenerated tissues, including the epidermal-dermal junction. This was attributed to the pro-angiogenesis, immunomodulation, and enhanced collagen synthesis provided by NO [110]. In another study, PEI-PO-NONOate polymer was applied to full-thickness excisional cutaneous wound models in mice to examine the impact of this formulation on wound healing. As compared to the control group, PEI-PO-NONOate polymer facilitated cutaneous wound healing and closure with increased granulation tissue development, collagen deposition, and angiogenesis [111]. Further work established that nitric oxide (NO)-releasing polymer can improve exosome-mediated proangiogenic potential by increasing their proangiogenic behavior. Exosomes generated from human placenta-derived MSCs (hP-MSCs) in response to NO stimulation enhance the angiogenic effects of human umbilical vein endothelial cells (HUVECs) in vitro. In a mouse model of hind limb ischemia, exosomes produced from hP-MSCs by NO stimulation had superior angiogenic effects and improved limb function. Improved VEGF and miR-126 levels in exosomes produced from hP-MSCs by NO stimulation were found as a novel mechanism contributing to these exosomes' increased capacity to stimulate angiogenic processes, according to further investigation [112]. Although exogenous NO compensation is a promising treatment method, the absence of stable NO molecules frequently leads to disappointing clinical outcomes. Controlling the disintegration of the CS-NO polymer, which is inhibited by galactose and only happens in the presence of glycosidase, allows for on-demand NO release. The CS-NO polymers can also be processed into supporting membranes or injectable hydrogels due to their great stability, proving their clinical promise. In the platelet-rich plasma (PRP) experiment, we found that the NO-releasing membrane hindered platelet adhesion and prolonged activated partial thromboplastin time (APTT). On the NO-contained membrane in vitro, we see increased human umbilical vein endothelial cell growth but decreased vascular smooth muscle cell proliferation. Furthermore, in diabetic mice with hind-limb ischemia, in vivo treatment of CS-NO solution greatly increased angiogenesis [112].

Similarly, Lee et al. used NO for the eradication of bacteria and to enhance wound healing by developing powder dressing capable of releasing NO in situ which becomes hydrogel when it's in contact with wounds, and found promising results [113]. Estes et al. tested different agents like nanoparticles (cerium oxide nanoparticles) and nitric oxide donor (S-nitroso-N-acetylpencillamine) and found good microbial

activity. Ghalei et al. added polylactic acid nanofibers to the above-mentioned materials to test their activity [114]. Different coatings and films combination can be used against microbial/bacterial infections as they fail the tissue engineering scaffolds and hinder the healing process. Chitosan has been used frequently for wound healing due to its antimicrobial properties [115,116]. It has also shown impressive results as NO-donor to be used in wound healing. Choi and their team used such composition in vitro and achieved 3-fold better activity as compared to the control group [117]. Lowe and coworkers successfully used acrylonitrile based terpolymers for wound dressing in vivo. They electro-spun them to form non-woven sheets in which NO binds to backbone polymer to form diazeniumdiolate group to enhance wound vascularity [117,118].

Zhao et al. explored polysaccharide-based biomaterial for controlled release of NO. Their chemical formation is stable under normal physiological conditions, but their release can be controlled by enzyme catalysis. The controlled release can be proven extremely beneficial for various medical purposes especially in diabetic patients [119].

Hydrogel scaffolds prepared from natural biomaterials are preferred for their better biocompatible properties as it is noticed that attachment of cells with these matrices along with their proliferation and migration into these materials is much higher [120]. Hydrogel matrices behave like extracellular matrices and thus favor the seeding of cells inside the 3D matrix [121]. In terms of physical characteristics, hydrogels display flexible microstructure, decent swelling capacity, and can be molded easily in different forms [122]. While in terms of biological responses, they are highly biocompatible to the human tissues, biodegradable, non-toxic, and can serve as a vehicle for the controlled release of drugs and proteins [123]. These properties of the hydrogels allow for wide-scale applicability in tissue engineering and regenerative medicines [124]. Among various kinds of biomaterials used in the encapsulation of NO-donors, chitosan is widely used for various types of scaffolds. Its scaffolds display good swelling properties along with antimicrobial activities. Chitosan is well known for its wound healing applications for its good, biocompatibility, and biodegradability [125]. Chitosan is mixed with other materials and used to fabricate different scaffolds, especially nanofibers, biofilm membranes, and hydrogels [126,127]. Synthetic polymers such as poly(vinyl) alcohol and polyethylene glycol (PEG) are hydrophilic materials and mixed.

with natural biomaterials to give them better mechanical strength and stability [129]. Among various kinds of biomaterials, PVA is a widely explored material for wound dressings as it is a cheap, elastic, and transparent material [107]. PVA has commonly been molded into hydrogels scaffolds by adopting various methods [130]. It is quite evident from all this discussion that NO plays an important part in the acceleration and promotion of the healing process as illustrated in Fig. 4.

3.3. Nitric oxide donors

NO is a very useful biologically relevant molecule with diverse therapeutic applications, but an extremely short half-life span of this molecule restricts its wide-scale applicability in several therapeutic applications. NO can be generated from various donor substances in the form of nitrosonium ions NO^+ and related signaling molecules. Exposure of healthy endothelium to endogenous NO inhibits platelet performance and consequently requires an exogenous supply of NO through donor compounds to prevent thrombus formation in vascular substitutes and other biomedical implants [94,131,132]. RSNOs are the most common carriers and donor molecules of NO gas in biological systems. Red blood cells release S-nitrosothiols into the bloodstream under low-oxygen conditions and are frequently used to dilate the blood vessels [96]. The RSNOs donors overcome the challenge of a short half-life span of NO gas by continuously supplying required amounts of NO in the physiological environment. The exogenous NO-donors display a promising ability to promote wound healing by enhancing collagen deposition, cell proliferation, granulation tissue formation, and angiogenic activity [111].

3.3.1. Nitroglycerin

Nitroglycerin is a useful NO-releasing organic nitrate that produces beneficial effects on patients suffering from hypertension and angina pain. Nitroglycerin produces a significant amount of NO (1 mol equivalent of NO) once it is bioactivated by an enzyme known as mitochondrial aldehyde dehydrogenase (mtALDH) [133]. However, patients receiving prolonged nitroglycerin treatment often develop complications like nitrate tolerance (tachyphylaxis). This can be ascribed to the generation of reactive oxygen species by this substance, subsequent oxidation of the thiol group of the mtALDH and the dysfunction of mtALDH [133]. In addition, the low bioavailability of nitroglycerin is a hindrance to its clinical effectiveness [134]. Since nitroglycerin is a contact explosive, formulation, transport, and storage are tightly regulated by federal laws.

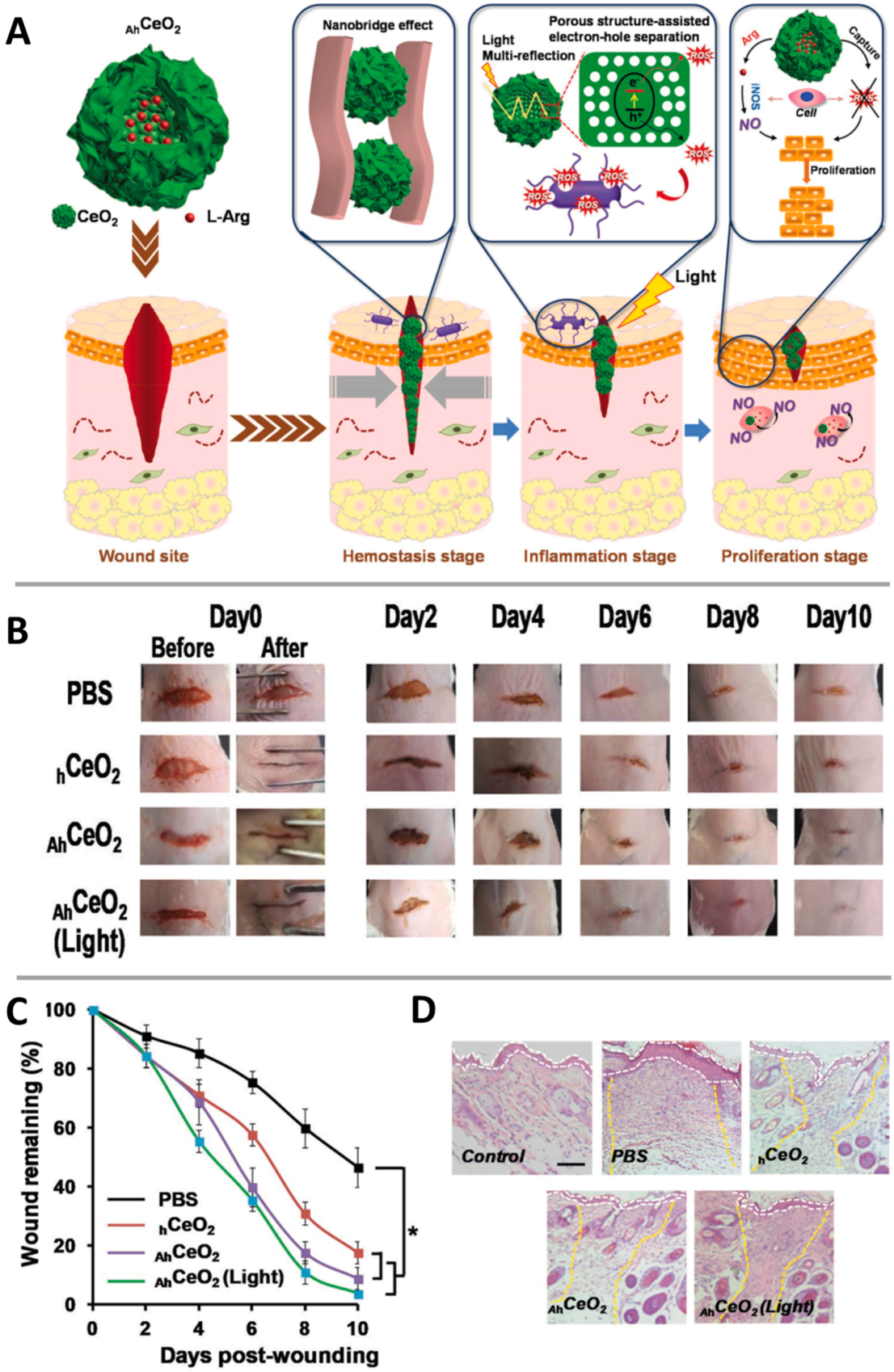
3.3.2. Metal nitric oxide complexes

Metal NO complexes (metal nitrosyls) are another class of NO-donors used in several pharmaceutical applications [107]. Metal nitrosyls are complexes of transition metals that contain NO bonded to them. A wide range of nitrosyl complexes has been developed and used in various applications, including those based on transition metals such as iron [135], manganese [136], and ruthenium [137]. Metal nitrosyls complexes are generally used as powerful vasodilators in hypertensive patients. For instance, sodium nitroprusside ($Na_2[Fe(CN)_5NO]$, SNP) based nitrosyl complexes can release NO in the presence of reducing agents such as glutathione and cysteine. In such complexes, NO release can also be achieved by photo-triggering approaches [131] and accomplish on-demand vasodilatory effects [138]. Metal nitrosyl can also be used as stimuli-responsive antimicrobial agents to manage wound infections. For instance, a photoactive manganese nitrosyl, namely [Mn(PaPy3)(NO)](ClO4)(Mn-NO) incorporated in an aluminosilicate based porous materials showed light-responsive NO release and excellent antimicrobial activity [138]. However, the potential release of toxic agents like cyanide and peroxynitrite from nitrosyl complexes results in serious concerns when considering them for clinical use [139] and this needs to be addressed properly by the detailed studies before the clinical translation of products containing nitrosyl complexes.

3.3.3. N-diazeniumdiolates (NONOates) and S-nitrosothiols (RSNOs)

N-diazeniumdiolates (NONOates) and RSNOs have extensively utilized NO-donors owing to their ability to naturally release NO under physiologically relevant conditions, good stability, and their higher bioavailability [131,132]. The RSNOs are thiols containing compounds and occur naturally in the bloodstream [140]. Both RSNOs and N-diazeniumdiolates (NONOates) are considered as suitable for prolonged delivery of NO to the target site. RSNOs are commonly found in the body but can be synthesized artificially by nitrosation of the sulfur atom of the thiol group [94,132]. However, NONOates are purely synthetic substances prepared by nitrosation of amine functional groups [141]. NONOates are highly unstable compounds and produce two molecules of NO upon decomposition [95]. In contrast, factors such as heat, light, copper ions affect the stability of RSNOs and generate only one molecule per RSNO.

NONOates can undergo hydrolysis under physiological conditions and release two-mole equivalents of NO per each mole of the donor. NONOates can also release NO upon triggering with stimuli such as changes in temperature, photochemical reactions, or by the help of suitable enzymes [132]. The half-lives of NONOates are determined by the structure of the amine precursors as well as the stabilization by hydrogen bond formation of available free amines present in the NONOate [142,143]. Studies indicated that more lipophilic NONOates release NO to a lesser extent than less-lipophilic ones [144]. Several additives like borate derivatives [144] have been incorporated in the organic polymeric phase to counter the effect of lipophilic amine



(caption on next page)

Fig. 3. Successive stages of the wound healing process carried out with L-arginine-loaded hollow cerium oxide nanoparticles. A) represents various stages of the closure of wound gap by nano bridge formation. Hollow cerium oxide nanoparticles absorb light due to their inherent property and produce ROS due to electron-hole separation ability from their porous shell which acts as an antimicrobial agent at the inflammatory stage. At the proliferation stage, L-arginine-loaded hollow cerium oxide nanoparticles (depending upon their SOD and catalase potential) absorb ROS from the wound. At the same time, L-arginine released from nanoparticles is converted to NO by iNOS pathway in macrophages that enhance the proliferation of cells; B) Images showing the wound closure after treatment with the L-arginine-cerium oxide nanoparticles during 10 days of the experiment; C) wound closure kinetics represented in percentage quantification from initial wound length (*t*-test, n = 5, mean ± SD, *P < 0.05); D) stained images of H&E have been acquired post-wounding at 10 Day; epidermal layer formation is evident from the white dashed lines while wound area that has been fully regenerated is marked with the yellow dashed lines. Scale bar: 100 μm [128].

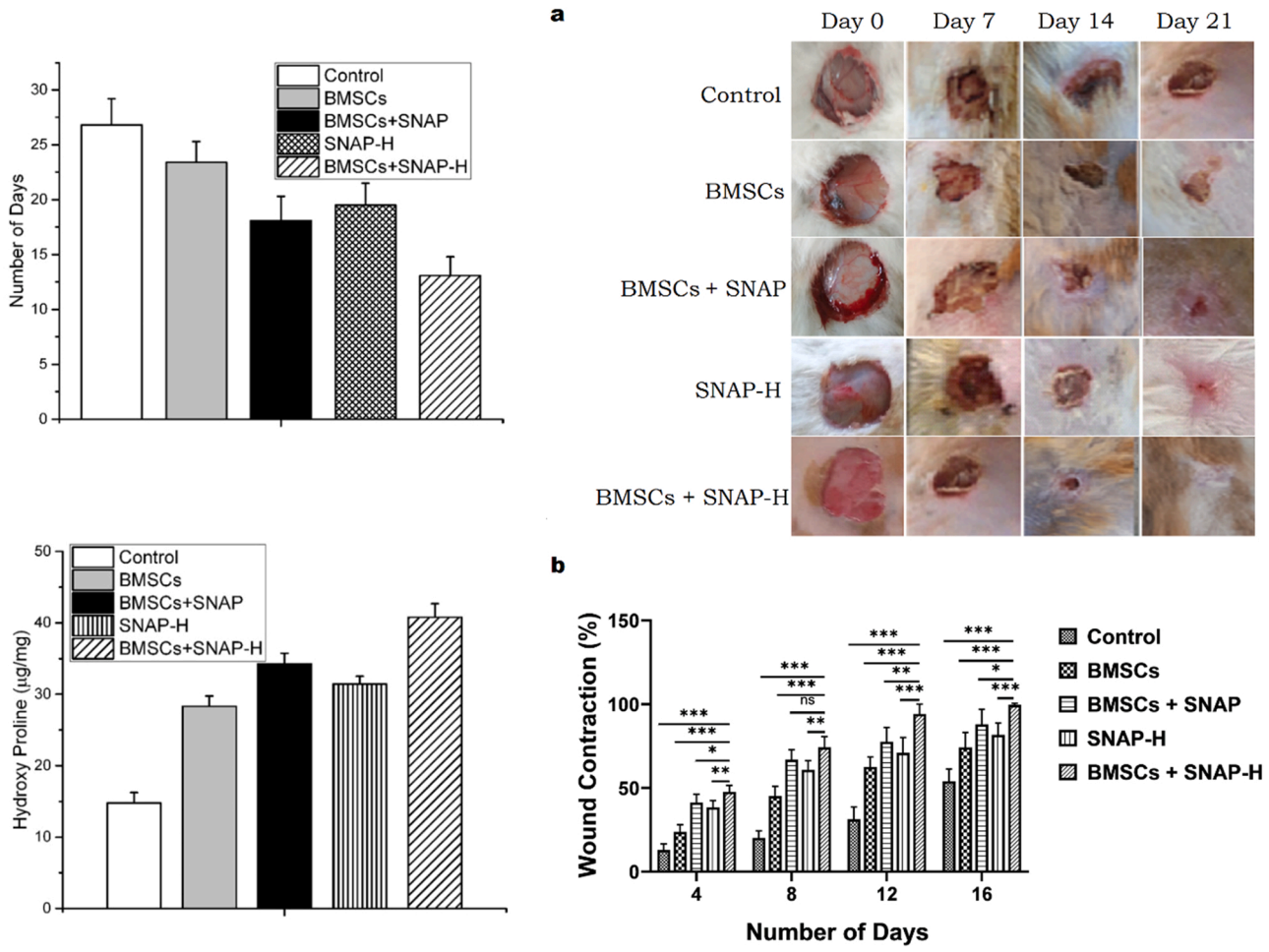


Fig. 4. (a) Day of complete recovery-re-epithelialization day. All the groups containing SNAP demonstrated more efficient healing than other groups but the groups containing SNAP-loaded hydrogel and BMSCs showed an accelerated process of wound healing due to NO signaling activity and regeneration potential of BMSCs. (b) The content of hydroxyproline in different treatment groups on day 14. SNAP-loaded hydrogel and also the BMSCs group demonstrated maximum hydroxyproline which can be due to the combined biological effects of bone marrow-derived MSCs and SNAP hydrogels released hydrogel. All values shown here are taken in triplicate and have been shown as mean ± SEM (n = 6). (c) Wound contraction after treatment of different groups in regards to specific intervals of time. a) Physical presence of wounds of different groups on different days e.g., 0, 7, 14, and 21. b) Wound contraction graphs are depicting improvement of wound healing when NO-releasing matrices were used. Measurements were taken through the scale and values obtained are in agreement with physical appearance. Reproduced from Ref. [175] with permission from IOPscience.

by-product generated during NO release that increases the pH of the organic phase. This can minimize the rise in pH within the organic polymer phase and extend the NO release period. Despite the wide range of potential applications, there is a concern that diazeniumdiolate compounds may induce carcinogenic effects upon higher exposure [143].

RSNO are low risk, naturally occurring NO-donors within tissues and blood [145] that can be produced by the thiol nitrosation reaction and include substances such as S-nitrosohemoglobin (SNO-Hb), GSNO, S-nitrosocysteine (SNO-Cys), and S-nitrosoalbumin (SNO-Alb) [139]. GSNO and S-Nitroso-N-acetyl-DL-penicillamine (SNAP) are two widely used RSNOs in the pharmaceutical and medical fields. GSNO is naturally produced in the body and thus inherently more safe for medical applications such as in supporting ulcer treatment and tissue regeneration [146,147].

SNAP is a commonly used NO-releasing agent belonging to S-nitrosothiols which is used for the relatively slow release of NO under physiologically relevant conditions [89,148]. SNAP releases NO over a longer duration at room temperature and is tried as an active ingredient to accelerate the overall healing of an injury [149]. SNAP is also encapsulated in polymeric biomaterials for the prolonged and sustained delivery of NO to the tissues of interest [23,150]. In such studies, one of the main purposes of incorporating SNAP in wound healing matrices is to attribute them with antimicrobial properties by continuously

supplying NO [151,152]. Studies indicated that SNAP develops chemical linkages with amino groups of other carrier materials [94] and the NO release can be controlled by using external stimuli like light [153]. Interestingly, SNAP-containing polymers exhibit slow and controlled release of NO which is one of the main requirements for its application in different therapeutic products. Thus, due to these properties, SNAP is regarded as one of the useful donor materials for NO delivery in various medical applications including wound healing.

3.4. Controlling release of nitric oxide from its donors

Exogenously, generation of NO occurs through a three-step reaction by reaction of nitrite with an acid. During the first step, the reaction between nitrite and reactive free hydrogen cations produces nitrous acid (HNO_2). This leads to the degradation of HNO_2 to form dinitrogen trioxide (N_2O_3) releasing water as a byproduct. Finally, N_2O_3 decomposes in aqueous conditions to generate nitrogen dioxide (NO_2) and NO [154]. This is the mechanism when NO-donors such as sodium nitrite ($NaNO_2$) are added in salts and creams for localized treatment of wounds. The nitrite applied directly to the wound site reacts immediately to acidic species in the tissue microenvironment resulting in the conversion of nitrite to NO [155]. Treatment of a combination of two creams to the skin area while one containing $NaNO_2$ and the latter one containing agents such as citric or ascorbic acid, which are acidic, to immediately generate NO. The payload carried by NO from creams and ointments can be adjusted by changing $NaNO_2$ concentration, and also the concentration of acid in these formulations. Zhu et al. evaluated the effect of acidified nitrite solutions for curing non-chronic wounds in rats and mice [156,157]. It was observed that treating non-chronic wounds with NO-donor proved more beneficial compared to chronic wounds as there was a 50% enhanced wound closure rate in non-chronic wounds than in control groups. The concentration of NO was measured electrochemically in wound fluid to assess the therapeutic response of this treatment. It was observed that a concentration of ($>5 \times 10^{-3}$ m) produced remarkable wound healing potential. The healing improvements diminished after 3 d of treatments with suspensions. NO promoted release of altering growth factor (TGFβ-1) secretion, and the interleukin-8 (IL-8) stimulated infiltration of inflammatory cells needed for removing impaired tissues and also pathogens. This study highlighted the significance of quantifying the timeline and doses of NO for getting a maximum therapeutic response. Another important point was elaborated that healthy and chronic wounds are quite different in their physiology. The healing of chronic wounds is so disturbed that they do not follow a specific healing timeline. Any enhancement in the healing process in these cases is considered a positive outcome. In this context, Weller et al. treated diabetic wounds to gauge the wound therapeutic effects of acidified nitrate-containing creams [158]. Application of acidified nitrite creams every day showed that after day 18, wound closure was 90% greater in incisional wounds compared to control groups which healed only 60%. The treatment of wounds with acidified nitrite greatly determined the outcome as it was seen that applying the cream on day 3 rather than on day one produces better outcomes. Several studies demonstrate the importance of treating human wounds with acidified nitrite creams. A group of 37 patients with Buruli ulcers (mycobacterial infection on long-lasting ulcers) were treated with acidified nitrite cream in 2004 first [158]. Two different doses of 6 and 9 wt% in aqueous cream of $NaNO_2$/ citric acid gels were used to treat Buruli ulcers for 6 weeks. There was a remarkable increase in the healing process in the patients treated with nitrite-based creams. Ulcer size was reduced by 56% with the acidified nitrite gel treatments compared to control groups. The results showed that acidified nitrite gel treatment is well allowed and highly efficient. After this study, Ormerod et al. carried out treatment of wounds contaminated with methicillin-resistant *S. aureus* (MRSA) bacteria using the same acidified nitrite creams as described in the former study [159]. Treatment of wounds contaminated with antibiotic-resistant bacteria is one of the major challenges as traditional antibiotics cannot remove entrenched bacteria. MRSA Wounds contaminated with MRSA are extremely difficult to respond to spontaneously or affected by contemporary pharmaceutical interventions [160]. To overcome this problem, Weller et al. reported that NO is very useful for controlling the growth of *S. aureus* in impaired wounds [160]. Several studies performed on the susceptibility of *S. aureus* to NO showed that these bacteria have a low resistance to NO [14,97,161]. Ormerod et al. showed that the daily application of 4.5 wt % nitrite creams for 5 days on the MRSA infected wounds fully removes these bacteria when the study was performed for increasing the wound closure on 15 wounds in eight patients [159]. Although a low dose of nitrite was used in the treatment of MRSA-contaminated wounds as aforementioned doses used for the treatment of Buruli ulcer which was 4.5 wt% vs 6 wt%, the results of acidified nitrite creams were the same for the cure of nonhealing wounds in humans. One thing worth will be to mention that dose discrepancy as observed in previous studies may be due to varying conditions of chronic wounds. Friedman and co-workers used another technology by encapsulating nitrate in a nanoparticle (NO-np) loaded hydrogel/glass composite system instead of suspending it in ointments [162]. The nanoparticles were prepared PEG, and chitosan using tetramethyl orthosilicate as a crosslinking agent. The nanoparticle hydrogel system containing nitrite was capable of releasing NO for an extended period of duration (24 h) [162]. The wound healing potential of nanoparticles containing acidified nitrite was assessed in several preclinical studies [14,97,163]. Initially, the efficacy of the bacterial NO-np system was tested against *Acinetobacter baumannii* and MRSA infected wounds in mouse models [14,97]. The wounds showed accelerated wound closure activity once NO-np system treatment was applied in both cases. Mihu et al. observed that full-thickness infected wounds completely recover within 7–8 days compared to control untreated wounds, where complete healing took place after 10–12 days [97]. The analysis of infected wounds revealed that the NO-np system drastically reduced the bacterial level. NO-np system also enhanced the collagen content which is an indicator of tissue remodeling in the mouse model [14,97]. NO-np treatments were applied to the human-sourced dermal fibroblasts and their behavior was evaluated by an in vitro scratch assay [162]. An accelerated fibroblast migration through scratch assay with the NO-np treatments compared to control groups (non--NO-releasing nanoparticles). The increase in fibroblast migration with the NO-np system was evidence for increased collagen content formation as this is the primary function of fibroblast cells. This was evidence of the biological mechanism involved in the increased collagen content formation with the NO-treated tissue. There was another study carried on to evaluate the wound healing effects of NO-np systems in noninfected mouse wounds [164]. Again, there was an increased collagen content formation observed in the NO-np-treated mice. There was an infiltration of macrophage-like cells in the infected area after NO treatment revealing that NO is involved in the movement of inflammatory cells to the wound area. After the treatment with the NO-np system, there was improved vascularization in the wound, which was responsible for greater oxygenation and enhanced, a factor in promoting wound healing [164]. Stimulating the migration of fibroblast/macrophage and angiogenesis with acidified nitrite to have great potential for using this treatment for faster healing compared to untreated wounds. From these studies, it can be inferred that nitrite-derived NO treatment can be applied for the nonobese, diabetic, and severe combined immunodeficiency mice [163]. The daily treatment with NO-np systems produced significant wound healing after day 7. Wound healing showed a better response (≈57%) with NO treatments compared to control groups (≈15%). The authors reported that treatments with NO-np were beneficial for all kinds of wounds such as healthy, infected, and immunocompromised chronic wounds. Furthermore, it was noted that NO-np systems can be combined with traditional wound dressings used for the treatment of diabetics struggling with nonhealing impaired wounds. The use of a high dose of acidified nitrite may cause severe itching or pain as noted by - Ormerod et al. in their

studies [165]. Extreme care should be taken during the development process of nitrite-containing creams as these may cause severe skin irritation. It is highly important to identify the lowest useful dose of acidified nitrite to achieve maximum healing potential. Additionally, the release kinetics of NO from acidified nitrites should be considered with immense care to ensure complete its therapeutic utility for healing activity. Anyhow, NO produced from any of the systems is a very viable, simpler, and highly useful method for accelerating the healing of impaired wounds as shown in Table 1.

3.5. Nitric oxide encapsulated polymeric matrices

SNAP and other NO-releasing agents are impregnated into polymer scaffolds for targeted and controllable release of this signaling molecule. NO-releasing agents are either covalently linked with biopolymers or blended directly into polymers to achieve physical encapsulation into the polymers [95,131,132]. The most common examples of NO-donor incorporated scaffolds include hydrogels, polyethylene oxide, PVC, silica particles, sol-gel, polyurethanes, polymethacrylates, silicone rubbers, and PEG. [95,131,132]. Li and Lee developed a very useful copolymer of PVP-PVMMA by blending poly (vinyl pyrrolidone) (PVP) and poly (vinyl methyl ether-co-maleic anhydride) (PVMMA) polymers. NO-donors in the form of RSNOs precursors consisting of S-nitroso (γ-Glu-Cys)5-Gly (PC5) and glutathione (GSH) were loaded in PVP-PVMMA copolymer [90]. The amount of NO-donor incorporated into PVP-PVMMA copolymer was calculated to be ≈ 45–80 nmol mg^{-1} and this donor copolymer was capable of releasing NO for 10 days upon applying the stimulus of light. The application of GSNOPVMMA/PVP powder on the wounds produced on the skin of diabetic rats showed a considerable improvement in wound closure on days 4, 7, and 10 but after day 10, wound closure slowed down coinciding with the fact of exhausting the NO supply from these copolymers. This study motivated the need for the development of scaffolds that can supply NO for extended periods [90]. Impressed by the former study, Vogt et al. showed that the covalent linking of SNAP compound with gelatin nanofibers can tremendously prolong the release of NO [166]. It was proved that exposure of 1654 cd light to the SNAP incorporated gelatin nanofibers results in the 24–59nmol mg^{-1} discharge of NO for a prolonged period.

Treatment of *Staphylococcus aureus* by exposing SNAP incorporated gelatin nanofibers with 54 cd light showed complete growth inhibition of these bacteria. Exposing *Staphylococcus aureus* at a higher concentration of NO was very useful for inhibition of growth of these bacteria but exposing mammalian cells to a higher concentration of NO proved toxic to their cells [167]. Later, it was established that encapsulating NO-donors such as GSNO within polymeric biofilms could be very useful for the treatment of chronic wounds. Kim et al. prepared NO-releasing chitosan biofilms by impregnating GSNO for wound closure applications as NO-loaded chitosan scaffolds could be more effective due to the synergistic effects of these two agents [168]. Chitosan is a well-known antibacterial agent proved in a few studies [169,170]. Treating *S. aureus* and *Pseudomonas aeruginosa* with GNSO loaded chitosan membranes significantly inhibited the growth of these bacteria compared to control chitosan biofilms in vitro studies [168]. Application of chitosan films on the wounds created on the epidermis of a rat model significantly increased the wound closure but treatment of skin wounds with GSNO loaded chitosan membranes immensely improved the wound closure potential compared to chitosan biofilms on day 15 [168].

NO-releasing chitosan films enhanced granulation of tissues and collagen content formation which was observed from the tissue sections of recovered wounds. The outcomes of this study revealed that NO-releasing chitosan biofilms are very useful for inhibiting the growth of

Table 1
Detailed information regarding nitric oxide-releasing biomaterial for wound healing applications.

Materials used	Date of publication	Experimental system	Animal model	Application	Work reported by
PEO-PPO-PEO (F127) micelles,poly(acrylic acid) (PAA), S-nitrosoglutathione (GSNO)	July 2018	Hydrogel	Mice	Wound healing	Champeau et al. [107]
Poly(ε-caprolactone) (PCL) and chitosan-based NO-releasing biomaterials	May 2017	Wound healing dressing	Mice	Wound Healing	Zhou et al. [108]
PEI-PO-NONOate polymer	March 2019	Nitric oxide donor polymer	Mice	Wound Healing	Zhang et al. [75]
Human placenta-derived MSCs (hP-MSCs) by NO stimulation	July 2017	Nitric oxide donor polymer	In vitro stem cells	Angiogenic activity	Du et al. [110]
$_{Ah}CeO_2$ NPs	July 2019	Nanoparticles	Mice	Wound healing	Ma et al. [110]
S-nitrosoglutathione (GSNO), alginate, pectin, and polyethylene glycol (PEG)	Oct 2019	Hydrogel	Mice	Antibacterial activity and wound healing	Lee et al. [112]
Pluronic F127 hydrogel containing S-nitrosoglutathione (GSNO)	June 2012	Hydrogel	Wistar rats	Inflammatory pain	Verceline et al. [143]
S-nitrosoglutathione (GSNO)	Jan 2020	Film membranes	Mice	Antibacterial and wound healing	Choi et al. [116]
Acrylonitrile (AN)-based terpolymers	Feb 2015	Electrospun nanofibers	Mice	Wound healing	Lowe et al. [117]
Glycosylated NO compound and chitosan	Nov 2013	Polysaccharide-based biomaterials	Mice	Wound healing	Zhao et al. [118]
Poly(*N*-isopropyl acrylamide) (PNIPAM)	2020	3D hydrogel		Cell proliferation and growth	Rivero et al. [121]
S-Nitroso-N-acetylpenicillamine (SNAP)And gelatin	Aug 2013	Nanofibrous matrix	In vitro	Antibacterial activity	Vogt et al. [159]
Honey, a nitric oxide (NO) donor, *S*-nitroso-*N*-acetyl-penicillamine (SNAP), and polylactic acid (PLA) nanofibers	Feb 2021	Electrospun membranes	In vitro	Antibacterial	Ghalei et al. [113]
Chitosan (CS), polyvinyl alcohol (PVA), and S-nitroso-N-acetyl-DL-penicillamine (SNAP)	June 2019	Hydrogel	Chick embryo	Angiogenic activity	Zahid et al. [22]
Pluronic F127, branched polyethylenimine (BPEI), and diazeniumdiolates (NONOates)	June 2011	Hydrogel	Cell culture	Promote cell proliferation	Kim et al. [97]
GSNO, SNAC and Synperonic F-127	Nov 2004	Hydrogels	Human skin	Promote vasodilation and blood flow in the skin	Seabra et al. [169]
Gelatin methacrylate (GelMA) and S-Nitroso-N-acetylpenicillamine (SNAP)	August 2021	Hydrogel	In vitro and in vivo rat model	Accelerate wound closure process	Zahid et al. [205]
Tetramethylorthosilicate, polyethylene glycol, chitosan, glucose, and sodium nitrite	Oct 2009	Nanoparticles	Mice	Accelerate wound closure process	Martinez et al. [17]

bacteria and tissue remodeling during wound healing applications [171]. In another study, it was demonstrated that hydrogel loaded with GSNO accelerates wound closure by supplying NO to the affected site and hydration. De Oliveira and coworkers impregnated S-nitroso N-acetylcysteine and GSNO (low molecular weight NO-donors such) into hydrogel scaffolds of Pluronic-F127 to evaluate their effect on blood flow [172]. The release of NO from these formulations was stabilized and extended for 3 h after the protection of GSNO from photothermal decomposition. The dermal flow of blood was tremendously enhanced for 3 h when NO-releasing hydrogel was applied over human forearms [173]. In the same direction, another study revealed that upon application of a NO-releasing hydrogel, blood supply around a wound site is improved substantially resulting in the accelerated wound healing process in diabetes-induced rats [173]. This was examined when two different doses (23×10^{-3} and 230×10^{-3} m) of GSNO impregnated hydrogels were put on the surfaces of the wound. The hydrogel with a high dose of GSNO produced an enhanced rate at which wound closes activity both in normal and diabetic rats. The hydrogel containing a low dose of NO-releasing donor did not show any effect on blood supply around the wound site suggesting a threshold amount of GSNO required enhancing perfusion. Interestingly, high dose treatment of NO did not exhibit any side effect on the systolic blood flow or heartbeat of rats suggesting NO is non-toxic and tolerable to the mammalian body. Amadeu et al. have seen that GSNO application which is loaded with hydrogel during both inflammatory and proliferative phases of the wound healing process produces better-wound closure results compared to using this formulation in one stage only [174].

GSNO loaded hydrogels were applied to the wounds produced on the skin of a rat for 8 h. It was noted that treatment of wounds over both proliferative and inflammatory stages resulted in a 50% recovery of wound area while treating the wound only in one phase showed a 16.6% recovery of wound size. The benefits of treatments with NO gas during proliferative and inflammatory phases were witnessed by enhanced granulation, re-epithelization, and collagen content formation. In another study, de Oliveira and co-workers used a combination of NO-releasing hydrogels and NO-releasing biofilms for the treatment of wounds created in the skin of a mouse model [147]. They developed GSNO impregnated Pluronic-F127 hydrogels and applied them over the skin wound during the early phase of the process of wound healing. Later, RSNO loaded biofilms formed from PVA and a polymer were applied over the hydrogel scaffolds as an additional covering. The covering of hydrogel with PVA biofilms prevented the burst NO release from the GSNO-donor which further resulted in an extended-release of NO for 24 h. The prolonged availability of NO from these formulations leads to enhanced wound closure by improved granulation, epithelization, and collagen content disposition. The improved wound closure was attributed to controlled, sustained, and prolonged release of NO from a combination of NO-releasing hydrogels and biofilms.

4. Nitric oxide and nitric oxide loaded patches in diabetic wound healing

Nitric oxide-releasing agents loaded in patches increase the wound closure process by increasing angiogenic activity, tissue remodeling, and regeneration.

4.1. Nitric oxide-releasing patches promote diabetic wound healing

During diabetes, the secretion of insulin by pancreatic β-cells, and the response of the body cells to the insulin are reduced considerably. Studies suggest that the secretion, release, and signaling of insulin are dependent specifically upon the levels of NO and therefore, any change in NO generation may affect the etiology of diabetes. An insufficient amount of NO is released when there is decreased synthesis of L-arginine endogenously and increased interaction of NO with O_2. During diabetes, the level of L-arginine drops drastically in plasma and there is a reduced generation and functioning of NO-associated pathways. Increasing the intake of arginine-enriched food supplements not only increases the amount of L-arginine in blood plasma but also improves the efficiency of various signaling pathways. Seifter et al. showed that arginine supplementation in the diet of animals improved wound healing as assessed through increased collagen deposition and wound breaking strength [176]. Furthermore, Witte et al. showed that administration of a NO-donor, molsidomine, to diabetic animals also significantly improved the wound breaking strength [177]. In a double-blinded clinical trial performed in 2014, oral dietary supplementation of arginine in diabetic patients with poor limb perfusion and/or albumin content of less than 40 g/l in plasma, were more likely to undergo wound healing than controls by approximately 1.7 times. However, the direct effects of NO on wound characteristics remained uncertain in that study [178]. Other than dietary arginine, direct administration of arginine to wounds has also yielded good results [179]. Heffernan et al. used the same principle of topical administration of arginine and examined tissue morphology to find out its impact on wound closure rates. They found keratinocytes (which are flatter, have thinner epidermis with cuboid morphology) in the arginine supplemented group. Researchers concluded that these morphological changes enhance the proliferation and differentiation of keratinocytes which explain accelerated wound healing rates in the arginine-administered group [180]. Reesi et al. found tremendous results by using nanofibrous hydrocolloid-based wound dressing for controlled release of arginine. Nanofibers are proved to have a high aspect ratio and a 3-D structure that absorbs wounds exudates and promotes skin regeneration, homeostasis, and cell proliferation [181]. Furthermore, increasing the amount of L-arginine in plasma also increases the β-cell mass/ functions and enhances insulin sensitivity through the excessive generation of NO. This provides enough evidence that NO has an important role in energy regulation, endothelium-dependent relaxation, and reduces vascular disturbances. Higher amounts of L-arginine result in increased regeneration of β-cells in the pancreas (Vasilijević, Buzadžić, et al. 2007) and thus, enhances insulin production and secretion (Lajoix, Reggio et al., 2001). Improved amounts of L-arginine through different routes result in the elimination of the endogenous NOS inhibitor ADMA (asymmetric dimethyl-L-arginine) through cationic anion transporter thus displaying the significance of L-arginine [182]. Furthermore, a decrease of ADMA amount in diabetes significantly reduces most of the vascular complications [183].

Another important factor is that reduced bioavailability of NO is linked to hyperglycemia and hyperlipidemia. During these conditions, O_2^- is produced continuously, and consequently, the rate of O^{2-} dismutation becomes quite low compared to NO interaction with O^{2-} which could be a contributing factor for the reduced bioavailability of NO. Under diabetic conditions, mimics of (superoxide dismutase) SOD can be used to control the levels of NO and O_2^-. The studies suggest that although SOD mimics were primarily designed to counter the abnormally high levels of O_2^-, subsequent investigations revealed that these mimics also decrease drastically $ONOO^-$ [186]. Insulin sensitivity can be increased and metabolic complications in diabetes can be reduced by using the compounds that restore the energy-producing capacity by improving the functionality of mitochondria [186,187].

4.2. Nitric oxide-releasing patches can enhance angiogenesis in diabetic wounds

NO is a vigorously active radicle with a broad range of physiological activities. As mentioned earlier depending on its concentration, NO can augment cell proliferation and promote angiogenesis leading to better and faster wound healing [188]. Angiogenesis is the complex process of production of new blood vessels from the pre-existing vascular bed and is organized by different growth factors and cytokines. It is extremely important in maintaining vascular integrity in the wound healing process as evident from Fig. 4. NO is produced by vascular endothelial cells to regulate vascular homeostasis hence, disturbance in this production

can lead to many adverse effects on the vascular system [189]. The part played by NO in angiogenesis was demonstrated in 1997 by Ziche et al. [170]. The role of VEGF (vascular endothelial growth factor) has been highlighted lately in inducing angiogenesis via an EDNO-dependent pathway [190]. Zhang et al. found promising results to promote angiogenesis after incorporating NO-releasing chitosan hydrogel to enhance the effectiveness of placenta-derived mesenchymal stem cells in hindlimb ischemia therapy in vivo [191]. Further research is required to understand and manipulate the role of NO in angiogenesis and its anti-cancer effects.

4.3. Nitric oxide-releasing agents improve tissue regeneration and remodeling

Clinical attention was drawn to the oral administration of L-arginine, which causes NO production, as it strengthens the patient's immune system as well as promotes rapid wound healing [192]. Reactive NO intermediates, as a consequence of arginine metabolism, found in the early inflammatory phase of the wound repair emphasized the importance of NO and its biological properties [193]. Further studies found a correlation between lowered NOS activity and impaired wound healing when there was a decrease in the deposition of the extracellular matrix [194]. Yamasaki et al. demonstrated that iNOS produces adequate amounts of NO that lead to important tissue functions during the inflammatory phase of wound healing [195]. In the repair process, inhibition of iNOS enzymatic activity leads to the loss of proliferation capacity of keratinocytes and hence delay in re-epithelialization. Studies show that the expression of eNOS mRNA is reduced in diabetic wounds tissues as compared to its expression in tissues present in normal wounds [196]. The consequences of reduced expression of eNOS mRNA results in a decrease in the production of growth factors that increase endothelial cell proliferation and thus angiogenic activity as shown in Fig. 5A. In addition, NO produced by iNOS in wounds increases VEGF and chemokines expression at the repair site [197]. Lee et al. found that mice with eNOS deficiency had delayed wound closure [198]. Stallmeyer et al. also confirmed that eNOS enzymatic activity contributes to epithelial regeneration. They also showed that subjects with eNOS deficiency had reduced wound margin epithelia [199]. Dou et al. [184] and Wan et al. [185] found that as NO-releasing poly(ε-caprolactone) (PCL)/sulfonated keratin (Fig. 5B) and S-nitrosated keratin (Fig. 5C) composite platforms can be potentially used for tissue engineering. In general, it seems that combining NO catalytic and vascular cell targeting can enhance the probability of wound healing in different tissues [200]. For example, it has been well-documented that NO-releasing chitosan-poly (vinyl alcohol) hydrogel could enhance angiogenesis *ex vivo* [194].

5. Challenges and prospects

It is a well accepted fact by the scientific community that NO plays important role in achieving the rapid healing of diabetic wounds and promoting blood vessel formation. However, there is still a lack of authentic data regarding the therapeutic window and side effects due to long-term use. Most of the higher oxides of nitrogen are eye, skin, and respiratory tract irritants [201,202]. NO can react with oxygen and form nitrogen dioxide (NO_2) which is a corrosive material that creates nitric

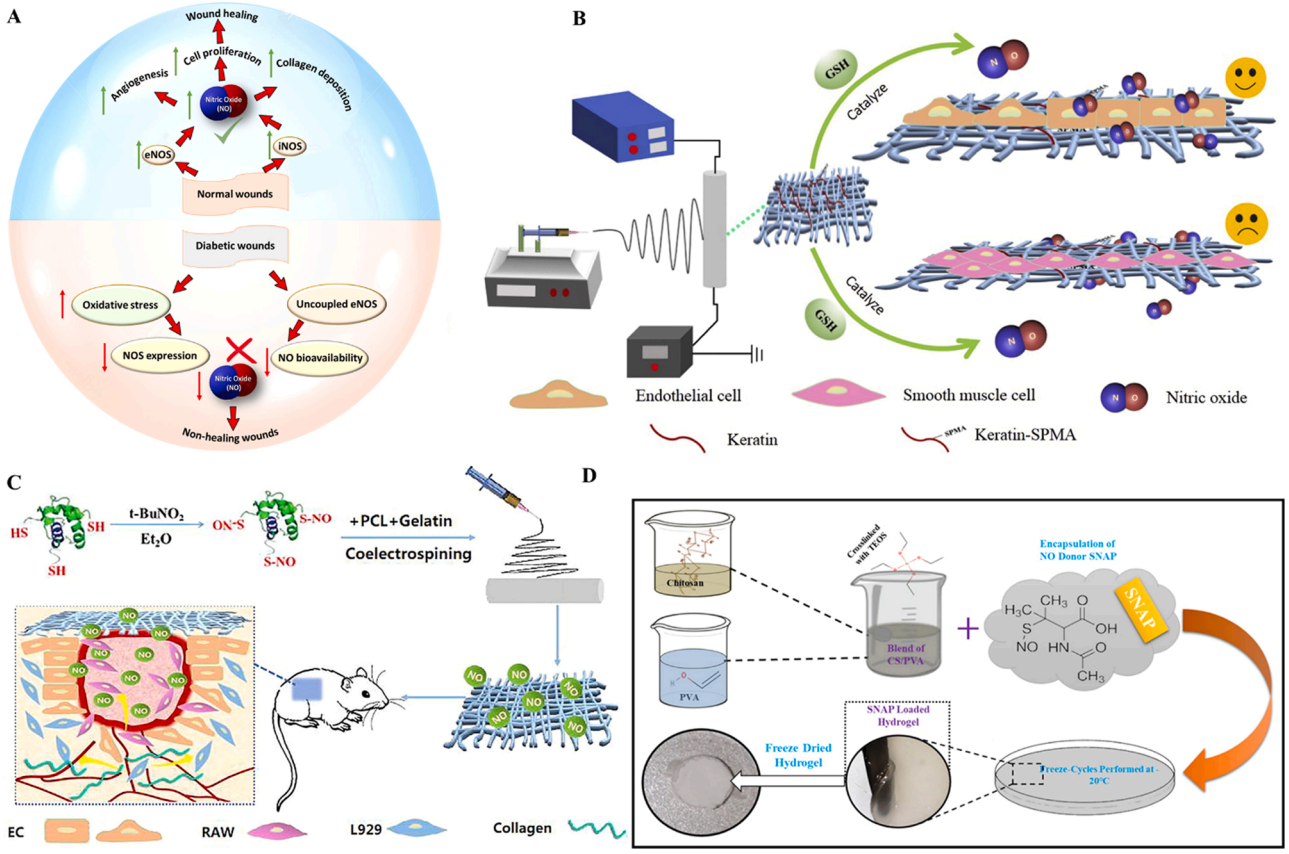


Fig. 5. (A) Differential expression of NO-related genes and production of NO in normal and diabetic wounds. Reduced expression of eNOS mRNA leads to a decrease in bioavailability of NO generation resulting in stalled angiogenic activity and wound healing process. (B) PCL/sulfonated keratin platforms for vascular tissue engineering based on a NO-releasing capability. Reproduced from Ref. [184] with permission from Elsevier. (C) S-nitrosated keratin platforms with NO release capability for wound healing. Reproduced from Ref. [185]. (D) NO-releasing chitosan-poly (vinyl alcohol) hydrogel for angiogenesis. Reproduced from Ref. [23] with the permission from Elsevier.

and nitrous acids upon reaction with water and is more toxic to the biological system than NO [203]. Nitric acid formed from NO_2 may lead to second-and third-degree skin burns [204]. NO, itself is a powerful and quick inducer of methemoglobinemia [205]. Methemoglobin inducers including NO_2 are fetotoxic and have affected behavior as well as growth statistics in newborn mice [206]. Exposure to nitrogen oxides may cause abnormalities in the pulmonary system such as pulmonary edema, bronchitis, bronchiolitis, pneumonitis, and emphysema [207]. NO, and NO_2 can also result in cough, hyperpnea, and dyspnea. Damage to, and subsequent scarring of, the bronchioles may result in life-threatening symptoms and inadequate oxygenation of the tissues. Above mentioned complications and side effects of NO-based agents and diabetic wound healing formulations or patches loaded with them should be carefully addressed before their clinical application. Future studies should also focus on strategies that help to minimize such complications such as controlling the release rate, the stimuli-responsive release of NO, incorporation of agents that can neutralize NO after achieving required therapeutic effects, and approaches to prevent the conversion of NO into NO_2.

Although a central role for NO on wound repair has been well-recognized by in vitro and in vivo experiments as well as by clinical studies to some extent [208], the application of NO-releasing agents in the treatment of diabetic wounds has not been very common in clinical practice. One possible reason for the failure of markedly accelerating the closure of chronic wounds may be due to fast clearance of released NO in the wound fluids, which may impair the ability of endogenous and exogenously applied NO to stimulate healing. The fast clearance is expected as NO is a short-lived gas molecule. The use of relatively stable NO-donors in hydrogel patches can solve this issue to some extent. In a recent study, we have demonstrated that loading of SNAP in GelMA hydrogel can ensure the slow release of NO and is effective in promoting wound healing in diabetic rats [209]. Nanoformulation approaches to protect NO-donors inside a relatively hydrophobic shell/capsule may be an efficient method for achieving sustained release of NO for a longer period on the wound site for achieving the healing of diabetic wounds. Some recent studies focused on the development of iNOS loaded nanoparticles for the osteogenic differentiation of embryonic stem cells. Lee and coworkers found that iNOS loaded with mineralized nanoparticles showed a significant increase in NO concentration without any adverse effects on cells [210]. Such efforts can be directed towards the development of wound healing patches loaded with iNOS releasing nanomaterials. Use of stable nanomaterials that can activate the iNOS pathway and induce the generation of NO in the biological system could be a very promising area of future research. Some initial studies show promising outcomes in this direction. For example, in one of the studies, the incorporation of iNOS inducible europium hydroxide nanorods in tissue engineering scaffolds showed pro-angiogenic properties as well as enhanced cell growth in vitro and in vivo systems. It has shown great potential in the fields of vascular disease therapy and tissue engineering [211]. Similarly, Arancibia et al. used copper nanoparticles and Liu et al. used gold nanoparticles with iNOS activity in vivo for their preliminary experiments and got promising results [212–214].

To harness the full potential NO in diabetic wound healing, multiple approaches along with NO delivery may be required. Recently drug delivery biomaterials with intrinsic or innate anti-diabetic activity and vasculoprotective effects have been identified [93,215]. The combined formulation of such materials together with NO-donors might offer an added benefit in the potential treatment of diabetic wounds. In addition, the use of anti-inflammatory drugs (e.g., NSAIDs) with NO generation potential may provide double benefit compared to the administration of other NO-releasing agents [216]. Along with the approaches for the effective delivery of NO through wound healing patches, gene manipulation to enhance the NO generation in the viable cells of the wound could be another promising approach to effectively utilize the potential of NO in diabetic wound healing. For instance, an interesting gene manipulation study showed that the systemic delivery of iNOS conjugated nanoparticles increased the nitrate levels in the blood [217]. Although this study was focused on cancer therapy, such approaches pave a direction for the effective use of genetic engineering and nanotechnology to achieve the manipulation of molecular pathways regulating NO generation for the treatment of diabetic wounds. Thus, despite the huge progress in the field of biomaterials science in developing NO generating biomaterials aiming at the rapid diabetic wound healing, future studies should focus on the approaches aiming to reduce the possible adverse effects, control the NO release and increase the stability of NO-donors. This can be achieved using advanced nanotechnology-based smart carriers, stimuli-responsive polymers, novel designs as well as gene manipulation techniques before clinical trials and the ultimate translation to the clinical settings for the management of unmet clinical challenges.

6. Conclusions

NO produced endogenously by endothelial cells under normal physiological conditions stimulates cell proliferation, cell migration, and differentiation of stem cells leading to enhanced vascularization and faster healing of wounds. In the case of diabetic wounds, the endogenous supply of NO is reduced or slowed down, and this insufficient supply of NO could not meet the critical challenges such as microbial infections, chronic inflammation, and nutrient deficiency to the growing cells mainly due to lack of angiogenic activity. Topical application of NO from exogenous sources such as NO-donors could fulfill these needs, however, fast degradation rates of NO-donors prevent smooth and sustained supply to impaired diabetic wounds. Thus, the use of hydrogels and other polymers to protect and facilitate the controlled release of NO-donors or NO is a promising approach in diabetic wound treatment. Although there is already has been considerable progress made, still future studies should focus on the approaches aiming to reduce the possible adverse effects, control the NO release and increase the stability of NO-donors.

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CRediT authorship contribution statement

Rashid Ahmed: Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, **Robin Augustine:** Investigation, Resources, Writing – original draft, Writing – review & editing, **Maryam Chaudhry:** Writing – original draft, Writing – review & editing, **Usman A. Akhtar**: Writing – original draft, Writing – review & editing, **Alap Ali Zahid:** Writing – original draft, Writing – review & editing, **Muhammad Tariq**: Conceptualization, Writing – review & editing, **Mojtaba Falahati**: Writing – review & editing, **Irfan S. Ahmad:** Investigation, Writing – review & editing, Visualizationg, **Anwarul Hasan:** Conceptualization, Supervision, Funding acquisition, **R. Ahmed**: Project coordination. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest statement

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

References

[1] Atlas, IDF Diabetes Atlas. International Diabetes Federation.
[2] Shraim, Moursi, Benter, Habib, Akhtar, The role of epidermal growth factor receptor family of receptor tyrosine kinases in mediating diabetes-induced cardiovascular complications, Front. Pharmacol. 12 (2021), 701390.
[3] Richmond, Maderal, Vivas, Evidence-based management of common chronic lower extremity ulcers. Dermatol. Ther. 26 (3) (2013) 187–196.
[4] Martinengo, Olsson, Bajpai, Soljak, Upton, Schmidtchen, Car, Jarbrink, Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies, Ann. Epidemiol. 29 (2019) 8–15.
[5] Zahid, Ahmed, Augustine and Hasan, Enhanced angiogenesis with nitric oxide releasing chitosan-pva hydrogel for wound healing. 2019.
[6] Augustine, Rehman, Joshy, Hasan, Stromal cell-derived factor loaded co-electrospun hydrophilic/hydrophobic bicomponent membranes for wound protection and healing, RSC Adv. 11 (1) (2021) 572–583.
[7] Bjarnsholt, Kirketerp-Møller, Jensen, Madsen, Phipps, Krogfelt, Høiby, Givskov and regeneration, Why chronic wounds will not heal: a novel hypothesis. 2008. 16(1): p. 2-10.
[8] Guo, Dipietro, Factors affecting wound healing, J. Dent. Res. 89 (3) (2010) 219–229.
[9] Pastar, Stojadinovic, Yin, Ramirez, Nusbaum, Sawaya, Patel, Khalid, Isseroff, Tomic-Canic, Epithelialization in wound healing: a comprehensive review, Adv Wound Care 3 (7) (2014) 445–464.
[10] Rittie, Cellular mechanisms of skin repair in humans and other mammals, J. Cell Commun. Signal. 10 (2) (2016) 103–120.
[11] Rippa, Kalabusheva, Vorotelyak, Regeneration of dermis: scarring and cells involved, Cells 8 (6) (2019) 607.
[12] Shpichka, Butnaru, Bezrukov, Sukhanov, Atala, Burdukovskii, Zhang, Timashev, Skin tissue regeneration for burn injury, Stem Cell. Res. Ther. 10 (1) (2019) 94.
[13] Sumpio, Contemporary evaluation and management of the diabetic foot, Scientifica 2012 (2012), 435487.
[14] Martinez, Han, Chacko, Mihu, Jacobson, Gialanella, Friedman, Nosanchuk, Friedman, Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against Staphylococcus aureus skin infection, J. Investig. Dermatol 129 (10) (2009) 2463–2469.
[15] Attinger, Janis, Steinberg, Schwartz, Al-Attar, Couch, Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants, Plast. Reconstr. Surg. 117 (7) (2006) 72s–109s.
[16] Frykberg, Franks, Edmonds, Brantley, Teot, Wild, Garoufalis, Lee, Thompson, Reach, Dove, Lachgar, Grotemeyer, Renton, Group, A multinational, multicenter, randomized, double-blinded, placebo-controlled trial to evaluate the efficacy of cyclical topical wound oxygen (TWO2) therapy in the treatment of chronic diabetic foot ulcers: the TWO2 study, Diab. Care (2020) 616–624.
[17] Kaur, Midha, Giri, Mohanty, Functional skin grafts: where biomaterials meet stem cells, Stem Cells Int. 2019 (2019) 1286054.
[18] Mariani, Lisignoli, Borzi, Pulsatelli, Biomaterials: foreign bodies or tuners for the immune response? Int. J. Mol. Sci. 20 (3) (2019) 636.
[19] Dhivya, Padma and Santhini, Wound dressings–a review. 2015. 5(4).
[20] Moradi, Mahdizadeh, Saric, Kim, Harati, Shahsavarani, Greber, Moore, Research and therapy with induced pluripotent stem cells (iPSCs): social, legal, and ethical considerations, Stem Cell. Res. Ther. 10 (1) (2019) 341.
[21] Akram, Zedan, AL-Ameri and Zedan, Establishment and Immunological Characterization of Mesenchymal Stem Cells Derived from Human Umbilical Cord.
[22] Cheng, He, Shen, Zhang, Yu, Hu, Nitric oxide (NO)-releasing macromolecules: rational design and biomedical applications, Front. Chem. 7 (2019) 530.
[23] Zahid, Ahmed, Raza Ur Rehman, Augustine, Tariq, Hasan, Nitric oxide releasing chitosan-poly (vinyl alcohol) hydrogel promotes angiogenesis in chick embryo model, Int. J. Biol. Macromol. 136 (2019) 901–910.
[24] Zahid, Fabrication of a Nitric Oxide Releasing Chitosan-PVA-SNAP, Qatar University, 2019.
[25] Ahmed, Afreen, Tariq, Zahid, Masoud, Ahmed, Ali, Akram and Hasan, Bone marrow mesenchymal stem cells preconditioned with nitric oxide releasing chitosan/PVA hydrogel attenuate diabetic wound healing in rabbits. 2020.
[26] Bahadoran, Carlström, Mirmiran and Ghasemi, Nitric oxide: To be or not to be an endocrine hormone? 2020. 229(1): p. e13443.
[27] Dawson, Dawson, Snyder, A novel neuronal messenger molecule in brain: the free radical, nitric oxide. Ann. Neurol. 32 (3) (1992) 297–311.
[28] Thomas, Ridnour, Isenberg, Flores-Santana, Switzer, Donzelli, Hussain, Vecoli, Paolocci, Ambs, Colton, Harris, Roberts, Wink, The chemical biology of nitric oxide: implications in cellular signaling, Free Radic. Biol. Med. 45 (1) (2008) 18–31.
[29] Miller, Megson, Recent developments in nitric oxide donor drugs, Br. J. Pharmacol. 151 (3) (2007) 305–321.
[30] Ibrahim, Wong, Mohamed, Mohamed, Chin, Ima-Nirwana, Shuid, Wound healing properties of selected natural products, Int. J. Environ. Res. Public Health 15 (11) (2018) 2360.
[31] Achuth, Moochhala, Mahendran, Tan, Nitrosoglutathione triggers collagen deposition in cutaneous wound repair, Wound Repair Regen. 13 (4) (2005) 383–389.
[32] Andriany, Tahir, Sjattar, Ake and Nuru, Wound healing angiogenesis: A perspective of nurse. 2019. 3(1): p. 1-3.
[33] Augustine, Rehman, Ahmed, Zahid, Sharifi, Falahati and Hasan, Electrospun chitosan membranes containing bioactive and therapeutic agents for enhanced wound healing. 2020.
[34] Augustine, Zahid, Hasan, Wang, Webster, CTGF loaded electrospun dual porous core-shell membrane for diabetic wound healing, Int. J. Nanomed. 14 (2019) 8573–8588.
[35] Augustine, Zahid, Wang, Webster and Hasan. Growth factor releasing core-shell polymeric scaffolds for tissue engineering applications. in 2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2019. IEEE.
[36] Veith, Henderson, Spencer, Sligar, Baker, Therapeutic strategies for enhancing angiogenesis in wound healing, Adv Drug Deliv Rev 146 (2019) 97–125.
[37] Das, Majid, Baker, Syndecan-4 enhances PDGF-BB activity in diabetic wound healing, Acta Biomater. 42 (2016) 56–65.
[38] Mandla, Davenport Huyer, Radisic, Review: multimodal bioactive material approaches for wound healing, APL Bioeng. 2 (2) (2018), 021503.
[39] Bussolati, Dunk, Grohman, Kontos, Mason, Ahmed, Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide, Am. J. Pathol. 159 (3) (2001) 993–1008.
[40] Yang, Qi, Yang, Huang, Nitric oxide based strategies for applications of biomedical devices, Biosurf. Biotribol. 1 (3) (2015) 177–201.
[41] Okur, Karantas, Senyigit, Ustundag Okur, Siafaka, Recent trends on wound management: new therapeutic choices based on polymeric carriers, Asian J. Pharm. Sci. 15 (6) (2020) 661–684.
[42] Marsh, Marsh, A short history of nitroglycerine and nitric oxide in pharmacology and physiology, Clin. Exp. Pharmacol. Physiol. 27 (4) (2000) 313–319.
[43] Marino, Flu pandemics: homeopathic prophylaxis and definition of the epidemic genius. 2009. 8(28): p. 100-109.
[44] Guthrie, X.X.—Contributions to the knowledge of the amyl group. 1859. 11(3): p. 245-252.
[45] Katsuki, Arnold, Mittal and Murad, Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. 1977. 3(1): p. 23-35.
[46] Arnold, Mittal, Katsuki and Murad, Nitric oxide activates guanylate cyclase and increases guanosine 3′: 5′-cyclic monophosphate levels in various tissue preparations. 1977. 74(8): p. 3203-3207.
[47] Furchgott, Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (5789) (1980) 373–376.
[48] Cherry, Furchgott, Zawadzki, Jothianandan, Role of endothelial cells in relaxation of isolated arteries by bradykinin, Proc. Natl. Acad. Sci. USA 79 (6) (1982) 2106–2110.
[49] Ignarro, Buga, Wood, Byrns, Chaudhuri, Endothelium-derived relaxing factor produced and released from artery and vein is nitric-oxide, Proc. Natl. Acad. Sci. USA 84 (24) (1987) 9265–9269.
[50] Ignarro, Byrns, Buga and Wood, Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. 1987. 61(6): p. 866-879.
[51] Jaffrey, Snyder, Nitric oxide: a neural messenger, Annu. Rev. Cell Dev. Biol. 11 (1) (1995) 417–440.
[52] Alderton, Cooper, Knowles, Nitric oxide synthases: structure, function and inhibition, Biochem. J. 357 (Pt 3) (2001) 593–615.
[53] Asano, Chee, Gaston, Lilly, Gerard, Drazen, Stamler, Constitutive and inducible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells, Proc. Natl. Acad. Sci. USA 91 (21) (1994) 10089–10093.
[54] Morrissey, McCracken, Kaneto, Vehaskari, Montani, Klahr, Location of an inducible nitric oxide synthase mRNA in the normal kidney, Kidney Int. 45 (4) (1994) 998–1005.
[55] Laughlin, Pollock, Amann, Hollis, Woodman, Price, Training induces nonuniform increases in eNOS content along the coronary arterial tree, J. Appl. Physiol. (1985) 90 (2) (2001) 501–510.
[56] McNaughton, Puttagunta, Martinez-Cuesta, Kneteman, Mayers, Moqbel, Hamid and Radomski, Distribution of nitric oxide synthase in normal and cirrhotic human liver. Proc. Natl. Acad. Sci. USA, 2002. 99(26): p. 17161-17166.
[57] Lam, Peterson, Richardson, Croatt, d'Uscio, Nath, Katusic, Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin, Am. J. Physiol. Heart Circ. Physiol. 290 (2) (2006) H786–H793.
[58] Kone, Molecular biology of natriuretic peptides and nitric oxide synthases, Cardiovasc. Res. 51 (3) (2001) 429–441.
[59] Lavnikova, Drapier, Laskin, A single exogenous stimulus activates resident rat macrophages for nitric oxide production and tumor cytotoxicity, J. Leukoc. Biol 54 (4) (1993) 322–328.
[60] Michel and Feron, Nitric oxide synthases: which, where, how, and why? 1997. 100(9): p. 2146-2152.
[61] Elfering, Sarkela, Giulivi, Biochemistry of mitochondrial nitric-oxide synthase, J. Biol. Chem. 277 (41) (2002) 38079–38086.
[62] Marletta, Nitric oxide synthase: aspects concerning structure and catalysis, Cell 78 (6) (1994) 927–930.
[63] Villanueva, Giulivi, Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease, Free Radic. Biol. Med. 49 (3) (2010) 307–316.
[64] Fang, Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity, J. Clin. Investig. 99 (12) (1997) 2818–2825.
[65] Witte, Barbul, Role of nitric oxide in wound repair, Am. J. Surg. 183 (4) (2002) 406–412.
[66] Albina, Mills, Barbul, Thirkill, Henry, Mastrofrancesco, Caldwell, Arginine metabolism in wounds, Am. J. Physiol 254 (4 Pt 1) (1988) E459–E467.

[67] Barbul, Lazarou, Efron, Wasserkrug, Efron, Arginine enhances wound healing and lymphocyte immune responses in humans, Surgery 108 (2) (1990) 331–336.
[68] Bulgrin, Shabani, Smith, Arginine-free diet suppresses nitric-oxide production in wounds, J. Nutr. Biochem. 4 (10) (1993) 588–593.
[69] SMITH and Dunphy, The influence of wound healing of urinary Nitrate levels in Rats. 1991.
[70] Shi, Most, Efron, Witte, Barbul, Supplemental L-arginine enhances wound healing in diabetic rats, Wound Repair Regen. 11 (3) (2003) 198–203.
[71] Shabani, Pulfer, Bulgrin, Smith, Enhancement of wound repair with a topically applied nitric oxide-releasing polymer, Wound Repair Regen. 4 (3) (1996) 353–362.
[72] Reichner, Meszaros, Louis, Henry, Mastrofrancesco, Martin, Albina, Molecular and metabolic evidence for the restricted expression of inducible nitric oxide synthase in healing wounds, Am. J. Pathol 154 (4) (1999) 1097–1104.
[73] Shi, Efron, Most, Tantry and Barbul, Supplemental dietary arginine enhances wound healing in normal but not inducible nitric oxide synthase knockout mice. 2000. 128(2): p. 374-378.
[74] Coneski, Schoenfisch, Nitric oxide release: part III. Measurement and reporting, Chem. Soc. Rev. 41 (10) (2012) 3753–3758.
[75] Vaughn, Kuo, Liao and Physiology, Estimation of nitric oxide production and reactionrates in tissue by use of a mathematical model. 1998. 274(6): p. H2163-H2176.
[76] Zhang, Chen, Li, Feng, Nie, Wei, Li, Han, Han, Kong, Guo, Zhao, Li, A nitric oxide-releasing hydrogel for enhancing the therapeutic effects of mesenchymal stem cell therapy for hindlimb ischemia, Acta Biomater. 113 (2020) 289–304.
[77] Chu, Lyu, Wang, Jin, Lu, Xing, Hu, Cocktail polyprodrug nanoparticles concurrently release cisplatin and peroxynitrite-generating nitric oxide in cisplatin-resistant cancers, Chem. Eng. J. 402 (2020), 126125.
[78] Gheibi, Samsonov, Gheibi, Vazquez, Kashfi, Regulation of carbohydrate metabolism by nitric oxide and hydrogen sulfide: Implications in diabetes, Biochem. Pharmacol. 176 (2020), 113819.
[79] Hakim, Sugimori, Camporesi, Anderson, Half-life of nitric oxide in aqueous solutions with and without haemoglobin, Physiol. Meas. 17 (4) (1996) 267–277.
[80] Thomas, Liu, Kantrow and Lancaster, The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O2. 2001. 98(1): p. 355-360.
[81] Liu, Samouilov, Lancaster Jr and Zweier, Nitric oxide uptake by erythrocytes is primarily limited by extracellular diffusion not membrane resistance. 2002. 277 (29): p. 26194-26199.
[82] Navati, Chung, Friedman, Trehalose-based glassy matrices as an effective tool to trap short-lived intermediates in the nitric oxide dioxygenation (NOD) reaction of hemoglobin, J. Phys. Chem. B 120 (20) (2016) 4529–4539.
[83] Massion, Feron, Dessy, Balligand, Nitric oxide and cardiac function: ten years after, and continuing, Circ. Res. 93 (5) (2003) 388–398.
[84] Gifford, Batchelor, Lee, Gokulrangan, Meyerhoff, Wilson, Mediation ofin vivo glucose sensor inflammatory response via nitric oxide release, Biomater. Biomater. 75 (4) (2005) 755–766.
[85] Hill, Dranka, Bailey, Lancaster Jr and Darley-Usmar, What part of NO don't you understand? Some answers to the cardinal questions in nitric oxide biology. 2010. 285(26): p. 19699-19704.
[86] Major, Brant, Reynolds, Bartlett, Meyerhoff, Handa, Annich, The attenuation of platelet and monocyte activation in a rabbit model of extracorporeal circulation by a nitric oxide releasing polymer, Biomaterials 31 (10) (2010) 2736–2745.
[87] Wo, Brisbois, Bartlett, Meyerhoff, Recent advances in thromboresistant and antimicrobial polymers for biomedical applications: just say yes to nitric oxide (NO), Biomater. Sci. 4 (8) (2016) 1161–1183.
[88] Isenberg, Ridnour, Espey, Wink, Roberts, Nitric oxide in wound-healing, Microsurgery 25 (5) (2005) 442–451.
[89] VanWagner, Rhadigan, Lancina, Lebovsky, Romanowicz, Holmes, Brunette, Snyder, Bostwick, Lee, Frost, Rajachar, S-nitroso-N-acetylpenicillamine (SNAP) derivatization of peptide primary amines to create inducible nitric oxide donor biomaterials, ACS Appl Mater Interfaces 5 (17) (2013) 8430–8439.
[90] Li, Lee, Controlled nitric oxide delivery platform based on S-nitrosothiol conjugated interpolymer complexes for diabetic wound healing, Mol. Pharm. 7 (1) (2010) 254–266.
[91] Schairer, Chouake, Nosanchuk, Friedman, The potential of nitric oxide releasing therapies as antimicrobial agents, Virulence 3 (3) (2012) 271–279.
[92] Kumar, Singh, Bhardwaj, Therapeutic role of nitric oxide as emerging molecule, Biomed. Pharmacother. 85 (2017) 182–201.
[93] Akhtar, Chandrasekhar, Yousif, Renno, Benter, El-Hashim, Chronic administration of nano-sized PAMAM dendrimers in vivo inhibits EGFR-ERK1/2-ROCK signaling pathway and attenuates diabetes-induced vascular remodeling and dysfunction, Nanomedicine 18 (2019) 78–89.
[94] Reynolds, Frost, Meyerhoff, Nitric oxide-releasing hydrophobic polymers: preparation, characterization, and potential biomedical applications, Free Radic. Biol. Med. 37 (7) (2004) 926–936.
[95] Frost, Reynolds, Meyerhoff, Polymers incorporating nitric oxide releasing/generating substances for improved biocompatibility of blood-contacting medical devices, Biomaterials 26 (14) (2005) 1685–1693.
[96] Damodaran, Reynolds, Biodegradable S-nitrosothiol tethered multiblock polymer for nitric oxide delivery, J. Mater. Chem. 21 (16) (2011) 5870–5872.
[97] Mihu, Sandkovsky, Han, Friedman, Nosanchuk and Martinez, The use of nitric oxide releasing nanoparticles as a treatment against Acinetobacter baumannii in wound infections. 2010. 1(2): p. 62-67.
[98] Kim, Lee, Singha, Kim, Shin, Jo, Han and Kim, NONOates–polyethylenimine hydrogel for controlled nitric oxide release and cell proliferation modulation. 2011. 22(6): p. 1031-1038.
[99] Sood, Granick, Tomaselli, Wound dressings and comparative effectiveness data, Adv. Wound Care 3 (8) (2014) 511–529.
[100] Mogosanu, Grumezescu, Natural and synthetic polymers for wounds and burns dressing, Int. J. Pharm. 463 (2) (2014) 127–136.
[101] Saghazadeh, Rinoldi, Schot, Kashaf, Sharifi, Jalilian, Nuutila, Giatsidis, Mostafalu, Derakhshandeh, Yue, Swieszkowski, Memic, Tamayol, Khademhosseini, Drug delivery systems and materials for wound healing applications, Adv. Drug Deliv. Rev. 127 (2018) 138–166.
[102] Mihai, Dima, Dima, Holban, Nanomaterials for wound healing and infection control, Materials 12 (13) (2019) 2176.
[103] Mir, Ali, Barakullah, Gulzar, Arshad, Fatima, Asad, Synthetic polymeric biomaterials for wound healing: a review, Prog. Biomater. 7 (1) (2018) 1–21.
[104] Calo, Khutoryanskiy, Biomedical applications of hydrogels: a review of patents and commercial products, Eur. Polym. J. 65 (2015) 252–267.
[105] Masood, Ahmed, Tariq, Ahmed, Masoud, Ali, Asghar, Andleeb, Hasan, Silver nanoparticle impregnated chitosan-PEG hydrogel enhances wound healing in diabetes induced rabbits, Int. J. Pharm. 559 (2019) 23–36.
[106] Ruseva, Nedkov, Alexandrova, Dinev, Shestakova, Hristov, Vassileva, Polyzwitterionic hydrogels as wound dressings with enzymatic debridement functionality for highly exuding wounds. Polym. Int. 68 (9) (2019) 1626–1635.
[107] Kamoun, Kenawy, Chen, A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings, J. Adv. Res. 8 (3) (2017) 217–233.
[108] El-Sherbiny, Yacoub, Hydrogel scaffolds for tissue engineering: progress and challenges, Glob. Cardiol. Sci. Pract. 2013 (3) (2013) 316–342.
[109] Champeau, Povoa, Militao, Cabrini, Picheth, Meneau, Jara, de Araujo, de Oliveira, Supramolecular poly(acrylic acid)/F127 hydrogel with hydration-controlled nitric oxide release for enhancing wound healing, Acta Biomater. 74 (2018) 312–325.
[110] Zhou Wang Zhang Li Wu Wei Ji Kong Zhao. Functional poly (ε-caprolactone)/chitosan dressings with nitric oxide-releasing property improve wound healing. 54 2017 128 137.
[111] Zhang, Tang, Chen, Zhou, Li, Liu, Yang, Lin, Zhang, He, A polyethylenimine-based diazeniumdiolate nitric oxide donor accelerates wound healing, Biomater. Sci. 7 (4) (2019) 1607–1616.
[112] Du, Zhang, Zhang, Wang, Nie, Tao, Han, Liang, Wang, Liu, Liu, Han, Kong, Zhao, Li, Enhanced proangiogenic potential of mesenchymal stem cell-derived exosomes stimulated by a nitric oxide releasing polymer, Biomaterials 133 (2017) 70–81.
[113] Lee, Hlaing, Cao, Hasan, Ahn, Song, Yoo, In situ hydrogel-forming/nitric oxide-releasing wound dressing for enhanced antibacterial activity and healing in mice with infected wounds, Pharmaceutics 11 (10) (2019) 496.
[114] Ghalei, Li, Douglass, Garren, Handa, Synergistic approach to develop antibacterial electrospun scaffolds using honey and s-nitroso-n-acetyl penicillamine, ACS Biomater. Sci. Eng. 7 (2) (2021) 517–526.
[115] Augustine, Rehman, Ahmed, Zahid, Sharifi, Falahati, Hasan, Electrospun chitosan membranes containing bioactive and therapeutic agents for enhanced wound healing, Int. J. Biol. Macromol. 156 (2020) 153–170.
[116] Barman, Mahmood, Augustine, Hasan, Thomas, Ghosal, Natural halloysite nanotubes/chitosan based bio-nanocomposite for delivering norfloxacin, an anti-microbial agent in sustained release manner, Int. J. Biol. Macromol. 162 (2020) 1849–1861.
[117] Choi, Hasan, Cao, Lee, Hlaing, Yoo, Chitosan-based nitric oxide-releasing dressing for anti-biofilm and in vivo healing activities in MRSA biofilm-infected wounds, Int. J. Biol. Macromol. 142 (2020) 680–692.
[118] Lowe, Bills, Verma, Lavery, Davis, Balkus, Electrospun nitric oxide releasing bandage with enhanced wound healing, Acta Biomater. 13 (2015) 121–130.
[119] Zhao, Zhang, Song, Ji, Yao, Cui, Shen, Wang, Kong, Polysaccharide-based biomaterials with on-demand nitric oxide releasing property regulated by enzyme catalysis, Biomaterials 34 (33) (2013) 8450–8458.
[120] Tibbitt, Anseth, Hydrogels as extracellular matrix mimics for 3d cell culture, Biotechnol. Bioeng. 103 (4) (2009) 655–663.
[121] Catoira, Fusaro, Di Francesco, Ramella, Boccafoschi, Overview of natural hydrogels for regenerative medicine applications, J. Mater. Sci. Mater. Med. 30 (10) (2019) 115.
[122] Rivero, Capella, Liaudat, Bosch, Barbero, Rodriguez, Rivarola, Mechanical and physicochemical behavior of a 3D hydrogel scaffold during cell growth and proliferation, RSC Adv. 10 (10) (2020) 5827–5837.
[123] Li, Mooney, Designing hydrogels for controlled drug delivery, Nat. Rev. Mater. 1 (12) (2016) 1–17.
[124] Hunt, Chen, van Veen, Bryan, Hydrogels for tissue engineering and regenerative medicine, J. Mater. Chem. B 2 (33) (2014) 5319–5338.
[125] Wu, Li, Lee, Chitosan-based composite hydrogels for biomedical applications, Macromol. Res. 25 (6) (2017) 480–488.
[126] Ahsan, Thomas, Reddy, Sooraparaju, Asthana, Bhatnagar, Chitosan as biomaterial in drug delivery and tissue engineering, Int. J. Biol. Macromol. 110 (2018) 97–109.
[127] Ahmed, Tariq, Ali, Asghar, Noorunnisa Khanam, Augustine, Hasan, Novel electrospun chitosan/polyvinyl alcohol/zinc oxide nanofibrous mats with antibacterial and antioxidant properties for diabetic wound healing, Int. J. Biol. Macromol. 120 (Pt A) (2018) 385–393.

[128] Ma, Cheng, Jian, Feng, Chang, Zheng, Wu, Wang, Li, Zhang, Hollow, rough, and nitric oxide-releasing cerium oxide nanoparticles for promoting multiple stages of wound healing, Adv. Healthc. Mater 8 (16) (2019), e1900256.
[129] Casey and Protection, Investigation of chitosan-PVA composite films and their adsorption properties. 2015. 3(02): p. 78.
[130] Kenawy, Kamoun, Eldin, El-Meligy, Physically crosslinked poly(vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: Synthesis and characterization for biomedical applications, Arab. J. Chem. 7 (3) (2014) 372–380.
[131] Jen, Serrano, van Lith, Ameer, Polymer-based nitric oxide therapies: recent insights for biomedical applications, Adv. Funct. Mater. 22 (2) (2012) 239–260.
[132] Riccio, Schoenfisch, Nitric oxide release: part I. Macromolecular scaffolds, Chem. Soc. Rev. 41 (10) (2012) 3731–3741.
[133] Divakaran, Loscalzo, The role of nitroglycerin and other nitrogen oxides in cardiovascular therapeutics, J. Am. Coll. Cardiol. 70 (19) (2017) 2393–2410.
[134] Noonan, Benet, The bioavailability of oral nitroglycerin, J. Pharm. Sci. 75 (3) (1986) 241–243.
[135] Grossi, D'Angelo, Sodium nitroprusside: mechanism of NO release mediated by sulfhydryl-containing molecules, J. Med. Chem. 48 (7) (2005) 2622–2626.
[136] Seabra, Duran, Nitric oxide-releasing vehicles for biomedical applications, J. Mater. Chem. 20 (9) (2010) 1624–1637.
[137] Nichols, Storm, Koh, Schoenfisch, Local delivery of nitric oxide: targeted delivery of therapeutics to bone and connective tissues, Adv. Drug Deliv. Rev. 64 (12) (2012) 1177–1188.
[138] Xiang, Guo and Liu, Transition-metal nitrosyls for photocontrolled nitric oxide delivery. 2017. 2017(12): p. 1586-1595.
[139] Wang, Xian, Tang, Wu, Wen, Cai, Janczuk, Nitric oxide donors: chemical activities and biological applications, Chem. Rev. 102 (4) (2002) 1091–1134.
[140] Giustarini, Milzani, Colombo, Dalle-Donne, Rossi, Nitric oxide and S-nitrosothiols in human blood, Clin. Chim. Acta 330 (1–2) (2003) 85–98.
[141] Hrabie and Keefer, Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. 2002. 102(4): p. 1135-1154.
[142] Wan, Gao, Li, Preparation and characterization of diazeniumdiolate releasing ethylcellulose films, J. Mater. Sci. Mater. Med. 20 (1) (2009) 321–327.
[143] Hong, Kim, Na, Park, Kim, Singha, Im, Han, Kim, Lee, Poly(norepinephrine): ultrasmooth material-independent surface chemistry and nanodepot for nitric oxide, Angew. Chem. Int. Ed. Engl. (2013) 9187–9191.
[144] Batchelor, Reoma, Fleser, Nuthakki, Callahan, Shanley, Politis, Elmore, Merz, Meyerhoff, More lipophilic dialkyldiamine-based diazeniumdiolates: synthesis, characterization, and application in preparing thromboresistant nitric oxide release polymeric coatings, J. Med. Chem. 46 (24) (2003) 5153–5161.
[145] Stamler, Simon, Osborne, Mullins, Jaraki, Michel, Singel, Loscalzo, S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds, Proc. Natl. Acad. Sci. USA 89 (1) (1992) 444–448.
[146] Vercelino, Cunha, Ferreira, Cunha, Ferreira, de Oliveira, Skin vasodilation and analgesic effect of a topical nitric oxide-releasing hydrogel, J. Mater. Sci. Mater. Med. 24 (9) (2013) 2157–2169.
[147] Schanuel, Raggio Santos, Monte-Alto-Costa, de Oliveira, Combined nitric oxide-releasing poly(vinyl alcohol) film/F127 hydrogel for accelerating wound healing, Colloids Surf B Biointerfaces 130 (2015) 182–191.
[148] Zahid, Ahmed, Ur Rehman, Augustine and Hasan. Reactive nitrogen species releasing hydrogel for enhanced wound healing. in 2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2019. IEEE.
[149] Kosutova, Mikolka, Kolomaznik, Balentova, Calkovska, Mokra, Effects of S-nitroso-N-acetyl-penicillamine (SNAP) on inflammation, lung tissue apoptosis and iNOS activity in a rabbit model of acute lung injury. Pulmonary Infection and Inflammation, Springer, 2016, pp. 13–23.
[150] Tsihlis, Kapadia, Vavra, Jiang, Fu, Martinez, Kibbe, Nitric oxide decreases activity and levels of the 11S proteasome activator PA28 in the vasculature, Nitric Oxide 27 (1) (2012) 50–58.
[151] Cariello, de Souza, Foronda, Yu, Hofling-Lima, de Oliveira, In vitro amoebicidal activity of S-nitrosoglutathione and S-nitroso-N-acetylcysteine against trophozoites of Acanthamoeba castellanii, J. Antimicrob. Chemother. 65 (3) (2010) 588–591.
[152] Lopez-Jaramillo, Ruano, Rivera, Teran and Salazar-Irigoyen, Treatment of cutaneous leishmanlasis with nitric-oxide donor. 1998. 351(9110): p. 1176-1177.
[153] Williams, The chemistry of S-nitrosothiols, Acc. Chem. Res. 32 (10) (1999) 869–876.
[154] Brasted, Reaction of sodium nitrite and sulfamic acid - indirect gravimetric determination of nitrites, Anal. Chem. 24 (7) (1952) 1111–1114.
[155] Dave, Joshi, Venugopalan, Biomedical evaluation of a novel nitrogen oxides releasing wound dressing, J. Mater. Sci. Mater. Med. 23 (12) (2012) 3097–3106.
[156] Zhu, Ka, Murad, Nitric oxide accelerates the recovery from burn wounds, World J Surg 31 (4) (2007) 624–631.
[157] Zhu, Wei, Bian, Murad, Effects of nitric oxide on skin burn wound healing, J. Burn Care Res. 29 (5) (2008) 804–814.
[158] Weller, Finnen, The effects of topical treatment with acidified nitrite on wound healing in normal and diabetic mice, Nitric Oxide 15 (4) (2006) 395–399.
[159] Ormerod, Shah, Li, Benjamin, Ferguson, Leifert, An observational prospective study of topical acidified nitrite for killing methicillin-resistant Staphylococcus aureus (MRSA) in contaminated wounds, BMC Res. Notes 4 (1) (2011) 458.
[160] Tentolouris, Petrikkos, Vallianou, Zachos, Daikos, Tsapogas, Markou, Katsilambros, Prevalence of methicillin-resistant Staphylococcus aureus in infected and uninfected diabetic foot ulcers, Clin. Microbiol. Infect. 12 (2) (2006) 186–189.
[161] Yang, Schoenfisch, Nitric oxide-releasing hyperbranched polyaminoglycosides for antibacterial therapy, ACS Appl. Bio Mater. 1 (4) (2018) 1066–1073.
[162] Friedman, Han, Navati, Chacko, Gunther, Alfieri, Friedman, Sustained release nitric oxide releasing nanoparticles: characterization of a novel delivery platform based on nitrite containing hydrogel/glass composites, Nitric Oxide 19 (1) (2008) 12–20.
[163] Blecher, Martinez, Tuckman-Vernon, Nacharaju, Schairer, Chouake, Friedman, Alfieri, Guha, Nosanchuk, Friedman, Nitric oxide-releasing nanoparticles accelerate wound healing in NOD-SCID mice, Nanomedicine 8 (8) (2012) 1364–1371.
[164] Han Nguyen Macherla Chi Friedman Nosanchuk Martinez. Nitric oxide–releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition 180 4 2012 1465 1473.
[165] Ormerod, van Voorst Vader, Majewski, Vanscheidt, Benjamin, van der Meijden, Evaluation of the efficacy, safety, and tolerability of 3 dose regimens of topical sodium nitrite with citric acid in patients with anogenital warts: a randomized clinical trial, JAMA Dermatol. 151 (8) (2015) 854–861.
[166] Vogt, Xing, He, Li, Frost, Zhao, Fabrication and characterization of a nitric oxide-releasing nanofibrous gelatin matrix, Biomacromolecules 14 (8) (2013) 2521–2530.
[167] Murphy, Nitric oxide and cell death, Biochim. Biophys. Acta 1411 (2-3) (1999) 401–414.
[168] Kim, Noh, Thapa, Hasan, Choi, Kim, Lee, Ku, Yoo, Nitric oxide-releasing chitosan film for enhanced antibacterial and in vivo wound-healing efficacy, Int. J. Biol. Macromol. 79 (2015) 217–225.
[169] Sudarshan, Hoover, Knorr, Antibacterial action of chitosan, Food Biotechnol. 6 (3) (1992) 257–272.
[170] Singh, Shitiz, Singh, Chitin and chitosan: biopolymers for wound management, Int. Wound J. 14 (6) (2017) 1276–1289.
[171] Koehler, Brandl, Goepferich, Hydrogel wound dressings for bioactive treatment of acute and chronic wounds, Eur. Polym. J. 100 (2018) 1–11.
[172] Shishido, Seabra, Loh, Ganzarolli de Oliveira, Thermal and photochemical nitric oxide release from S-nitrosothiols incorporated in Pluronic F127 gel: potential uses for local and controlled nitric oxide release, Biomaterials 24 (20) (2003) 3543–3553.
[173] Seabra, Fitzpatrick, Paul, De Oliveira and Weller, Topically applied S-nitrosothiol-containing hydrogels as experimental and pharmacological nitric oxide donors in human skin. 2004. 151(5): p. 977-983.
[174] Amadeu, Seabra, de Oliveira, Monte-Alto-Costa, Nitric oxide donor improves healing if applied on inflammatory and proliferative phase, J. Surg. Res. 149 (1) (2008) 84–93.
[175] Ahmed, Afreen, Tariq, Zahid, Masoud, Ahmed, Ali, Akram, Hasan, Bone marrow esenchymal stem cells preconditioned with nitric-oxide-releasing chitosan/PVA hydrogel accelerate diabetic wound healing in rabbits, Biomed. Mater. 16 (3) (2021), 035014.
[176] Seifter, Rettura, Barbul, Levenson, Arginine: an essential amino acid for injured rats, Surgery 84 (2) (1978) 224–230.
[177] Witte, Kiyama, Barbul, Nitric oxide enhances experimental wound healing in diabetes, Br. J. Surg. 89 (12) (2002) 1594–1601.
[178] Armstrong, Hanft, Driver, Smith, Lazaro-Martinez, Reyzelman, Furst, Vayser, Cervantes, Snyder, Moore, May, Nelson, Baggs, Voss, Diabetic Foot Nutrition Study, Effect of oral nutritional supplementation on wound healing in diabetic foot ulcers: a prospective randomized controlled trial, Diabet. Med. 31 (9) (2014) 1069–1077.
[179] Shearer, Richards, Mills, Caldwell, Differential regulation of macrophage arginine metabolism: a proposed role in wound healing, Am. J. Physiol. 272 (2 Pt 1) (1997) E181–E190.
[180] Heffernan, Dudley, McNeil, Howdieshell, Local arginine supplementation results in sustained wound nitric oxide production and reductions in vascular endothelial growth factor expression and granulation tissue formation, J. Surg. Res. 133 (1) (2006) 46–54.
[181] Liu, Zhou, Gao, Zhai, Electrospun nanofibers as a wound dressing for treating diabetic foot ulcer, Asian J. Pharm. Sci. 14 (2) (2019) 130–143.
[182] Strobel, Muller, Zolk, Endress, Konig, Fromm, Maas, Transport of asymmetric dimethylarginine (ADMA) by cationic amino acid transporter 2 (CAT2), organic cation transporter 2 (OCT2) and multidrug and toxin extrusion protein 1 (MATE1), Amino Acids 45 (4) (2013) 989–1002.
[183] Liu, Li, Chen, He, Ma, Ma, Zhao, Tian, Relationship between Serum Asymmetric Dimethylarginine Level and Microvascular Complications in Diabetes Mellitus: A Meta-Analysis, 2019, Biomed Research International, 2019.
[184] Dou, Wang, Jin, Li, Wang, Yuan, Shen, PCL/sulfonated keratin mats for vascular tissue engineering scaffold with potential of catalytic nitric oxide generation, Mater. Sci. Eng. C Mater. Biol. Appl. 107 (2020), 110246.
[185] Wan, Liu, Xin, Li, Dou, Han, Kang, Yuan, Chi, Shen, S-nitrosated keratin composite mats with NO release capacity for wound healing, Chem. Eng. J. 400 (2020), 125964.
[186] Batinic-Haberle, Reboucas, Spasojevic, Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential, Antioxid. Redox Signal. 13 (6) (2010) 877–918.
[187] Sivitz, Yorek, Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities, Antioxid. Redox Signal. 12 (4) (2010) 537–577.
[188] Yang, Zelikin, Chandrawati, Progress and promise of nitric oxide-releasing platforms, Adv. Sci. 5 (6) (2018) 1701043.
[189] Murohara, Asahara, Nitric oxide and angiogenesis in cardiovascular disease, Antioxid. Redox Signal. 4 (5) (2002) 825–831.

[190] Papapetropoulos, Garcia-Cardena, Madri, Sessa, Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells, J. Clin. Investig. 100 (12) (1997) 3131–3139.
[191] Gronbach, Mitrach, Lidzba, Muller, Moller, Rother, Salbach-Hirsch, Hofbauer, Schnabelrauch, Hintze, Hacker, Schulz-Siegmund, Scavenging of Dickkopf-1 by macromer-based biomaterials covalently decorated with sulfated hyaluronan displays pro-osteogenic effects, Acta Biomater. 114 (2020) 76–89.
[192] Albina, Mills, Henry, Caldwell, Temporal expression of different pathways of 1-arginine metabolism in healing wounds, J. Immunol. 144 (10) (1990) 3877–3880.
[193] Schaffer, Tantry, Gross, Wasserkrug, Barbul, Nitric oxide regulates wound healing, J. Surg. Res. 63 (1) (1996) 237–240.
[194] Yamasaki, Edington, McClosky, Tzeng, Lizonova, Kovesdi, Steed, Billiar, Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer, J. Clin. Investig. 101 (5) (1998) 967–971.
[195] Sharma, Singh, Warren, Thangapazham, Maheshwari, Differential regulation of angiogenic genes in diabetic wound healing, J. Investig. Dermatol 126 (10) (2006) 2323–2331.
[196] Frank, Stallmeyer, Kampfer, Kolb, Pfeilschifter, Nitric oxide triggers enhanced induction of vascular endothelial growth factor expression in cultured keratinocytes (HaCaT) and during cutaneous wound repair, FASEB J. 13 (14) (1999) 2002–2014.
[197] Lee, A.N. Salyapongse, G.A. Bragdon, L.L. Shears, S.C. Watkins, H.D. Edington, T. R. Billiar, Impaired wound healing and angiogenesis in eNOS-deficient mice, Am. J. Physiol. Heart Circ. Physiol. 277 (1999) H1600–H1608.
[198] Stallmeyer, Anhold, Wetzler, Kahlina, Pfeilschifter, Frank, Regulation of eNOS in normal and diabetes-impaired skin repair: implications for tissue regeneration, Nitric Oxide 6 (2) (2002) 168–177.
[199] Li, Liu, Yang, Qiu, Lu, Tu, Xiong, Huang, Yang, Mussel-inspired “built-up” surface chemistry for combining nitric oxide catalytic and vascular cell selective properties. 241 (2020), 119904.
[200] Nieder, Benbi, Reactive nitrogen compounds and their influence on human health: an overview, Rev. Environ. Health (2021).
[201] Covello, Merkhoher, Risk Assessment Methods: Approaches for Assessing Health and Environmental Risks, Springer Science & Business Media, 1993.
[202] Skalska, Miller, Ledakowicz, Trends in NOx abatement: a review, Sci. Total Environ. 408 (19) (2010) 3976–3989.
[203] Kolios, Striepling, Kolios, Rudolf, Dresing, Dorges, Sturmer, Sturmer, The nitric acid burn trauma of the skin, J. Plast. Reconstr. Aesthet. Surg. 63 (4) (2010) e358–e363.
[204] Sinisterra, Miravet, Alfonso, Soliz, Papazian, Methemoglobinemia in an infant receiving nitric oxide after the use of eutectic mixture of local anesthetic, J. Pediatr. 141 (2) (2002) 285–286.
[205] Fan, Steinberg, Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity, Regul. Toxicol. Pharmacol. 23 (1 Pt 1) (1996) 35–43.
[206] Pandey, Singh, Nitrogen dioxide: risk assessment, environmental, and health hazard. Hazardous Gases, Elsevier, 2021, pp. 273–288.
[207] Silva, Rueda, Marquez, Lopez, Smith, Calderon, Castillo, Matute, Rueda-Clausen, Orduz, Silva, Kampeerapappun, Bhide, Lopez-Jaramillo, Double blind, randomized, placebo controlled clinical trial for the treatment of diabetic foot ulcers, using a nitric oxide releasing patch: PATHON, Trials 8 (1) (2007) 1–8.
[208] Zahid, Augustine, Dalvi, Reshma, Ahmed, Raza Ur Rehman, Marei, Alfkey, Hasan, Development of nitric oxide releasing visible light crosslinked gelatin methacrylate hydrogel for rapid closure of diabetic wounds, Biomed. Pharmacother. 140 (2021), 111747.
[209] Lee, Lee, Lee, Lee, Heo, Osteogenic effect of inducible nitric oxide synthase (iNOS)-loaded mineralized nanoparticles on embryonic stem cells, Cell. Physiol. Biochem. 51 (2) (2018) 746–762.
[210] Augustine, Nethi, Kalarikkal, Thomas, Patra, Electrospun polycaprolactone (PCL) scaffolds embedded with europium hydroxide nanorods (EHNs) with enhanced vascularization and cell proliferation for tissue engineering applications, J. Mater. Chem. B 5 (24) (2017) 4660–4672.
[211] Liu, Li, Wang, Sun, Wang, Wang, Sun, Enhancement of lipopolysaccharide-induced nitric oxide and interleukin-6 production by PEGylated gold nanoparticles in RAW264.7 cells, Nanoscale 4 (22) (2012) 7135–7142.
[212] Arancibia, Barrientos, Torrejón, Escobar, Beltrán, Copper oxide nanoparticles recruit macrophages and modulate nitric oxide, proinflammatory cytokines and PGE2 production through arginase activation, Nanomedicine 11 (10) (2016) 1237–1251.
[213] Grosse, Stenvik, Nilsen, Iron oxide nanoparticles modulate lipopolysaccharide-induced inflammatory responses in primary human monocytes, Int. J. Nanomed. 11 (2016) 4625–4642.
[214] Kheraldine, Rachid, Habib, Al Moustafa, Benter, Akhtar, Emerging innate biological properties of nano-drug delivery systems: a focus on PAMAM dendrimers and their clinical potential, Adv. Drug Deliv. Rev. 178 (2021), 113908.
[215] Keeble, Moore, Pharmacology and potential therapeutic applications of nitric oxide-releasing non-steroidal anti-inflammatory and related nitric oxide-donating drugs, Br. J. Pharmacol. 137 (3) (2002) 295–310.
[216] McCrudden, McBride, McCaffrey, Ali, Dunne, Kett, Coulter, Robson, McCarthy, Systemic RALA/iNOS nanoparticles: a potent gene therapy for metastatic breast cancer coupled as a biomarker of treatment, Mol. Therapy-Nucleic Acids 6 (2017) 249–258.