CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION

In the course of daily activities, individuals are prone to various wounds and injuries, ranging from minor damage to tissue as well as organ loss, where skin cells necessitating simple treatment and time for recovery while in case of tissue or organ loss specific regeneration assistive technology will be applied. further, wounds may commonly manifests as superficial injuries confined toouter skin layers, while some wound extend deeper, affecting underlying tissues and organs, with severity varying from minor to life-threatening. Therefore, wound care is a critical facet of global healthcare, encompassing a spectrum of injuries from minor cuts to chronic ulcers [1].

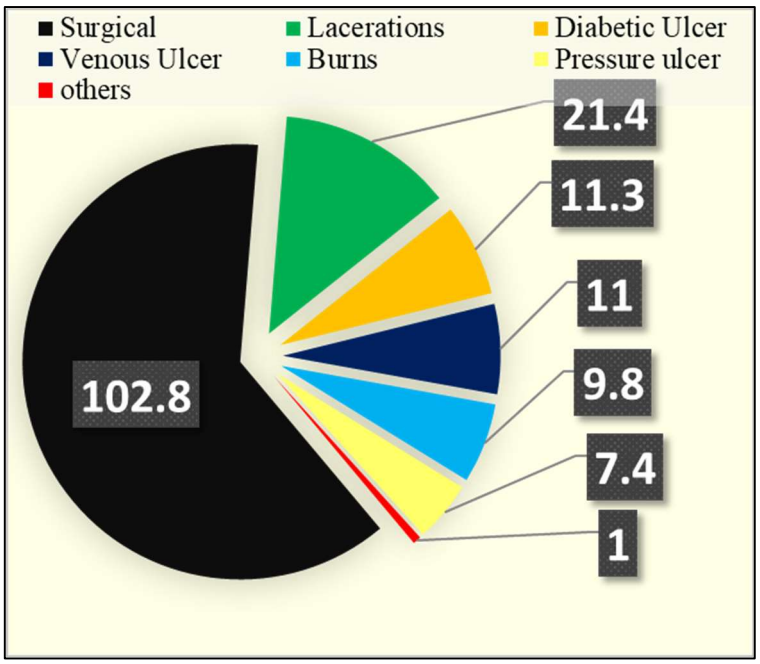

Figure 1.1: Worldwide wound statistics (in millions)[2].

The global impact of wounds and injuries on health care system is profound, with an estimated 2.5 million people experiencing chronic wounds worldwide [1] and recent report (Figure 1.1) shades light on the categorization and estimation of the number of patients suffering from various types of wounds worldwide. This necessitates the demand for effective strategies in the treatment and management of wound to prevent

complications and to enhance overall well-being. From the World Health Organization (WHO) perspective, proper wound care is fundamental to achieving universal health coverage. The WHO underscores the importance of accessible and affordable wound care services, emphasizing their significant contribution to reducing the burden of disease and improving health outcomes [3].

Although all people are vulnerable to injury, certain groups face a greater risk such as children, the elderly, alcoholics, individuals with narcotic addiction, those with mental illness or disability, and individuals in hazardous or high-risk occupations. Patients with chronic illnesses or weakened immune systems and those in low-resource settings are at an elevated risk of developing wounds. Studies indicate that up to 9.7% of the global population may suffer from chronic wounds, with diabetic foot ulcers affecting approximately 6.3% of individuals with diabetes [1, 4]. It is imperative to tailor wound care strategies to address the specific needs of these populations, as highlighted by the WHO's global health agenda [5]. In alignment with the global burden of wounds, the WHO places particular emphasis on education and awareness campaigns to promote preventive measures. Adhering to optimal safety measures, especially in unfamiliar environments, is paramount for preventing various types of wounds. Vigilance when handling sharp objects or hazardous materials is essential to minimize the risk of injury. Apart from above, simple practices like proper hygiene, timely wound cleaning, and the use of appropriate dressings are underscored as crucial elements in reducing infections and complications, contributing to the overall improvement of wound care outcomes [6].

1.2 ORIGINS AND CATEGORIES OF WOUNDS

Wounds are originates from numerous events and can be categorized in many ways. An example is acute and chronic wounds, which are distinguished by the length of time takes to heal. Acute wounds heal normally and without complications within the anticipated

amount of time, while, chronic wounds take longer to heal and may cause difficulties [7]. In addition to above, wounds can also be categorized as closed or open. Closed wounds don't reveal underlying tissues, like non-penetrating injuries, whereas open wounds, especially penetrating wounds, expose underlying tissues and organs to the outside world [8]. An alternative classification assesses wounds for cleanliness. Clean wounds lack foreign materials, contrasting with contaminated or infected wounds, which may harbour dirt, causative agents, bacteria, or other foreign substances [7, 8].

In terms of where they originate, wounds can be either internal or exterior. Internal wounds are frequently linked to chronic medical problems and are caused by compromised immune and nervous system processes as well as decreased blood, oxygen, or nutrition delivery. A number of factors, such as both piercing and non-penetrating trauma, can create external wounds. Non-penetrating wounds include abrasions, lacerations, contusions, and concussions. They are frequently caused by blunt trauma or friction. Penetrating wounds, such as gunshot, surgical, and stabbing wounds, occur when trauma penetrates the whole thickness of the skin. Thermal, chemical, electrical, and bite/sting wounds are examples of miscellaneous wounds [7, 8]. Wounds can appear with many signs and symptoms, such as discomfort, swelling, redness, bleeding, and functional impairment, which vary depending on the site, depth, and causing agent. Infections, inflammation, pyrexia, scarring, and total loss of function are a few possible complications.

Chronic wounds, including pressure ulcers and diabetic foot ulcers, pose significant challenges on a global scale. The WHO advocates different treatment strategies for different types of wounds which are the collaborative efforts among healthcare professionals, policymakers, and communities to develop comprehensive wound care programs that are specifically tailored to the unique needs of individuals grappling with

chronic wounds. Such an approach aligns with the broader objective of achieving equitable healthcare access [9]. Recognizing the transformative potential of technology in healthcare, the WHO highlights innovations such as telemedicine and mobile health applications. These technological advancements hold the promise of enhancing access to wound care expertise, particularly in underserved areas where resources may be limited. The integration of technology into wound care aligns with the WHO's commitment to leveraging innovation for improved healthcare delivery [10].

Wound care extends beyond the treatment of existing wounds to encompass preventive measures. The National Wound Care Center (NWCC) advocates for a holistic approach that includes lifestyle modifications, regular screenings, and early intervention to minimize the impact of wounds on individuals and communities. This approach also resembles to the WHO's comprehensive understanding of healthcare, considering not only treatment but also prevention and health promotion [11, 12]. Health disparities in wound care outcomes highlight the need for a comprehensive approach. The WHO emphasizes considering social determinants, cultural factors, and economic conditions while developing strategies to address the disparities. Adopting a holistic perspective helps healthcare systems reduce inequities in wound care globally [13, 14].

Research and development in wound care play a pivotal role in the WHO's strategy to make it real. Continuous efforts to enhance therapeutic interventions, promote evidence-based practices, and develop cost-effective solutions are crucial for improving outcomes and reducing the overall burden of wounds. This research-focused approach aligns with the WHO's commitment to advancing healthcare through innovation and evidence-based practices [15, 16]. In conclusion, addressing the global challenge of wound care requires a concerted effort to prioritize it within healthcare systems. By aligning policies, fostering innovation, and ensuring equitable access to quality services, significant strides can be

made in improving the well-being of populations affected by wounds worldwide.

1.3 WOUND HEALING (WH) PROCESS

Skin is the biggest organ of the human body, which acts as a chemical,physical as well as mechanical barrier to shield the body from germs, radiations, and mechanical rips [17]. The epidermis, dermis, and subcutaneous layer make up the layers of skin. These layers are in dynamic equilibrium, constantly regenerating skin organ and contains different types of cells. The skin's uppermost or outer layer is called the epidermis which protects underlaying layers from UV damage and infections, dermis is the supportive layer of skin, made of fibrous connective tissue and collagen fibers and its deepest layer is called the subcutis layer or hypodermis, connects the skin to the fascia of bones and muscles.

Keratinocytes make up the majority of the epidermis, which is further divided into layers based on the maturity stage of the cells. The loss of the keratinocytes' nucleus, known as corneocytes, indicates that the keratinocyte maturation process is terminal [18]. The maturing keratinocytes migrates from the stem-cell-containing basal membrane toward the outer layer, where they shed [19]. Desmosomes firmly bind the keratinocytes in the skin, and together form the anatomical barrier [20]. Because epidermis lacks its own blood supply, for nutrition and oxygen it depends on the dermis [21]. The subcutaneous layer is primarily composed of fat and performs several functions, such as securing the muscle to the top layers, shielding the bones and muscles, and regulating body temperature [22]. Since a wide variety of microorganisms, including bacteria, fungus, and viruses, naturally reside on the skin, therefore, skin's chemical barrier is crucial [23]. The management and regulation of pH is essential for the activation of many enzymes and the inhibition of bacterial growth [24, 25] as well as for the production of host defence peptides (HDPs) [20, 26] which together form the chemical barrier. Because of their anti-pathogenic properties, HDPs are secreted via the skin as one of the first lines of defence

[27].

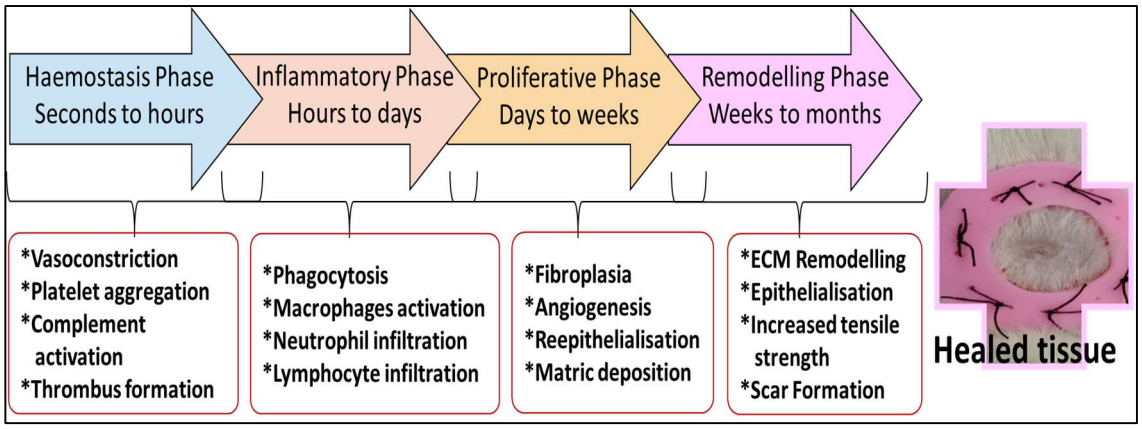

Figure 1.2: Wound healing phases – Wound healing unfolds through distinct phases: Haemostasis, inflammation, proliferation and migration, and remodelling. Commencing immediately, the first phase involves blood clotting, while the second phase focuses on wound cleansing, marked by immune cell infiltration and activation. Days later, the third phase sees cell proliferation, migration, and differentiation, particularly in epithelial, endothelial, and fibroblast cells reconstructing new tissue. The concluding maturation phase, lasting weeks to months, entails the strengthening and remodelling of the newly formed tissue.

The keratinocyte cells of the skin generate a variety of chemokines, cytokines, and HDPs, including LL-37 [28], MCP-1 [29], IL-6, IL-8, and tumor necrosis factor α (TNFα) [30]. These chemicals attract neutrophils, monocytes, and leukocytes as part of the innate immune response [31, 32] and play a critical role in starting the healing process when a skin injury takes place, they stop fluid loss and opportunistic microorganisms from invading the wound by repairing the injury [33]. Four stages comprise the WH process: haemostasis, inflammation, proliferation, and tissue remodelling [34] (Figure 1.2). The stages happen in a timely manner and overlap to eachother [35].

1.3.1 Haemostasis Phase

As soon as the wound emerges, the haemostasis phase gets started, during which the blood vessels contract to cease the blood loss and a fibrin clot forms to cover the wound [36]. Platelets attach to the exposed collagen and create an unstable fibrin clot. Clotting factors promote fibrin production and strengthen the clot even more [37]. At this stage growth factors, chemokines, and pro-inflammatory cytokines are released when the skin is

injured [38]. Various immune cells such as neutrophils and monocytes migrate into the injured region when chemokines and pro-inflammatory chemicals are secreted to commence the inflammatory phase [38, 39].

1.3.2 Inflammatiory Phase

In order to eradicate pathogens and cellular debris, neutrophil, lymphocyte, and macrophage infiltration occurs at the wound site, and this phase of WH is characterizing the inflammatory phase [40]. One of the first immune cells that infiltrate at this stage are neutrophil, these are interacting and encounters the site of damage and their infiltration depends on pro-inflammatory cytokines [41] and damage-associated molecular patterns (DAMP), which include DNA, RNA, and other cellular components [42]. By phagocytosing of bacteria at the wound site, neutrophils destroy pathogens. This process also enhances the releases growth factors, cytokines, ROS, proteolytic enzymes, and HDPs, which further aid in the destruction and elimination of infectious organisms [43, 44]. Numerous other cells, including fibroblasts, endothelial cells, and keratinocytes, also produce and releases these factors at site of wound, and the release of these factors stimulates and activates other immune cells [45]. Macrophages are one of the most common immune cells found at the site of wound, predominately and depending on the cytokine secretion profile, classified into two subgroups: M1 and M2 macrophages [46]. Pro-inflammatory cytokines, pathogen-associated modifying proteins (PAMPs), DAMPs and other materials alter the macrophage phenotype during the inflammatory phase, causing them to become M1-macrophages [47]. Pro-inflammatory cytokines such as TNFα, IL-6, and IL-8, are secreted by M1 type macrophages and play a crucial role in drawing more immune cells to the site of injury [48]. During the inflammatory phase, primary function of immune cells is to eradicate intracellular infections; nevertheless, macrophages also play a crucial role in the phagocytosis of apoptotic neutrophils.

Regulated and controlled elimination of aged neutrophils is necessary for continuous healing. If the phagocytosis of neutrophils is not controlled, they will generate DAMPs, which exacerbates inflammation and damage the regenerated tissue [49]. This removal of neutrophils from wound site will alters the wound microenvironmental and cause M1 macrophages to polarize and transform into M2 type macrophages [50]. Anti-inflammatory cytokines, such IL-10, TGF-β, IL-4, IL-13 and IL-1RA, are secreted mainly by M2 type macrophages and are typical for the start of the proliferation and migration phase [51].

1.3.3 Cell Proliferation and Migration

The proliferative and migratory phase encompasses three distinct components: re-epithelialization, neovascularization, and formation of granulation tissue. This phase is predominantly characterized by the migration and proliferation of skin cells such as keratinocytes, endothelial cells, and fibroblasts [36]. At this stage, the epithelial layer sealed the affected area, re-vascularized, and damaged tissue is replaced by these entities working together through a fibroblast-mediated mechanism [52]. These three components occur in a sequential but in overlapping manner. Re-epithelialization occurs beneath the fibrin clot as keratinocytes migrate in response to epidermal growth factor (EGF). Cell migration is a dynamic process supported by actin filaments, resulting in cellular overhang, adhesion, contraction, and detachment [53]. The initiation of this process is brought about by the polymerization of actin at the leading edge, resulting in the formation of an overhang that is stabilized on the surface through the presence of adhesion proteins [54]. The process of cell contraction, which enables forward motion, is achieved through actin-myosin polymerization [55, 56]. Forward movement of cells has done with the help of certain enzymes such as matrix metalloproteinase (MMPs) which detaches cells from the matrix [57].

Angiogenesis plays a pivotal role in the proper healing of wounds by supplying vital oxygen and nutrients for energy production [58]. During WH energy consumption is increased because various metabolism process is occurring actively, including cell proliferation, migration, and collagen production etc. In this scenario, without sufficient oxygen, the mitochondrial ATP synthesis will be compromised, leading to delayed WH [59]. Angiogenesis in new tissue completes in four steps: matrix degradation, migration towards angiogenic stimuli, endothelial cell proliferation, and reorganization while the sprouting angiogenesis is guided by tip cells, which respond to vascular endothelial growth factor (VEGF) stimuli [60]. These tip cells possess filopodia that secrete proteolytic enzymes [61], necessary for the degradation of the basement membrane of endothelial cells and the subsequent sprouting into new tissue regions [62]. When an injury penetrates the dermis, granulation tissue starts to grows, which serves as new connective tissue. Fibroblasts are the major cells, produce collagen and extracellular matrix (ECM) to strengthen the regenerated tissue. Collagen III, is the prominent protein present in the granulation tissue [63]. But this newly regenerated tissue is initially weaker than unwounded skin, until remodelling takes place to reinforce the tissue.

1.3.4 Remodelling of Tissues

It may take several years for the remodelling process to be completed and for the new tissue to reach maturity and resemble the old tissue [64]. Collagen rearrangement and devascularization of the newly formed tissue are the hallmarks of this phase. Additionally, by attaching to collagen at the borders of the wound and constricting the wound region, the differentiated form of fibroblasts known as myofibroblasts decreases the size of the newly created tissue [65]. During this phase crucial ECM components are broken down and replaced throughout the delicate process of tissue development, here collagen III is replaced by collagen I [65, 66]. and makes wound region stronger and more tense. After

full wound contraction, myofibroblast experiences apoptosis, [67] with quantity reduction of immune cells, [68] while the blood vessels remain intact [69]. Compared to healthy skin, the regenerated skin is different because its collagen is disordered and its fibers are thicker. The fibers become more arranged and interconnected throughout the remodelling phase, which further strengthens the tissue [70].

1.4 MATERIALS FOR WOUND DRESSING

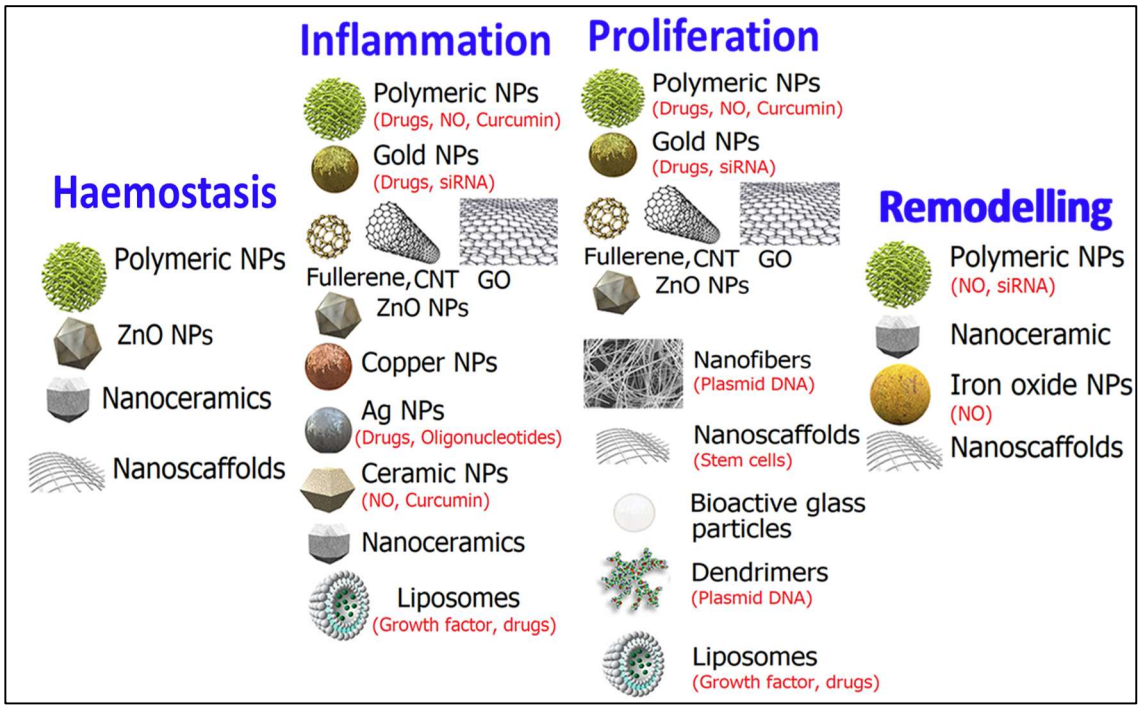

Figure 1.3: Biomaterials based nanotherapeutics exerts their regenerating effect at different stages of wound healing [72].

WH is a complex biological process involving a cascade of cellular and molecular events that restore the integrity of damaged tissues [71]. Biomaterials from natural or synthetic sources have revolutionized WH by providing a supportive scaffold for tissue regeneration and promoting various aspects of the healing process [72, 73]. These materials are designed typically, in such a way that they interact with living cells and wound microenvironment during different phases of WH (Figure 1.3). Materials for WH can be derived from a variety of materials such as polymers [71, 74], ceramics [75, 76], inorganic materials [77], metal oxides [78, 79], graphene [80, 81], and carbon nanotubes

[82]. Each biomaterial has its own unique properties and can be tailored to address specific tissue repair needs. Here, literature has been reviewed consisting of the diverse array of biomaterials currently employed in WH applications.

1.4.1 Nanoparticles and their application in wound healing

NPs are ultrafine particles of matter with at least one dimension of between 1-100 nm [83] and some times bigger particles up to 500 nm or more in size or fibers and tubes that have at least one dimension smaller than 100 nm, are extensively explored in the biomedical and TE fields [84]. NPs can be made from a variety of materials, including metals [83, 85], ceramics [75], polymers [86], and organic molecules [72]. They can be synthesized in a variety of ways, including chemical [87], physical, and biological methods [88]. The properties of NPs can be vary different from the properties of the same material in bulk form [89]. This is because the small size of NPs affected by their surface area, which in turn affects their chemical reactivity, optical, and electrical properties. The following are potential application of NPs

(i) Medicine: NPs can be used to deliver drugs to the specific parts of the body, to improve the solubility of drugs, and to reduce the side effects of drugs [90].

(ii) Electronics: NPs can be used to make transistors, solar cells, and other electronic devices [91].

(iii) Materials science: NPs can be used to make stronger, lighter, and more durable materials [92].

(iv) Environmental remediation: NPs can be used to clean up pollution and to remove toxins from the environment [93].

(v) Food and cosmetics: NPs can be used to improve the taste, texture, and appearance of food and cosmetics [94].

1.4.1.1 Organic nanoparticles

(i) Lipid nanocarriers: Lipid NPs serve as effective carriers for pharmaceuticals, growth hormones, and siRNA [95, 96]. Two categories of lipid NPs, namely solid lipids nanocarriers (SLNs) and nanostructured lipid carriers (NLCs) have been developed for wound care. These carriers safeguard pharmaceuticals from degradation and ensure sustained drug release. Solid lipids and NLCs outperform traditional delivery methods due to their small size, facilitating potential diffusion within biofilms and proximity to bacterial cells [97].

(ii) Polymeric nanocarriers: Polymers consist of monomers, which are large repeating units. These biomaterials (Table 1.1) offer a wide range of properties that can be tailored to specific WH needs. Their biocompatibility, tuneable porosity, and ability to incorporate bioactive molecules along with the ability to mimic the native extracellular matrix make them versatile and effective in promoting tissue regeneration [98]. In regenerative medicine, polymer-based scaffolds support cell growth and tissue regeneration. The controlled release of bioactive molecules from the polymer aids tissue regeneration, addressing the physical and biological aspects of wound closure [99]. Polymer's dynamic platform addresses emerging healthcare challenges, from chronic wounds to degenerative diseases, reshaping the landscape of modern medicine. Polymers can be broadly classified into two groups based on their source of origin: natural polymers and synthetic polymers.

a. Nanocarriers fabricated from Natural polymers: Polymers of natural origin originate from biological entities, including plants, animals, and microorganisms. Numerous natural polymers, commonly employed in the creation of polymeric wound dressings, are documented in the literature. Examples of these polymers include cellulose, chitosan, alginates, chitin, heparin, chondroitin, agar, carrageenan, collagen, gelatin,

fibrin, keratin, silk fibroin, albumin, and fucoidan. These polymers have advantages such as biocompatibility, biodegradability, bioactivity, and low toxicity. They can also mimic the extracellular matrix of the skin and interact with the cells and growth factors involved in the healing process. Some of these polymers can also possess antibacterial, anti-inflammatory, or proliferative properties, which can enhance the WH outcome [71].

Cellulose and chitosan, natural polysaccharides, have excellent biocompatibility and haemostatic properties, creating a favorable environment for cell adhesion and proliferation [73, 100]. Hyaluronic acid, a major extracellular matrix component, promotes cell migration and tissue regeneration [101, 102]. Sodium alginate and carrageenan, extracted from seaweed, form biocompatible hydrogels suitable for WH [73, 74, 103, 104]. Collagen, hyaluronic acid and gelatin scaffold enhance bioactivity [105]. Protein-based polymers such as gelatin, collagen, silk fibrin, and keratin provide structural support and facilitate cell attachment. Collagen, a major connective tissue component, promotes angiogenesis and accelerates wound closure. Silk fibrin provides a scaffold for cell growth, and keratin exhibits excellent biocompatibility, resembling the native extracellular matrix [100, 106-108].

b. Nanocarriers fabricated from synthetic polymers: Synthetic polymers are artificially created by chemical reactions, such as polymerization or condensation. In scientific literature, numerous synthetic polymers are commonly employed in the fabrication of polymeric wound dressings, such as polyethylene glycol [109], polyvinyl alcohol [110-112], polyurethane [113], polyacrylamide, polyacrylic acid, polyethylene oxide [109], polylactic acid [114] polyglycolic acid, poly-lactide-co-glycolides (PLGA) [115], poly(amido amine) and polycaprolactone [116]. These polymers have advantages such as tunability [74], stability, versatility, and cost-effectiveness. They can also be modified to achieve desired properties, such as porosity, hydrophilicity, mechanical

strength, degradation rate, and drug release. Some of these polymers can also be functionalized with bioactive molecules, such as drugs, peptides, proteins, or NPs, to achieve controlled delivery and targeted therapy. However, synthetic polymers may also have some drawbacks, such as poor biocompatibility, immunogenicity, toxicity, or environmental impact [74].

PVA, PCL, PLGA, PLA and PU offer tunable mechanical properties and controlled degradation rates [74]. PVA forms transparent films [117], aiding wound monitoring; PCL ensures long-term support for tissue regeneration [116]; and PLGA and PLA balance mechanical strength with biodegradability. Polyurethane (PU) exhibits elasticity, making it suitable for joint wound dressings [74, 102, 111, 114-116, 118]. In a study polylactic acid (PLA)-based scaffolds are used for TE applications due to their biodegradability and ability to mimic the mechanical properties of natural tissues. These scaffolds provide a supportive structure for cell growth and differentiation, facilitating tissue regeneration [102]. Polyethylene oxide/polyethylene glycol (PEO/PEG) provides hydrophilicity while reducing swelling [109]. Hydrogels derived from polyethylene glycol (PEG) find extensive application in WH owing to their capacity to sustain a moist wound environment and facilitate cell migration. These PEG hydrogels can be additionally customized by incorporating growth factors or drugs to augment the process of WH [98].

c. Nanopolymers/Polymer Nanoparticles: Nanopolymers are polymers with at least one dimension in the nanoscale (<100 nanometers). They can be made from a variety of polymers, including natural polymers, synthetic polymers, and hybrid polymers. Nanopolymers have a number of potential applications, including drug delivery, TE, and imaging.

Dextran, a polysaccharide, promotes cell adhesion and nutrient transport. Fibrin, a natural

polymer that forms blood clots, acts as a wound sealant. The combination of these polymers produces synergistic effects [119]. A mixture of chitosan and PVA forms a hydrogel with increased strength and antibacterial properties [112]. Collagen and PLGA in a scaffold enable sustained release of bioactive molecules, which promote tissue regeneration [107].

Both natural and synthetic polymers have been widely used in manufacturing of wound dressing materials as they can offer various benefits and functionalities such as protection, absorption, adhesion, hydration, haemostasis, infection control, and tissue regeneration [71, 74]. Depending on the type and source of the polymer, they can have different advantages and disadvantages. Therefore, the selection and design of the polymer should be based on the specific requirements and characteristics of the wound and the patient. By optimizing the properties and performance of the polymers, they can potentially improve the WH process and outcome.

Collaboration between researchers, engineers, and health care professionals is essential to advancing polymer-based technologies. In this scenario, smart materials have been developed by incorporating sensors in responsive polymer wound dressings which enable real-time monitoring [120]. The synergy between polymers and stem cell therapy holds promise for optimizing regenerative capacity [121]. Challenges regarding the use of polymers for WH include understanding polymer-biological interactions, long-term biocompatibility, immunogenic responses, and toxicity. Despite the challenges, a diverse range of polymers (Table 1.1) are driving innovation in WH and regenerative medicine.

Table 1.1: Polymers used in wound healing

A. Natural polymers		
Polymer Name	**Role in wound healing**	**Reference**
Cellulose and its derived products	Promote tissue regeneration and lesion closure, provide moist environment as absorbs wound	[122, 123]

	exudates, provide a scaffold support	
Chitosan	Having antimicrobial activity, biodegradability, scaffolding support, absorbancy and moisture retention, modifiability: supports WH	[123, 124]
Hyaluronic acid	Having biocompatible, moisture retention, anti-inflammatory, biodegradable, angiogenic, absorbant activity, supports WH	[125]
Sodium alginate	Due to good absorbancy, haemostatic property, biocompatibility, conformbility, supports WH.	[104]
Carrageenan	Due to geling and film forming property, biocompatibility, antiviral and antimicrobial, and haemostatic activity, supports WH.	[73]
Gelatin	Promotes cell growth, biodegradable, haemostatic, moisture retention, ease of modification, film forming capability	[108]
Collagen	Being biocompatibe, biodegradable, low immunogenicity, haemostatic, and ease of modification as well as promotes cell migration and proliferation, angiogenic effect; supports WH.	[107]
Silk fibrin	Due to biocompatibe, biodegradabe, excellent mechanical strength, promotes cell migration and proliferation, angiogenic activity, promotes WH.	[100]
Keratin	Due promotes cell proliferation, regulates wound moisture, biodegradable, immune response modulation, haemostatic properties supports WH.	[106]
B. Synthetic polymers		
Polymer Name	**Role in wound healing**	**Reference**
Polyvinyl alcohol (PVA)	Due to moisture retention, film forming capacity, biodegradability, low antigenicity, favours WH.	[110-112]
Polycaprolactone (PCL)	Due to tissue compatibility, biodegradability, mechanical strength, versatility in formulation, slow degradation, ease of processing widely used for fabrication of WH scaffolds.	[116]
Polyactic acid (PLA)	Due to biocompatibility, biodegradation, versatility in formulation, mechanical strength, ease of processing, tunable degradation rate, tissue compatibility, low antigenicity PLA used in WH.	[114]
Polyactic-co-glycolic	Due to biocompatibility, biodegradability, versatility in formulation, tunable degradation	[115]

acid (PLGA)	rate, mechanical strength, ease of processing, tissue compatibility, drug delivery options, PLGA used as WH and supportive materials	
Polyurethane (PU)	Due to biocompatibility, mechanical strength, moisture retention, versatility in formulation, gas permeability, ease of modification, biastability, haemostatic property, PU used as WH material.	[113]
Polyethylene oxide/ polyethylene glycols (PEO/PEG)	Having goof biocompatibility, hydrophilicity, versatility in formulation, drug delivery option, minimal inflammatory response, ease of modification, biodegradability, gas permeability, PEO/PEG applied vastlt in WH.	[109]

1.4.1.2 Inorganic nanocarriers

Utilizing NPs composed of metals and metal oxides for wound treatment is more favorable in comparison to conventional methods [126]. Their inherent nature is the basis for this preference, as these NPs demonstrate comparable properties in WH and antibacterial effects [77]. The performance of metallic NPs in biological contexts is often impacted by various factors, including surface functionalization, zeta potential, dimensions, morphology, hydrolytic stability, and porosity [126, 127]. Gold [79, 128, 129], silver [85, 88, 130, 131], and zinc oxide (ZnO) [132, 133] are among the most extensively studied metallic NPs. Table 1.2 provides a detailed explanation of the role of inorganic nanomaterials in WH.

(i) Ceramics: The application of ceramic materials is becoming increasingly popular due to its diverse properties such as high biocompatibility, chemical stability, antimicrobial properties, osseointegration, osteoconduction, osteoinduction, increased cell adhesion and improved characteristics such as stiffness, mechanical strength, wear resistance, etc and other property such as the ability to adapt to three-dimensional geometry or conform to various shapes easily [75]. These materials are particularly used for the regeneration of bone tissue or repair of bone wound by providing suitability for

long-term implantation. Their inert nature reduces the risk of immune reactions, while their bioactive properties can promote bone growth and tissue regeneration. Hydroxyapatite (HA)-based bio-ceramics are commonly used in bone TE due to their close resemblance to the mineral component of natural bone. HA scaffolds provide a biomimetic environment for osteoblast growth and differentiation, facilitating bone regeneration [76]. Bioactive glasses are a type of ceramic biomaterial that can dissolve and release ions, such as silicon and calcium, which stimulate bone growth and angiogenesis. Bioactive glasses are used for bone defect repair and periodontal regeneration [134].

(ii) Metals and metal oxides: Inorganic materials (Table 1.2), including minerals and metals, offer unique properties that can be harnessed for WH applications. Their antibacterial, antioxidant, and pro-healing properties make them valuable in promoting tissue regeneration.

Zinc oxide NPs (ZnO NPs) exhibit antibacterial properties and promote cell proliferation, making them effective in treating chronic wounds. ZnO dressings have been shown to reduce bacterial load and accelerate wound closure [78, 135].

Silver NPs (Ag NPs) possess potent antimicrobial activity against a broad spectrum of bacteria, making them ideal for treating infected wounds. Ag-impregnated dressings are widely used as antiseptic wound dressings to control infection and promote WH including bone wound. Apart from WH Ag NPs are also used in anticancer therapy, vaccine adjuvant, antidiabetic agent and as biosensors [85, 131].

Gold NPs (Au NPs) have gained attention in biomedical research due to their unique properties, including their biocompatibility and tunable surface chemistry [136]. Au NPs and their conjugates are used in imaging, analytics, phototheral and photodynamic therapeutics and biomedical diagnostics because they can be easily detected by light.

Apart from these as a carrier for delivering target molecules and as immunological and toxicological properties [128]. Studies have shown that gold NPs possess considerable potential in different facets of WH. It is possible to finely adjust their surface plasmon resonance properties to augment antimicrobial and wound-healing effects [79]. Au NPs impede pathogenic growth by adhering to bacterial DNA, hindering replication, and potentially altering energy metabolism. Additionally, they function as antioxidants, aiding WH and inhibiting reactive oxygen species [137].

The incorporation of Au NPs benefits materials like collagen and chitosan, offering biocompatibility and biodegradability [129]. Combining Au NPs with other materials such as chitosan significantly boosts wound-healing capacity. In various models, the nanocomposite accelerates tissue growth, stops bleeding, and improves burn WH. The coadministration of Au NPs (Au NPs) and chitosan in diabetic mice is shown to augment the healing of wounds, stimulate angiogenesis, and demonstrate antimicrobial characteristics [88, 137-139]. Hydrogel, combining chitosan with silver NPs, effectively prevents bacterial infection and reduces inflammation when incorporating Au NPs, promoting faster WH and minimizing scarring [140].

Table 1.2: Inorganic nanomaterials used in wound healing

Type of nanomaterial	Role in wound healing	Reference
Ceramic NPs	Serves as a superior substance for the regeneration of bone tissue by gradually releasing silicon (Si) and calcium (Ca) ions at the sites of wounds, thereby boosting cell proliferation and angiogenesis.	[141]
ZnO NPs	Demonstrate bactericidal and bacteriostatic properties, Show heightened permeability through skin and lipid membranes,Manifest minimal levels of toxicity	[133]
Ag NPs	Antimicrobial properties exhibited, Modulation of immune response to reduce inflammation, Promotion of angiogenesis, Disruption of biofilm, Stimulation of cell proliferation and migration	[142, 143]

Au NPs	Uses electrical stimulation to promote tissue regeneration in wounds, Applied in photothermal therapy to boost tissue regeneration, Acts as nanocarriers for delivering antibiotics, Utilized in gene nanotherapy.	[79]
TiO_2 NPs	TiO_2 NPs exhibit a hydrophilic nature and demonstrate elevated biocompatibility while also showcasing anti-inflammatory and antibacterial properties.	[144]
Cu NPs	Modulates the metabolic activities of cells and regulates growth factors and cytokines crucial for WH, thereby expediting the closure of wounds. Stimulates the proliferation of keratinocytes, promotes epithelialization, angiogenesis and augments collagen synthesis, thereby optimizing and accelerating the overall healing process.	[145, 146]
Si NPs	Si plays vital role in promoting topical WH, facilitating migration of fibroblasts. Nanocomposites leads to heightened mechanical strength and stability through the inclusion of Si.	[147]
Fe NPs	Having a broad antibacterial spectrum, Enabling easy combination and surface modification, Exhibiting improved biodegradability with decreased toxicity	[148]
GO NPs	The synergistic incorporation of graphene with chitosan, PVA, and hyaluronic acid expedites the recovery process of bacterial wounds, demonstrating notable antibacterial effectiveness. Additionally, it exhibits favorable angiogenic characteristics.	[80, 81]
CNT	A noteworthy antibacterial action upon amalgamation with (CNTs), Ag NPs, TiO2 NPs, and Hg composites. Affects the integrity of the bacterial cell membrane.	[82]
Fullerenes	Enhancing the healing of wounds through the control of the immune response and the release of growth factors, while exhibiting potent antibacterial effects against both *E. coli* and *Bacillus subtilis*.	[149]

(iii) Graphene and carbon nanotubes: Graphene and carbon nanotubes, having unique properties, such as high surface area, conductivity, and mechanical strength, offers promising potential in WH. Graphene-based biomaterials promote cell adhesion, proliferation, differentiation, making them suitable for TE and WH [81]. Graphene's electrical conductivity can also facilitate electrical stimulation to enhance WH [150]. Carbon nanotubes find application in electronics and composites,

owing to their exceptional strength and low weight. In the fields of pharmacy and medicine, CNTs have proven their effectiveness due to their larger surface area, enabling them to adsorb or conjugate with a diverse range of therapeutics and diagnostic entities such as drugs, genes, vaccines, antibodies, biosensors, and more [151]. Carbon nanotube-based scaffolds provide a highly porous structure that facilitates cell infiltration and nutrient transport, along with exhibiting antibacterial properties. Carbon nanotubes can also be used to deliver growth factors and drugs to promote WH [152].

1.4.2 Nanoparticles Employed in Wound Healing Therapy

For WH purpose, NPs made from following classes of biomaterials are used:

(i) NPs with innate wound-healing potential: The therapeutics developed for WH without use of drugs, cytokines, gene and stem cells are attractive because they have lower formulation and fabrication cost and low pharmaceutical development cost. In this context metallic NPs made up of silver (silver oxide), gold, zinc (ZnO), copper (copper oxide), iron (iron oxide), aluminium (aluminium oxide), titanium (titanium oxide) and gallium (gallium oxide) have been proved excellence in WH due to their intrinsic antibacterial activity [153]. Currently, these NPs are produced through the green synthesis procedure which assures reduced toxicity and improved safety during application along with combined effect of natural extracts [154, 155]. They exert their action through the production of reactive oxygen species (ROS) which interacts with cellular components such as RNAs, DNA and essential enzymes, and theses all events collectively provoke antibacterial effect [156]. In this line other materials which having innate WH activity are cerium, bioactive glass (BG), carbon based materials such as carbon dots [157] and grapheme [158] and materials bearing and releasing nitric oxide (NO).

(ii) NPs acts as carrier and delivers medication to the site of wound: NPs, as carriers in wound therapeutics, represent a paradigm shift in drug delivery strategies

owing to their unique physicochemical attributes [159]. Engineered from diverse materials such as polymeric matrices (e.g., PLGA [160]), lipids, or metallic composites (e.g., gold and silver), these nanoscale entities exhibit exceptional surface area-to-volume ratios, affording heightened drug loading capacities.

The carrier functionality is predicated on tailored encapsulation methodologies, whereby therapeutic agents—comprising growth factors, antimicrobials, and anti-inflammatory compounds—are entrapped within the nanoparticle matrix [153, 161, 162]. This encapsulation not only shields fragile bioactive molecules from degradation but also facilitates a controlled and sustained release profile, circumventing issues associated with conventional drug formulations. The multifaceted roles of NPs extend beyond mere drug carriers, encompassing an active participation in wound microenvironments [163]. NPs, with tunable surface chemistry, engage in selective interactions with cellular moieties, fostering targeted drug delivery to specific cell populations critical for regenerative processes [164]. Additionally, their inherent biocompatibility ensures minimal cytotoxicity, a critical consideration in WH applications.

The nanoscale dimensions of these carriers enable facile penetration into the wound site, addressing challenges associated with conventional therapeutics' limited spatial distribution [165]. Furthermore, the emergence of stimuli-responsive NPs heralds a new era in precision medicine. Photo-responsive NPs, activated under specific wavelengths, and thermo-responsive counterparts, responsive to changes in temperature, confer spatiotemporal control over drug release dynamics [166]. Such advancements empower clinicians with unprecedented precision in tailoring medication release to match the dynamic phases of WH. While challenges persist, including the elucidation of long-term biocompatibility and the standardization of manufacturing processes, the convergence of nanotechnology and wound therapeutics propels the field towards efficacious, patient-

centric interventions. As investigations into nanoparticle-mediated drug delivery systems burgeon, the potential for transformative modalities in wound care remains palpable, auguring a new frontier in the trajectory of medical interventions.

1.5 EXPLORING NANOTHERAPEUTICS: ADVANCING WOUND HEALING INNOVATIONS

Nanotherapeutics such as NPs enhance WH by leveraging the distinctive characteristics of the materials used. Various essential processes are involved in how nanomaterials impact the overall wound-healing mechanism.

1.5.1 Improvement in Antimicrobial action

Numerous nanomaterials, including zinc oxide (ZnO) NPs and silver (Ag) NPs, possess potent antibacterial properties, directly inhibiting microbial growth [167]. In WH, they create an environment that prevents infections, with varying antibacterial effects. Metallic NPs disrupt microbial processes efficiently, expediting healing [168]. Nanomaterials facilitate the precise release of antimicrobial agents, thereby improving effectiveness and accelerating the healing process. Cerium oxide NPs generate potent antibacterial reactive oxygen species (ROS), effectively diminishing microbial load and promoting wound disinfection [83]. In cases of persistent wound infections characterized by biofilms, nanomaterials infiltrate the matrix, thwarting the formation of antibiotic-resistant biofilms. Additionally, these nanomaterials interact with immune cells, thereby modulating the immune response throughout the process of WH [169].

1.5.2 Controlled delivery of drug candidates

Nanomaterials optimize drug delivery to wounds, ensuring controlled administration of medications and therapeutic agents for sustained therapeutic effects. Various nanocarriers such as polymeric NPs, liposomes, or dendrimers enable precise drug targeting,

enhancing bioavailability and reducing systemic side effects [170]. Nanomaterials protect drugs during storage, preserving stability, and extending shelf life by shielding them from environmental factors [171]. They enhance solubility, improving the bioavailability of poorly water-soluble pharmaceuticals. Modified nanocarrier features allow for tailored drug release, accommodating WH needs and minimizing administration frequency [172]. Functionalized nanomaterials, with ligands like antibodies, enable targeted drug binding to molecular wound targets, enhancing therapeutic efficacy and minimizing side effects [173]. Nanomaterials simplify combination therapy by co-encapsulating multiple agents, promoting synergistic effects or sequential drug release [174].

1.5.3 Modulation of cellular responses such as inflammation

Nanomaterials uniquely modulate the inflammatory response crucial for WH. Some nanomaterials, like gold NPs (Au NPs) and silver NPs (Ag NPs), inherently reduce inflammation by interacting with immune cells, curbing excessive cytokine release [140]. Silver NPs induce anti-inflammatory macrophages, aiding tissue healing, while modulating dendritic and T-lymphocytes for balanced immune responses [175]. Cerium oxide NPs (CeO_2 NPs) regulate reactive oxygen species (ROS), maintaining balance and reducing oxidative stress in the wound microenvironment [176]. Nanomaterials influence cytokine and chemokine production through signaling pathways, ensuring a controlled inflammatory response for effective tissue healing. Fibrosis which hinders healing, is managed by nanomaterials like graphene oxide NPs (GO NPs), controlling collagen production and fostering a conducive environment for WH [130].

1.5.4 Stimulation of cellular activities (proliferation and migration)

Nanomaterials that promote cell proliferation and migration play a crucial role in facilitating WH [177]. Hydrogels and polymeric nanofibers mimic the extracellular

matrix (ECM), serving as a substrate for cell adhesion, growth, and migration [173]. They mimic the ECM, promoting tissue regeneration and wound closure [178]. Nanomaterials, with suitable surface characteristics, engage cell receptors and activate pathways for migration and proliferation. Functionalized NPs, like those activating integrin receptors, enhance cellular responses [179]. Nanomaterials facilitate the movement and growth of cells by transporting bioactive chemicals and growth factors [127]. Controlled release mechanisms ensure localized distribution, activating cells crucial for WH [180]. Some nanomaterials promote angiogenesis, facilitating the development of a vascular network through the stimulation of endothelial cell functions. Scaffolds constructed with nanomaterials, featuring a three-dimensional framework, support cell adhesion, proliferation, and migration. Enhancing porosity, surface texture, and mechanical strength expedites the process of WH. The mechanisms of action differ depending on the type of nanomaterial, its characteristics, and cellular processes involved [127, 181].

1.5.5 Improvement in Angiogenic potential

The nanomaterials which facilitate angiogenesis, crucial for new blood vessel formation and simultaneously in WH [173]. They promote the functions of endothelial cells, supporting the development of functional blood vessels in the wound site. This amplifies blood circulation, thereby improving the delivery of oxygen and nutrients, ultimately facilitating enhanced WH and tissue regeneration. Nanomaterials, mirroring the extracellular matrix (ECM), initiate signaling pathways such as integrin receptors, stimulating cellular responses like proliferation and migration [182, 183]. Enhanced blood flow supports cell functions by increasing oxygen and nutrient. Nanomaterial scaffolds, acting as 3D frameworks, optimize cell behavior, expediting WH [78].

1.5.6 Remodelling Induction of Extracellular matrix

Nanomaterials significantly influence the extracellular matrix (ECM) remodeling which is very crucial for proer WH [173]. Nanomaterials have the capability to regulate ECM production, can align collagen fibers, and enhance tissue architecture, improving mechanical wound strength [132, 183]. Treatment efficacy with nanomaterials depends on their type, surface properties, and target tissue. A nuanced understanding of diverse nanomaterial mechanisms is vital for optimized WH [184]. Moreover, nanomaterials also controls ECM-degrading enzyme activity, notably matrix metalloproteinases (MMPs). They modulate MMPs to promote a balanced ECM remodeling process, preventing excessive degradation and enhancing healing outcomes [173, 184-186]. Nanomaterials also impact fibronectin and elastin production and organization within the ECM. Physicochemical properties and surface features of nanomaterials control ECM component creation and assembly [187].

The potential benefits of NPs are great, but there are also some concerns about their safety. Some studies have shown that NPs can be harmful to human health, but more research is needed to fully understand the risks. Overall, NPs are a rapidly developing field with a wide range of potential applications. As research continues, the information on the benefits and risks of NPs and how to use them safely will be explored. NPs are a promising new technology with the potential to revolutionize many industries. However, it is important to continue research to ensure that they are safe for human health and the environment.

1.6 GASEOUS INNOVATIONS: PIONEERING WOUND HEALING STRATEGIES

The utilization of various gases in WH represents a cutting-edge frontier in regenerative

medicine, leveraging their diverse physiological effects to expedite the intricate processes of tissue repair [188, 189].

Oxygen, a quintessential element in aerobic cellular respiration, assumes a pivotal role in WH dynamics [190]. Hyperbaric oxygen therapy (HBOT), involving the administration of pure oxygen at pressures exceeding atmospheric levels, enhances tissue oxygenation, mitigates hypoxia, and promotes collagen synthesis [191].

Nitric oxide (NO), an endogenously synthesized diatomic gas, emerges as a potent vasodilator with pivotal roles in angiogenesis and WH [192]. NO-releasing compounds, designed to release NO at controlled rates, exhibit therapeutic potential in augmenting tissue perfusion and fostering an optimal microenvironment for healing [193, 194].

Hydrogen, although traditionally recognized as an inert gas, has recently garnered attention for its potential therapeutic applications [195]. Molecular hydrogen (H_2), administered in various formulations, possesses anti-inflammatory and antioxidant properties, attenuating oxidative stress and inflammation in the wound milieu. Its ability to modulate signaling pathways implicated in tissue regeneration positions hydrogen as an intriguing candidate for adjuvant WH interventions [196].

Carbon dioxide (CO_2), predominantly associated with cellular metabolism and waste elimination, also plays a nuanced role in WH. Carbon dioxide-enriched environments, achieved through carbogen therapy or topical administration, stimulate vasodilation and improve tissue oxygenation, fostering an environment conducive to healing [197].

Carbon monoxide (CO), hitherto recognized as a toxic gas, has emerged as a signaling molecule with cytoprotective and anti-inflammatory attributes. Controlled administration of low concentrations of CO via inhalation or CO-releasing molecules has shown promise in enhancing angiogenesis and tissue repair [189].

Beyond these traditional gases, noble gases, such as helium and argon, have exhibited therapeutic potential in WH applications. Helium-neon laser, exerts analgesic effects and may attenuate inflammation, while argon, an inert noble gas, demonstrates anti-inflammatory properties and has been explored for its capacity to modulate immune responses [198, 199].

In this context the incorporation of gases into WH strategies underscores the dynamic interplay between elemental components and cellular processes in orchestrating tissue repair. From oxygen's crucial role in cellular metabolism and collagen synthesis to the diverse signaling functions of nitric oxide, hydrogen's antioxidant properties, and the nuanced effects of carbon dioxide and monoxide, each gas imparts unique contributions to the intricate tapestry of WH. The following table enlists the gases explored for WH along with their mechanisms of action:

Table 1.3 Various gases used as wound healing therapeutics and their respective mechanism

Gas	Role in wound healing	Ref.
O_2	Increases oxygen supply, cellular respiration and promotes collagen synthesis, supports angiogenesis at the wound beds.	[190]
NO	Acts as a signaling molecule. Dilates blood vessels, improving blood flow. Exhibits antimicrobial properties and aids in immune response.	[192]
O_3	Antimicrobial effects, reducing bacterial load. Modulates inflammation. Promotes tissue oxygenation.	[200]
CO_2	Hyperbaric oxygen therapy (HBO) using increased atmospheric pressure of oxygen. Enhances oxygen delivery to tissues. Reduces inflammation and edema.	[197]
H_2	Exhibits antioxidant properties. Modulates inflammation and oxidative stress. May enhance WH by reducing tissue damage.	[196]
He	Used in mixtures for hyperbaric oxygen therapy. Facilitates tissue oxygenation and promotes healing.	[201]
Ar	Potential neuroprotective and anti-inflammatory effects.	[198]
SO_2	Antioxidant and anti-inflammatory effects.	[189]

1.7 NITRIC OXIDE

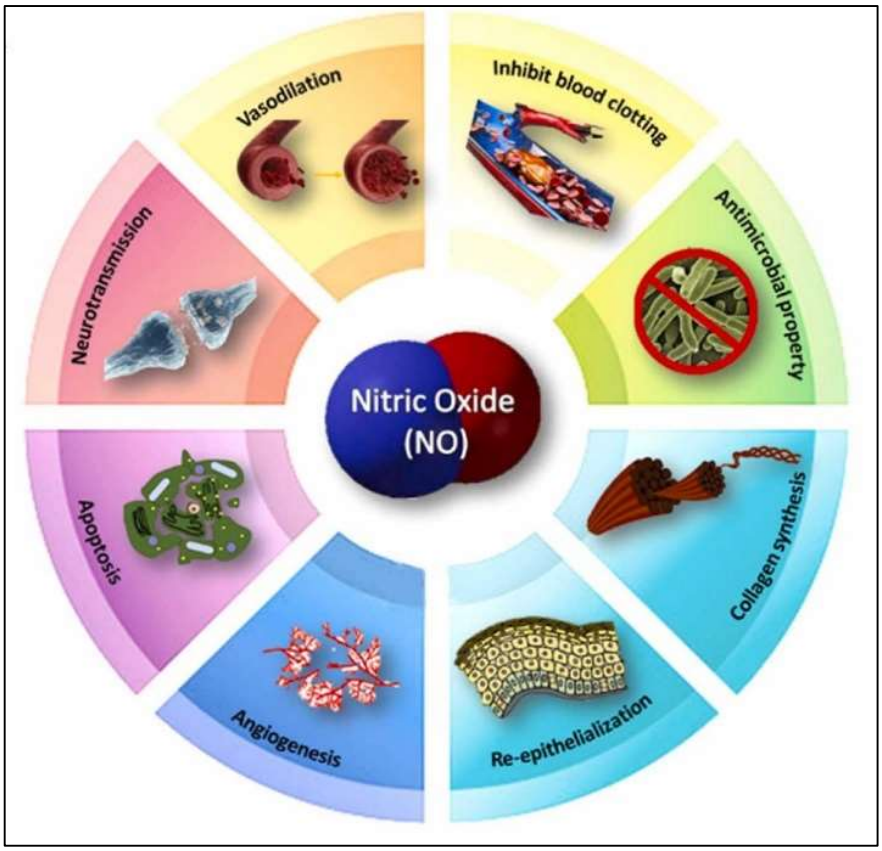

Figure 1.4: The biological attributes of nitric oxide (NO) play pivotal roles in vasodilation, blood clotting, neurotransmission, apoptosis, angiogenesis, re-epithelization, collagen synthesis, and antimicrobial functions. Notably, among these biological characteristics, the angiogenic activity, re-epithelization, and collagen synthesis prove beneficial in facilitating the wound healing process[207].

Nitric oxide (NO) is a potent gasotransmitter that plays a pivotal role in various physiological processes (Figure 1.4) and in the intricate process of WH by exerting influence over various aspects such as inflammatory and immune responses, angiogenesis, cellular metabolism, extracellular matrix (ECM) formation, and remodelling [202]. The efficacy of NO therapy hinges on maintaining an optimal concentration within the wound. At lower concentrations (1 µM to 1 mM), NO functions as a signalling molecule, binding to soluble guanylate cyclase (sGC) and initiating the PKG pathway by converting guanosine 5-triphosphate (GTP) to cGMP. This cascade regulates angiogenesis through endothelial nitric oxide synthase (eNOS)-derived NO,

contributing to VEGF expression and facilitating blood vessel dilation [203, 204]. The resulting enhancement of immune cell activity expedites WH with minimal adverse effects. Conversely, elevated NO concentrations (>1 mM) during neutrophil respiratory bursts lead to mitochondrial dysfunction, enzyme inactivation, and lipid peroxidation for pathogen elimination [205, 206]. In diabetic wounds, reduced NO production impedes normal healing. NO exerts the following actions, therefore favoured to use for WH over other gasotransmitter.

1.7.1 NO for Vasodilation and Increased Blood Flow

NO promotes vasodilation, leading to increased blood flow to the wounded area. This enhanced blood supply ensures the delivery of oxygen, nutrients, and immune cells essential for effective WH [208]. Other gasotransmitters may not exhibit such pronounced vasodilatory effects.

1.7.2 NO for Anti-Inflammatory Properties

NO possesses anti-inflammatory properties, helping to modulate the inflammatory response during WH. By inhibiting pro-inflammatory cytokines and promoting anti-inflammatory signals, NO aids in creating an optimal environment for tissue repair [209]. This anti-inflammatory effect distinguishes NO from other gasotransmitters that may not have comparable regulatory effects.

1.7.3 NO for Angiogenesis Stimulation

NO plays a pivotal role in angiogenesis, the formation of new blood vessels from existing one [210]. This is critical for supplying nutrients and oxygen to the regenerating tissue. The ability of NO to stimulate angiogenesis sets it apart as a valuable gasotransmitter for WH compared to others.

1.7.4 NO for Collagen Synthesis and Tissue Remodeling

NO has been shown to promote collagen synthesis [211], an essential component of WH that contributes to tissue strength and integrity. Additionally, NO influences the remodeling of the extracellular matrix [212], facilitating proper tissue repair. This impact on collagen and tissue remodeling distinguishes NO from other gasotransmitters in promoting structural integrity during WH.

1.7.5 Antimicrobial Effects

NO exhibits antimicrobial properties [206, 213] helping to control infection at the wound site. By directly targeting and inhibiting the growth of microorganisms, NO contributes to a cleaner wound environment. This antimicrobial action is a unique advantage of NO over other gasotransmitters in WH.

1.7.6 Cell Proliferation and Migration

NO facilitates cell proliferation and migration [214], essential processes in tissue regeneration. By promoting the movement and replication of cells involved in wound repair, NO accelerates the overall healing process. Other gasotransmitters may not have as significant an impact on these cellular activities.

1.7.7 NO for the Regulation of Matrix Metalloproteinases (MMPs)

NO helps in the regulation of the activity of matrix metalloproteinases (MMPs) enzymes involved in tissue remodeling [215]. Proper control of MMPs is crucial for preventing excessive tissue breakdown and promoting a balanced healing response. NO's ability to modulate MMP activity sets it apart in supporting controlled tissue remodeling.

1.7.8 NO for the Neurotransmitter Function

NO serves as a neurotransmitter in the nervous system [216], contributing to the

communication between nerve cells. This neuronal role can have positive effects on WH by influencing neurogenic inflammation and pain perception. The modulation of neurotransmitter function by NO may provide additional benefits not seen with other gasotransmitters in the context of WH.

1.7.9 Ischemia-Reperfusion Injury Protection

NO has been recognized for its ability to protect tissues from ischemia-reperfusion injury [217]. In situations where blood supply is temporarily interrupted and then restored, as in surgical procedures, NO can mitigate the damage caused by the reperfusion phase. This protective effect may not be as pronounced with other gasotransmitters.

1.7.10 Low Toxicity and Versatility of NO Delivery

NO is relatively low in toxicity, making it suitable for therapeutic applications. It can be delivered in various forms, such as through inhalation [218] topical application,[219] or as part of biomaterials [207]. This versatility in delivery methods allows for customized approaches based on the specific wound type and patient needs, setting NO apart from other gasotransmitters.

1.7.11 Synergistic Effects of NO with Other Growth Factors

NO has been demonstrated to synergistically [220] interact with different growth factors crucial for WH, including vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β). This collaboration amplifies the overall regenerative capability and efficiency of the WH process, showcasing a distinct advantage compared to other gasotransmitters.

1.7.12 Modulation of Oxidative Stress by NO

NO can modulate oxidative stress by interacting with reactive oxygen species (ROS) [221]. Excessive oxidative stress can impede WH [222], and NO's ability to balance this process contributes to its effectiveness in promoting a favorable healing environment. This regulatory role distinguishes NO from other gasotransmitters in managing oxidative stress during wound repair.

1.7.13 Therapeutic Potential of NO in Chronic Wounds

Chronic wounds often pose significant challenges in healing. NO has shown promise in addressing chronic wounds [223] by promoting angiogenesis, reducing inflammation, and combating microbial infections. The therapeutic potential of NO in chronic WH surpasses that of some other gasotransmitters.

1.7.14 Regulation of stem cell microenvironment in regenerative medicine

Midgley, Wei, and colleagues found that nitric oxide (NO) can influence stem cell behavior, affecting aspects such as survival, migration, differentiation, and the secretion of pro-regenerative factors. They also highlighted that combining biomaterials capable of controlling NO regeneration with stem cell delivery could offer a comprehensive and synergistic strategy to greatly enhance tissue regeneration [224].

1.7.15 Clinical Evidence and Established Therapies Using NO

There is a growing body of clinical evidence supporting the efficacy of NO in WH [225, 226]. NO-releasing dressings and topical applications have been developed and utilized in clinical settings, demonstrating the practical application and success of NO-based therapies [227]. The established use of NO in clinical practice reinforces its superiority over other gasotransmitters.

In summary, NO is unique and multifaceted combination impacts on vasodilation, anti-inflammatory properties, angiogenesis stimulation, collagen synthesis, antimicrobial effects, cell proliferation, and other distinctive attributes make it a compelling choice for promoting effective WH. The extensive range of benefits and the growing body of clinical evidence contribute to the superiority of NO over other gasotransmitters in the context of wound repair and regeneration. Therefore, NO stands out as a versatile and effective gasotransmitter for WH and these unique properties make NO a promising candidate for therapeutic interventions aimed at enhancing the WH process.

1.8 MOTIVATION FOR DEVELOPMENT OF ADVANCED NANO-THERAPEUTICS AS REGENERATIVE MEDICINE

Regenerative nanomedicine represents a frontier in scientific research with the potential to revolutionize healthcare and address critical challenges in WH. The motivation behind my project stems from a multifaceted perspective, encompassing social, economic, and medical considerations.

1.8.1 Medical Need and Social Impact

Addressing global health burden: Chronic wounds and slow-healing injuries pose a significant global health burden, affecting millions of individuals worldwide. By developing innovative solutions, we aim to alleviate suffering, enhance quality of life, and reduce the socioeconomic impact of chronic wounds.

Improving patient outcomes: The project's primary objective is to contribute to developing advanced WH nanomedicine. Success in this endeavour improves physical well-being and has profound implications for mental health, fostering a sense of well-being and confidence in patients dealing with chronic wounds.

1.8.2 Economic Considerations

Reducing healthcare costs: Chronic wounds often result in prolonged hospital stays, frequent medical interventions, and increased healthcare costs. The development of effective regenerative nanomedicine has the potential to significantly reduce these economic burdens by expediting the healing process and minimizing the need for extensive medical care.

Catalysing pharmaceutical/biotechnology industry: Innovations in nanomedicine open new avenues for the pharmaceutical/biotechnology industry. The successful translation of research into practical solutions not only benefits patients but also stimulates economic growth through the development and commercialization of novel medical technologies.

1.8.3 Scientific Advancement

Pushing boundaries of nanotechnology: The synthesis of polymeric NPs from glycine and the subsequent enhancement of their properties through nitric oxide loading represents a significant leap in the application of nanotechnology to address medical challenges.

Contribution to regenerative medicine: This research contributes to the broader field of regenerative medicine, offering insights into the design and optimization of nanomaterials for therapeutic purposes. These advancements pave the way for future breakthroughs in treating a variety of medical conditions.

1.8.4 Ethical Considerations

Minimizing animal testing: Using rat models to test WH potential underscores a commitment to ethical research practices. The ultimate goal is to translate laboratory successes into clinically viable treatments, minimizing the reliance on animal testing in

the long run.

In conclusion, the motivation behind the doing research work on regenerative nanomedicine lies in its potential to address a pressing medical need, improve patient outcomes, reduce economic burdens associated with chronic wounds, advance scientific understanding of nanotechnology applications, and adhere to ethical research practices. The success of this project holds promise for transforming healthcare and making a positive impact on individuals and societies around the world.

1.9 STATEMENT OF PROBLEM (EXISTING CHALLENGES AND RESEARCH GAPS)

WH is a complex physiological process crucial for the restoration of tissue integrity and functionality [71]. While significant progress has been made in understanding the mechanisms involved in wound repair, achieving optimal WH remains a challenging endeavor [64]. This problem definition aims to address the existing challenges and identify the research gap in the pursuit of enhancing and expediting the WH process.

1.9.1 Existing Challenges

1.9.1.1 Chronic wounds

Chronic wounds, such as diabetic ulcers and venous ulcers, pose a substantial challenge due to impaired healing processes [228]. Factors contributing to chronicity include prolonged inflammation, microbial colonization, and compromised tissue repair mechanisms [131].

1.9.1.2 Infection and microbial resistance

Wound infections, both acute and chronic, hinder the healing process [158]. The emergence of antimicrobial resistance further complicates treatment options, necessitating innovative approaches to control infections and promote healing [229].

1.9.1.3 Scar formation

Excessive or abnormal scar formation remains a significant concern [229]. Keloid and hypertrophic scarring can lead to functional impairment and aesthetic issues, emphasizing the need for interventions that modulate the scarring process [66, 167, 230].

1.9.1.4 Limited therapeutic options

Current therapeutic interventions, while effective to some extent, exhibit limitations. Traditional wound care methods, such as dressings and topical agents, may not be sufficient for complex wounds. There is a need for novel, targeted therapies that address specific aspects of the WH cascade [11].

1.9.1.5 Patient-specific variability

The response to WH interventions varies among individuals. Factors such as age, comorbidities, and genetic predispositions contribute to patient-specific variability, necessitating personalized approaches for optimal outcomes [11, 37].

1.9.2 Research Gap

Despite ongoing research efforts, several critical gaps persist in the understanding and application of WH strategies.

1.9.2.1 Mechanistic insights

Comprehensive elucidation of the intricate molecular and cellular mechanisms governing WH is lacking. A more in-depth understanding of signal transduction pathways, immune responses, and the interplay of various cell types is essential for targeted therapeutic development [64].

1.9.2.2 Advanced biomaterials and drug delivery

Exploration of advanced biomaterials and innovative drug delivery systems for

regenerative medicine and wound care is an area with untapped potential [224]. Developing smart materials and delivery systems such as self-healing hydrogel that respond dynamically to the wound environment and shows antiadhesion property and controlled drug release systems can revolutionize treatment modalities [120, 231].

1.9.2.3 Limitations in regenerative medicine approaches

Harnessing the full potential of regenerative medicine, including stem cell therapy and TE, requires further exploration [224]. Optimizing these approaches for practical clinical applications and addressing associated ethical and regulatory challenges are critical for advancing WH strategies [134, 167].

1.9.2.4 Limitations in integrated multidisciplinary approaches

Integrating knowledge from diverse fields such as immunology, microbiology, bioengineering, and data science is crucial [7, 36, 126]. Collaborative efforts that bridge the gap between basic research and clinical application can lead to holistic and effective WH solutions [188].

These problem definition underscores the complexity of achieving optimum WH and emphasizes the need for multidisciplinary research efforts to address existing challenges and bridge the current gap in knowledge and therapeutic options. Therefore, current health care demands a suitable materials and methods further to achieve rapid healing of the wounded skin/tissues and hence there is a huge scope to research in this direction.

As discussed ealier, WH is a dynamic and intricate biological process crucial for tissue restoration. Despite significant advancements in understanding the fundamental mechanisms, achieving optimal WH remains a complex challenge. Literature review revealed the insights into the existing challenges, research gap, and explore polymers exhibiting intrinsic WH activity, focusing on their effect on WH is based on the following

points.

Improvement in the treatment of chronic wounds: Chronic wounds, characterized by prolonged inflammation and impaired healing, continue to present a significant clinical challenge. Factors such as the dysregulation of growth factors, extracellular matrix components, and cellular senescence can contribute to the repair of chronicity the wounds [1]. Huang, Yang et al. developed a multifunctional sprayable NO releasing nanogel which can treat infected diabetic wound [232] but having the Ag, prolonged used can arise Ag resistant microbes and can stain the area of application.

Reduction of infection and microbial resistance: Infections significantly impede WH, and the rise of antimicrobial resistance poses an additional hurdle. Biofilm formation by bacteria in chronic wounds further complicates treatment [228]. There is a pressing need for novel antimicrobial strategies that combat resistant strains. Peng, Li et al. developed an strategy which synergistically can release NO and eradicates biofilm formed by infection causing bacteria by the use of photothermal mechanism [233]. Phototheramal therapy requires laser light, can cause damage to newly regenerated tissue.

Repair the scar formation: Abnormal scar formation, leading to keloid or hypertrophic scars, remains a concern. Mechanisms regulating fibroblast activity and collagen synthesis need further exploration to develop targeted interventions [230]. NO induced wound closure leads to no or negligible scar formation [234].

Increase the therapeutic options: Current wound care methods, including dressings and topical agents, may not be sufficient for complex wounds. Therefore, the demands for advanced therapeutics capable of addressing specific aspects of the WH cascade that can be developed [181].

Improve the patient-specific variability: Variability in patient response to WH

interventions emphasizes the need for personalized approaches. The integration of genetic and clinical data may provide insights into individualized treatment strategies [235].

Molecular signalling of wound healing: A profound grasp of the molecular and cellular mechanisms that regulate the healing of wounds is essential. Progress in technologies like single-cell sequencing and approaches in systems biology can aid in deciphering the intricacies of these processes [236].

Development of advanced biomaterials and drug delivery: Developing smart biomaterials and targeted drug delivery systems is an evolving field. Research should focus on designing materials that respond dynamically to the wound microenvironment, releasing therapeutic agents in a controlled manner [237].

Advancement in regenerative medicine: Regenerative medicine holds promise for WH, but translating these approaches into clinical settings requires further investigation. Overcoming challenges related to the scalability, safety, and ethical considerations of stem cell therapies is imperative [238].

Integration of therapeutic approaches: Collaborative efforts across disciplines are essential. Integrating immunology, microbiology, bioengineering, and data science can lead to a more holistic understanding of WH and facilitate the development of effective therapeutic strategies [239].

Development of polymers with intrinsic regenerative potentials: Certain polymers have demonstrated inherent WH properties, making them a prime focus for rapid WH. These polymers exhibit antimicrobial activity, accelerate wound closure, provide a moist environment, and offer high water content and biocompatibility with suitable mechanical strength stability in the living body.

(i) Polymer Exhibits antimicrobial activity and accelerates wound closure.

Drawbacks include poor mechanical properties and variability in sourcing [240].

(ii) They Provide a moist environment for WH. Challenges include limited structural integrity and the potential for rapid degradation [103].

(iii) Polymeric hydrogels offer high water content and biocompatibility with suitable mechanical strength stability in living body [241].

Thus on the basis of above literature review, achieving optimal WH is a complex challenge that requires multidisciplinary research efforts to address existing challenges and bridge current gaps in knowledge and therapeutic options. Current healthcare demands suitable materials and methods to achieve rapid healing of wounded skin/tissues, creating a significant scope for research in this direction.

1.10 OBJECTIVES

On the basis of above challenges observed in WH and identified research gap, we have formulated the following objectives, which are further subdivided into major and minor objectives. These major and minor objectives collectively form a comprehensive research framework aimed at advancing the understanding of WH, addressing existing challenges, and optimizing therapeutic strategies for improved patient outcomes.

1.10.1 Objective 1:

"Development and evaluation of biopolymeric PNAG NPs for advanced skin regeneration in wound healing"

Justification: The objective is to design and assess poly(N-acryloyl glycine) NPs (PNAG NPs) as a biocompatible and biodegradable platform for skin regeneration. The inherent WH and regenerative properties of PNAG make it an ideal candidate for fostering tissue repair.

1.10.2 Objective 2:

"Potentiation of wound healing activity of PNAG NPs with NO for enhanced skin regeneration"

Justification: This objective aims to augment the WH potential of PNAG NPs by incorporating NO, known for its regenerative effects. The loaded NPs, referred to as SP NPs, are expected to exhibit superior skin regeneration properties.

1.10.3 Objective 3:

"Comprehensive biological activity assessment of metal oxides in wound healing"

Justification: This objective seeks to investigate the biological activity of various metal oxides (ZnO, ZnO-SnO_2, ZnO-Ag_2O/Ag, ZnO-CuO) with a focus on haemolysis, cell migration, viability, and angiogenesis. Understanding the impact of these metal oxides on WH processes is crucial for potential therapeutic applications.

This research addresses the imperative need for advanced WH solutions by developing and enhancing biopolymeric PNAG NPs, potentiating them with NO, and comprehensively evaluating the biological activity of metal oxides. The objectives aim to contribute to the field of skin regeneration, providing potential therapeutic avenues for WH applications. Our research work will delve into the intricacies of WH mechanisms, employing an exhaustive literature review and analysis to elucidate the interplay of molecular and cellular players in this vital process.

Identification and addressing of existing challenges also will be addresses. Existing challenges in WH, including chronic wounds, infection management, scar formation, and patient-specific variability will be identify and critically evaluated.

1.11 REFERENCES

1. Sen, C.K., et al., *Human skin wounds: a major and snowballing threat to public health and the economy.* Wound Repair Regen, 2009. **17**(6): p. 763-71. DOI: 10.1111/j.1524-475X.2009.00543.x.
2. Chakraborty, C., B. Gupta, and S.K. Ghosh, *Mobile metadata assisted community database of chronic wound images.* Wound Medicine, 2014. **6**: p. 34-42. DOI: 10.1016/j.wndm.2014.09.002.
3. Rastein, D. *Wounded: The Other Ongoing Global Pandemic*. 2020 09-07-2023 [cited 2023 16-11-2023]; Available from: https://www.thestreet.com/csuiteadvisors/health-and-wellness/daniel-rastein-csa-world-health-organization-says-were-in-aa-wound-pandemic.
4. Broughton, G., 2nd, J.E. Janis, and C.E. Attinger, *Wound healing: an overview.* Plast Reconstr Surg, 2006. **117**(7 Suppl): p. 1e-S-32e-S. DOI: 10.1097/01.prs.0000222562.60260.f9.
5. WHO. *10 global health issues to track in 2021*. 2020 [cited 2023 16-11-2023]; Available from: https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021.
6. WHO. *Protocol for surgical site infection surveillance with a focus on settings with limited resources*. 2018 [cited 2023 10/11/2023]; Available from: https://cdn.who.int/media/docs/default-source/integrated-health-services-(ihs)/ipc/ssi-surveillance-protocol.pdf?sfvrsn=d24a1d1c_3&download=true.
7. Raziyeva, K., et al., *Immunology of Acute and Chronic Wound Healing.* Biomolecules, 2021. **11**(5). DOI: 10.3390/biom11050700.
8. Onyekwelu, I., et al., *Surgical Wound Classification and Surgical Site Infections in the Orthopaedic Patient.* J Am Acad Orthop Surg Glob Res Rev, 2017. **1**(3): p. e022. DOI: 10.5435/JAAOSGlobal-D-17-00022.
9. WHO. *Prevention and management of wound infection*. 2013 [cited 2023 15/11/2023]; Available from: https://www.who.int/publications/i/item/prevention-and-management-of-wound-infection.
10. WHO. *Global strategy on digital health 2020-2025*. 2021 [cited 2023 15/11/2023]; Available from: https://www.who.int/docs/default-source/documents/gs4dhdaa2a9f352b0445bafbc79ca799dce4d.pdf.
11. Lindholm, C. and R. Searle, *Wound management for the 21st century: combining effectiveness and efficiency.* Int Wound J, 2016. **13 Suppl 2**(Suppl 2): p. 5-15. DOI: 10.1111/iwj.12623.
12. EC, *Horizon Europe - Work Programme 2023-2024 Health*, in *(European Commission Decision C(2023) 2178 of 31 March 2023)*. 2023, European Commission.
13. Andermann, A. and C. Collaboration, *Taking action on the social determinants of health in clinical practice: a framework for health professionals.* CMAJ, 2016. **188**(17-18): p. E474-E483. DOI: https://doi.org/10.1503/cmaj.160177.

14. WHO. *Social Determinants of Health*. [cited 2023 16-11-2023]; Available from: https://www.who.int/health-topics/social-determinants-of-health#tab=tab_1. DOI: https://doi.org/.

15. Kolimi, P., et al., *Innovative Treatment Strategies to Accelerate Wound Healing: Trajectory and Recent Advancements.* Cells, 2022. **11**(15). DOI: 10.3390/cells11152439.

16. WHO. *Global partners commit to advance evidence-based traditional, complementary and integrative medicine*. 2023 [cited 2023 16-11-2023]; Available from: https://www.who.int/news/item/19-08-2023-global-partners-commit-to-advance-evidence-based-traditional--complementary-and-integrative-medicine.

17. Zaidi, Z. and S.W. Lanigan, *Skin: Structure and Function*, in *Dermatology in Clinical Practice*, S.W. Lanigan and Z. Zaidi, Editors. 2010, Springer London: London. p. 1-15. DOI: 10.1007/978-1-84882-862-9_1.

18. Matsui, T. and M. Amagai, *Dissecting the formation, structure and barrier function of the stratum corneum.* Int Immunol, 2015. **27**(6): p. 269-80. DOI: 10.1093/intimm/dxv013.

19. Cichorek, M., et al., *Skin melanocytes: biology and development.* Postepy Dermatol Alergol, 2013. **30**(1): p. 30-41. DOI: 10.5114/pdia.2013.33376.

20. Afshar, M. and R.L. Gallo, *Innate immune defense system of the skin.* Vet Dermatol, 2013. **24**(1): p. 32-8 e8-9. DOI: https://doi.org/10.1111/j.1365-3164.2012.01082.x.

21. Burr, S. and R. Penzer, *Promoting skin health.* Nurs Stand, 2005. **19**(36): p. 57-65; quiz 66. DOI: 10.7748/ns2005.05.19.36.57.c3871.

22. Lai-Cheong, J.E. and J.A. McGrath, *Structure and function of skin, hair and nails.* Medicine, 2017. **45**(6): p. 347-351. DOI: 10.1016/j.mpmed.2017.03.004.

23. Schommer, N.N. and R.L. Gallo, *Structure and function of the human skin microbiome.* Trends Microbiol, 2013. **21**(12): p. 660-8. DOI: 10.1016/j.tim.2013.10.001.

24. Schmid-Wendtner, M.H. and H.C. Korting, *The pH of the skin surface and its impact on the barrier function.* Skin Pharmacol Physiol, 2006. **19**(6): p. 296-302. DOI: 10.1159/000094670.

25. Fluhr, J.W. and P.M. Elias, *Stratum corneum pH: Formation and Function of the 'Acid Mantle'.* Exogenous Dermatology, 2002. **1**(4): p. 163-175. DOI: 10.1159/000066140.

26. Basler, K., et al., *The role of tight junctions in skin barrier function and dermal absorption.* J Control Release, 2016. **242**: p. 105-118. DOI: https://doi.org/10.1016/j.jconrel.2016.08.007.

27. Jenssen, H., P. Hamill, and R.E. Hancock, *Peptide antimicrobial agents.* Clin Microbiol Rev, 2006. **19**(3): p. 491-511. DOI: 10.1128/CMR.00056-05.

28. Erdag, G. and J.R. Morgan, *Interleukin-1alpha and interleukin-6 enhance the antibacterial properties of cultured composite keratinocyte grafts.* Ann Surg, 2002. **235**(1): p. 113-24. DOI: 10.1097/00000658-200201000-00015.

29. Mai, W., et al., *MCP-1 produced by keratinocytes is associated with leucocyte recruitment during elicitation of nickel-induced occupational allergic contact dermatitis.* Toxicol Ind Health, 2018. **34**(1): p. 36-43. DOI: 10.1177/0748233717738633.

30. Tuzun, Y., et al., *Keratinocyte cytokine and chemokine receptors.* Dermatol Clin, 2007. **25**(4): p. 467-76, vii. DOI: 10.1016/j.det.2007.06.003.

31. Dorschner, R.A., et al., *Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus.* J Invest Dermatol, 2001. **117**(1): p. 91-7. DOI: 10.1046/j.1523-1747.2001.01340.x.

32. Grone, A., *Keratinocytes and cytokines.* Vet Immunol Immunopathol, 2002. **88**(1-2): p. 1-12. DOI: 10.1016/s0165-2427(02)00136-8.

33. Grice, E.A. and J.A. Segre, *The skin microbiome.* Nat Rev Microbiol, 2011. **9**(4): p. 244-53. DOI: 10.1038/nrmicro2537.

34. Young, A. and C.-E. McNaught, *The physiology of wound healing.* Surgery (Oxford), 2011. **29**(10): p. 475-479. DOI: 10.1016/j.mpsur.2011.06.011.

35. Thiruvoth, F., et al., *Current concepts in the physiology of adult wound healing.* Plastic and Aesthetic Research, 2015. **2**(5): p. 250-256. DOI: 10.4103/2347-9264.158851.

36. Ellis, S., E.J. Lin, and D. Tartar, *Immunology of Wound Healing.* Curr Dermatol Rep, 2018. **7**(4): p. 350-358. DOI: 10.1007/s13671-018-0234-9.

37. Dryden, S.V., W.G. Shoemaker, and J.H. Kim, *Wound management and nutrition for optimal wound healing.* Atlas Oral Maxillofac Surg Clin North Am, 2013. **21**(1): p. 37-47. DOI: 10.1016/j.cxom.2012.12.008.

38. Beldon, P., *Basic science of wound healing.* Surgery (Oxford), 2010. **28**(9): p. 409-412. DOI: https://doi.org/10.1016/j.mpsur.2010.05.007.

39. Kurahashi, T. and J. Fujii, *Roles of Antioxidative Enzymes in Wound Healing.* Journal of Developmental Biology, 2015. **3**(2): p. 57-70. DOI: 10.3390/jdb3020057.

40. Guo, S. and L.A. Dipietro, *Factors affecting wound healing.* J Dent Res, 2010. **89**(3): p. 219-29. DOI: 10.1177/0022034509359125.

41. Futosi, K., S. Fodor, and A. Mocsai, *Neutrophil cell surface receptors and their intracellular signal transduction pathways.* Int Immunopharmacol, 2013. **17**(3): p. 638-50. DOI: 10.1016/j.intimp.2013.06.034.

42. Kono, H. and K.L. Rock, *How dying cells alert the immune system to danger.* Nat Rev Immunol, 2008. **8**(4): p. 279-89. DOI: 10.1038/nri2215.

43. Zhao, R., et al., *Inflammation in Chronic Wounds.* Int J Mol Sci, 2016. **17**(12). DOI: 10.3390/ijms17122085.

44. Borregaard, N., O.E. Sorensen, and K. Theilgaard-Monch, *Neutrophil granules: a library of innate immunity proteins.* Trends Immunol, 2007. **28**(8): p. 340-5. DOI: 10.1016/j.it.2007.06.002.

45. Wilgus, T.A., S. Roy, and J.C. McDaniel, *Neutrophils and Wound Repair: Positive Actions and Negative Reactions.* Adv Wound Care (New Rochelle), 2013. **2**(7): p. 379-388. DOI: 10.1089/wound.2012.0383.

46. Koh, T.J. and L.A. DiPietro, *Inflammation and wound healing: the role of the macrophage.* Expert Rev Mol Med, 2011. **13**: p. e23. DOI: 10.1017/S1462399411001943.

47. Kotwal, G.J. and S. Chien, *Macrophage Differentiation in Normal and Accelerated Wound Healing.* Results Probl Cell Differ, 2017. **62**: p. 353-364. DOI: 10.1007/978-3-319-54090-0_14.

48. Mosser, D.M., *The many faces of macrophage activation.* J Leukoc Biol, 2003. **73**(2): p. 209-12. DOI: 10.1189/jlb.0602325.

49. Greenlee-Wacker, M.C., *Clearance of apoptotic neutrophils and resolution of inflammation.* Immunol Rev, 2016. **273**(1): p. 357-70. DOI: 10.1111/imr.12453.

50. Bratton, D.L. and P.M. Henson, *Neutrophil clearance: when the party is over, clean-up begins.* Trends Immunol, 2011. **32**(8): p. 350-7. DOI: 10.1016/j.it.2011.04.009.

51. Landen, N.X., D. Li, and M. Stahle, *Transition from inflammation to proliferation: a critical step during wound healing.* Cell Mol Life Sci, 2016. **73**(20): p. 3861-85. DOI: 10.1007/s00018-016-2268-0.

52. Midwood, K.S., L.V. Williams, and J.E. Schwarzbauer, *Tissue repair and the dynamics of the extracellular matrix.* Int J Biochem Cell Biol, 2004. **36**(6): p. 1031-7. DOI: 10.1016/j.biocel.2003.12.003.

53. Ilina, O. and P. Friedl, *Mechanisms of collective cell migration at a glance.* J Cell Sci, 2009. **122**(Pt 18): p. 3203-8. DOI: 10.1242/jcs.036525.

54. Webb, D.J., J.T. Parsons, and A.F. Horwitz, *Adhesion assembly, disassembly and turnover in migrating cells -- over and over and over again.* Nat Cell Biol, 2002. **4**(4): p. E97-100. DOI: 10.1038/ncb0402-e97.

55. Li, B. and J.H. Wang, *Fibroblasts and myofibroblasts in wound healing: force generation and measurement.* J Tissue Viability, 2011. **20**(4): p. 108-20. DOI: 10.1016/j.jtv.2009.11.004.

56. Hopkinson, S.B., et al., *Focal Contact and Hemidesmosomal Proteins in Keratinocyte Migration and Wound Repair.* Adv Wound Care (New Rochelle), 2014. **3**(3): p. 247-263. DOI: 10.1089/wound.2013.0489.

57. Ronfard, V. and Y. Barrandon, *Migration of keratinocytes through tunnels of digested fibrin.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4504-9. DOI: 10.1073/pnas.071631698.

58. DiPietro, L.A., *Angiogenesis and wound repair: when enough is enough.* J Leukoc Biol, 2016. **100**(5): p. 979-984. DOI: 10.1189/jlb.4MR0316-102R.

59. Schreml, S., et al., *Oxygen in acute and chronic wound healing.* Br J Dermatol, 2010. **163**(2): p. 257-68. DOI: 10.1111/j.1365-2133.2010.09804.x.

60. Betz, C., et al., *Cell behaviors and dynamics during angiogenesis.* Development, 2016. **143**(13): p. 2249-60. DOI: https://doi.org/10.1242/dev.135616.

61. Chappell, J.C., D.M. Wiley, and V.L. Bautch, *Regulation of blood vessel sprouting.* Semin Cell Dev Biol, 2011. **22**(9): p. 1005-11. DOI: 10.1016/j.semcdb.2011.10.006.

62. van Hinsbergh, V.W. and P. Koolwijk, *Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead.* Cardiovasc Res, 2008. **78**(2): p. 203-12. DOI: 10.1093/cvr/cvm102.

63. Tracy, L.E., R.A. Minasian, and E.J. Caterson, *Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound.* Adv Wound Care (New Rochelle), 2016. **5**(3): p. 119-136. DOI: 10.1089/wound.2014.0561.

64. Velnar, T., T. Bailey, and V. Smrkolj, *The wound healing process: an overview of the cellular and molecular mechanisms.* J Int Med Res, 2009. **37**(5): p. 1528-42. DOI: 10.1177/147323000903700531.

65. Darby, I.A., et al., *Fibroblasts and myofibroblasts in wound healing.* Clin Cosmet Investig Dermatol, 2014. **7**: p. 301-11. DOI: 10.2147/CCID.S50046.

66. Gonzalez, A.C., et al., *Wound healing - A literature review.* An Bras Dermatol, 2016. **91**(5): p. 614-620. DOI: 10.1590/abd1806-4841.20164741.

67. Desmouliere, A., et al., *Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar.* Am J Pathol, 1995. **146**(1): p. 56-66.

68. Hinz, B. and G. Gabbiani, *Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling.* Thromb Haemost, 2003. **90**(6): p. 993-1002. DOI: 10.1160/TH03-05-0328.

69. DiPietro, L.A., *Angiogenesis and scar formation in healing wounds.* Curr Opin Rheumatol, 2013. **25**(1): p. 87-91. DOI: 10.1097/BOR.0b013e32835b13b6.

70. Xue, M. and C.J. Jackson, *Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring.* Adv Wound Care (New Rochelle), 2015. **4**(3): p. 119-136. DOI: 10.1089/wound.2013.0485.

71. Alven, S., et al., *Polymer-Based Wound Dressing Materials Loaded with Bioactive Agents: Potential Materials for the Treatment of Diabetic Wounds.* Polymers (Basel), 2022. **14**(4): p. 724. DOI: https://doi.org/10.3390/polym14040724.

72. Nandhini, J., E. Karthikeyan, and S. Rajeshkumar, *Nanomaterials for wound healing: Current status and futuristic frontier.* Biomedical Technology, 2024. **6**: p. 26-45. DOI: https://doi.org/10.1016/j.bmt.2023.10.001.

73. Sheokand, B., et al., *Natural polymers used in the dressing materials for wound healing: Past, present and future.* Journal of Polymer Science, 2023. **61**(14): p. 1389-1414. DOI: 10.1002/pol.20220734.

74. Xu, R., et al., *Recent Advances in Biodegradable and Biocompatible Synthetic Polymers Used in Skin Wound Healing.* Materials, 2023. **16**(15). DOI: 10.3390/ma16155459.

75. Mohapatra, B. and T.R. Rautray, *Ceramic coatings for wound healing applications*, in *Advanced Ceramic Coatings for Biomedical Applications*, R.K. Gupta, et al., Editors. 2023, Elsevier. p. 269-292. DOI: 10.1016/B978-0-323-99626-6.00004-4.

76. Tavoni, M., et al., *Bioactive Calcium Phosphate-Based Composites for Bone Regeneration.* Journal of Composites Science, 2021. **5**(9). DOI: 10.3390/jcs5090227.

77. Liang, Y., et al., *Antibacterial biomaterials for skin wound dressing.* Asian Journal of Pharmaceutical Sciences, 2022. **17**(3): p. 353-384. DOI: https://doi.org/10.1016/j.ajps.2022.01.001.

78. Nosrati, H., et al., *Nanocomposite scaffolds for accelerating chronic wound healing by enhancing angiogenesis.* J Nanobiotechnology, 2021. **19**(1): p. 1. DOI: 10.1186/s12951-020-00755-7.

79. Batool, Z., et al., *Hydrogel assisted synthesis of gold nanoparticles with enhanced microbicidal and in vivo wound healing potential.* Scientific Reports, 2022. **12**(1): p. 6575. DOI: https://doi.org/10.1038/s41598-022-10495-3.

80. Ur Rehman, S.R., et al., *Graphene Oxide Loaded Hydrogel for Enhanced Wound Healing in Diabetic Patients.* Annu Int Conf IEEE Eng Med Biol Soc, 2019. **2019**: p. 3943-3946. DOI: 10.1109/embc.2019.8857341.

81. Mukherjee, S., et al., *Graphene Oxides Show Angiogenic Properties.* Adv Healthc Mater, 2015. **4**(11): p. 1722-32. DOI: 10.1002/adhm.201500155.

82. Kittana, N., et al., *Enhancement of wound healing by single-wall/multi-wall carbon nanotubes complexed with chitosan.* Int J Nanomedicine, 2018. **13**: p. 7195-7206. DOI: 10.2147/ijn.S183342.

83. Elderdery, A.Y., et al., *Structural, Optical, Antibacterial, and Anticancer Properties of Cerium Oxide Nanoparticles Prepared by Green Synthesis Using Morinda citrifolia Leaves Extract.* Bioinorganic Chemistry and Applications, 2022. **2022**: p. 6835625. DOI: 10.1155/2022/6835625.

84. Haleem, A., et al., *Applications of nanotechnology in medical field: a brief review.* Global Health Journal, 2023. **7**(2): p. 70-77. DOI: 10.1016/j.glohj.2023.02.008.

85. Xu, L., et al., *Silver nanoparticles: Synthesis, medical applications and biosafety.* Theranostics, 2020. **10**(20): p. 8996-9031. DOI: 10.7150/thno.45413.

86. Wang, W., et al., *Nano-drug delivery systems in wound treatment and skin regeneration.* J Nanobiotechnology, 2019. **17**(1): p. 82. DOI: 10.1186/s12951-019-0514-y.

87. Gupta, P.S., et al., *In vivo potential of polymeric N-acryloyl-glycine nanoparticles with anti-inflammatory activities for wound healing.* Materials Advances, 2023. **4**(20): p. 4718-4731. DOI: 10.1039/D3MA00378G.

88. Singh, H., et al., *Ecofriendly synthesis of silver and gold nanoparticles by Euphrasia officinalis leaf extract and its biomedical applications.* Artificial Cells, Nanomedicine, and Biotechnology, 2018. **46**(6): p. 1163-1170. DOI: 10.1080/21691401.2017.1362417.

89. Guisbiers, G., S. Mejía-Rosales, and F. Leonard Deepak, *Nanomaterial Properties: Size and Shape Dependencies.* Journal of Nanomaterials, 2012. **2012**: p. 1-2. DOI: 10.1155/2012/180976.

90. Mitchell, M.J., et al., *Engineering precision nanoparticles for drug delivery.* Nat Rev Drug Discov, 2021. **20**(2): p. 101-124. DOI: 10.1038/s41573-020-0090-8.

91. Payal and P. Pandey, *Role of Nanotechnology in Electronics: A Review of Recent Developments and Patents.* Recent Pat Nanotechnol, 2022. **16**(1): p. 45-66. DOI: 10.2174/1872210515666210120114504.

92. Stark, W.J., et al., *Industrial applications of nanoparticles.* Chem Soc Rev, 2015. **44**(16): p. 5793-805. DOI: 10.1039/c4cs00362d.

93. Patil, S.S., et al., *Nanoparticles for environmental clean-up: A review of potential risks and emerging solutions.* Environmental Technology & Innovation, 2016. **5**: p. 10-21. DOI: 10.1016/j.eti.2015.11.001.

94. Kouhi, M., M.P. Prabhakaran, and S. Ramakrishna, *Edible polymers: An insight into its application in food, biomedicine and cosmetics.* Trends in Food Science & Technology, 2020. **103**: p. 248-263. DOI: 10.1016/j.tifs.2020.05.025.

95. Choi, J.U., et al., *Preparation and in vivo evaluation of cationic elastic liposomes comprising highly skin-permeable growth factors combined with hyaluronic acid for enhanced diabetic wound-healing therapy.* Acta Biomaterialia, 2017. **57**: p. 197-215. DOI: https://doi.org/10.1016/j.actbio.2017.04.034.

96. Rabbani, P.S., et al., *Novel lipoproteoplex delivers Keap1 siRNA based gene therapy to accelerate diabetic wound healing.* Biomaterials, 2017. **132**: p. 1-15. DOI: https://doi.org/10.1016/j.biomaterials.2017.04.001.

97. de Souza, M.L., et al., *Lipid Nanoparticles as a Skin Wound Healing Drug Delivery System: Discoveries and Advances.* Curr Pharm Des, 2020. **26**(36): p. 4536-4550. DOI: 10.2174/1381612826666200417144530.

98. Chen, H., et al., *An injectable self-healing coordinative hydrogel with antibacterial and angiogenic properties for diabetic skin wound repair.* NPG Asia Materials, 2019. **11**(1): p. 3. DOI: 10.1038/s41427-018-0103-9.

99. Dhandayuthapani, B., et al., *Polymeric Scaffolds in Tissue Engineering Application: A Review.* International Journal of Polymer Science, 2011. **2011**: p. 1-19. DOI: 10.1155/2011/290602.

100. Narayana, S., et al., *Potential benefits of using chitosan and silk fibroin topical hydrogel for managing wound healing and coagulation.* Saudi Pharm J, 2023. **31**(3): p. 462-471. DOI: 10.1016/j.jsps.2023.01.013.

101. Paswan, S.K., et al., *Designing and Formulation Optimization of Hyaluronic Acid Conjugated PLGA Nanoparticles of Tamoxifen for Tumor Targeting.* Pharm Nanotechnol, 2021. **9**(3): p. 217-235. DOI: 10.2174/2211738509666210310155807.

102. Ilomuanya, M.O., et al., *Polylactic acid-based electrospun fiber and hyaluronic acid-valsartan hydrogel scaffold for chronic wound healing.* Beni-Suef University Journal of Basic and Applied Sciences, 2020. **9**(1): p. 31. DOI: 10.1186/s43088-020-00057-9.

103. Lee, K.Y. and D.J. Mooney, *Alginate: properties and biomedical applications.* Prog Polym Sci, 2012. **37**(1): p. 106-126. DOI: 10.1016/j.progpolymsci.2011.06.003.

104. Cao, Y., et al., *A review on the synthesis and development of alginate hydrogels for wound therapy.* J Mater Chem B, 2023. **11**(13): p. 2801-2829. DOI: 10.1039/d2tb02808e.

105. Singh, D., et al., *Synthesis of composite gelatin-hyaluronic acid-alginate porous scaffold and evaluation for in vitro stem cell growth and in vivo tissue integration.* Colloids Surf B Biointerfaces, 2014. **116**: p. 502-9. DOI: 10.1016/j.colsurfb.2014.01.049.

106. Wang, L., et al., *Recent advances in keratin for biomedical applications.* Adv Colloid Interface Sci, 2023. **321**: p. 103012. DOI: 10.1016/j.cis.2023.103012.

107. Shi, S., et al., *Recent progresses of collagen dressings for chronic skin wound healing.* Collagen and Leather, 2023. **5**(1): p. 31. DOI: 10.1186/s42825-023-00136-4.

108. Ndlovu, S.P., et al., *Gelatin-Based Hybrid Scaffolds: Promising Wound Dressings.* Polymers, 2021. **13**(17). DOI: 10.3390/polym13172959.

109. Prete, S., et al., *Natural and Synthetic Polymeric Biomaterials for Application in Wound Management.* J Funct Biomater, 2023. **14**(9): p. 455. DOI: 10.3390/jfb14090455.

110. Zhang, X., et al., *Advances in wound dressing based on electrospinning nanofibers.* Journal of Applied Polymer Science, 2023. **141**(1): p. e54746. DOI: 10.1002/app.54746.

111. Dorkhani, E., et al., *Biomedical properties and hemostatic efficacy of polyvinyl alcohol (PVA) based hydrogel in experimental rat liver injury model.* J Appl Biomater Funct Mater, 2023. **21**: p. 22808000231198803. DOI: 10.1177/22808000231198803.

112. Grande-Tovar, C.D., et al., *Chitosan–Polyvinyl Alcohol Nanocomposites for Regenerative Therapy.* Polymers, 2023. **15**(23): p. 4595. DOI: 10.3390/polym15234595.

113. Teng, Q., et al., *Self-healing polyurethane elastomer with ultra-high mechanical strength and enhanced thermal mechanical properties.* Polymer, 2024. **290**: p. 126579. DOI: 10.1016/j.polymer.2023.126579.

114. Castaneda-Rodriguez, S., et al., *Recent advances in modified poly (lactic acid) as tissue engineering materials.* J Biol Eng, 2023. **17**(1): p. 21. DOI: 10.1186/s13036-023-00338-8.

115. Lu, Y., et al., *Properties of Poly (Lactic-co-Glycolic Acid) and Progress of Poly (Lactic-co-Glycolic Acid)-Based Biodegradable Materials in Biomedical Research.* Pharmaceuticals (Basel), 2023. **16**(3): p. 454. DOI: 10.3390/ph16030454.

116. Dobrzański, L.A., et al., *Biodegradable and antimicrobial polycaprolactone nanofibers with and without silver precipitates.* Archives of materials science and engineering, 2015. **76**.

117. Zhang, S., et al., *Transparent, High-Strength, and Antimicrobial Polyvinyl Alcohol/Boric Acid/Poly Hexamethylene Guanidine Hydrochloride Films.* Coatings, 2023. **13**(6). DOI: 10.3390/coatings13061115.

118. Deng, X., M. Gould, and M.A. Ali, *A review of current advancements for wound healing: Biomaterial applications and medical devices.* J Biomed Mater Res B Appl Biomater, 2022. **110**(11): p. 2542-2573. DOI: 10.1002/jbm.b.35086.

119. Sun, G. and J.J. Mao, *Engineering dextran-based scaffolds for drug delivery and tissue repair.* Nanomedicine (Lond), 2012. **7**(11): p. 1771-84. DOI: 10.2217/nnm.12.149.

120. Pang, Q., et al., *Smart wound dressing for advanced wound management: Real-time monitoring and on-demand treatment.* Materials & Design, 2023. **229**: p. 111917. DOI: 10.1016/j.matdes.2023.111917.

121. Das, P., et al., *Polymeric biomaterials-based tissue engineering for wound healing: a systemic review.* Burns Trauma, 2023. **11**: p. tkac058. DOI: 10.1093/burnst/tkac058.

122. Abazari, M.F., et al., *Recent Advances in Cellulose-Based Structures as the Wound-Healing Biomaterials: A Clinically Oriented Review.* Applied Sciences, 2021. **11**(17). DOI: https://doi.org/10.3390/app11177769.

123. Oliveira, C., et al., *Polymeric biomaterials for wound healing.* Front Bioeng Biotechnol, 2023. **11**: p. 1136077. DOI: 10.3389/fbioe.2023.1136077.

124. Croisier, F. and C. Jérôme, *Chitosan-based biomaterials for tissue engineering.* European Polymer Journal, 2013. **49**(4): p. 780-792. DOI: 10.1016/j.eurpolymj.2012.12.009.

125. Frenkel, J.S., *The role of hyaluronan in wound healing.* Int Wound J, 2014. **11**(2): p. 159-63. DOI: 10.1111/j.1742-481X.2012.01057.x.

126. Sánchez-López, E., et al., *Metal-Based Nanoparticles as Antimicrobial Agents: An Overview.* Nanomaterials, 2020. **10**(2). DOI: 10.3390/nano10020292.

127. Maťátková, O., et al., *Antimicrobial properties and applications of metal nanoparticles biosynthesized by green methods.* Biotechnology Advances, 2022. **58**: p. 107905. DOI: https://doi.org/10.1016/j.biotechadv.2022.107905.

128. Dykman, L.A. and N.G. Khlebtsov, *Gold nanoparticles in biology and medicine: recent advances and prospects.* Acta Naturae, 2011. **3**(2): p. 34-55.

129. Hashem, A.H., et al., *Synthesis of Chitosan-Based Gold Nanoparticles: Antimicrobial and Wound-Healing Activities.* Polymers, 2022. **14**(11). DOI: 10.3390/polym14112293.

130. Wierzbicki, M., et al., *Graphene Oxide in a Composite with Silver Nanoparticles Reduces the Fibroblast and Endothelial Cell Cytotoxicity of an Antibacterial Nanoplatform.* Nanoscale Research Letters, 2019. **14**(1): p. 320. DOI: 10.1186/s11671-019-3166-9.

131. Haidari, H., et al., *Eradication of Mature Bacterial Biofilms with Concurrent Improvement in Chronic Wound Healing Using Silver Nanoparticle Hydrogel Treatment.* Biomedicines, 2021. **9**(9). DOI: 10.3390/biomedicines9091182.

132. Husain, F.M., et al., *Biosynthesized Zinc Oxide Nanoparticles Disrupt Established Biofilms of Pathogenic Bacteria.* Applied Sciences, 2022. **12**(2). DOI: 10.3390/app12020710.

133. Le, V.A.T., et al., *Evaluation of the Performance of a ZnO-Nanoparticle-Coated Hydrocolloid Patch in Wound Healing.* Polymers (Basel), 2022. **14**(5). DOI: 10.3390/polym14050919.

134. Hollý, D., et al., *Stem Cells and Their Derivatives—Implications for Alveolar Bone Regeneration: A Comprehensive Review.* International Journal of Molecular Sciences, 2021. **22**(21). DOI: 10.3390/ijms222111746.

135. Pino, P., et al., *Antimicrobial Nano-Zinc Oxide Biocomposites for Wound Healing Applications: A Review.* Pharmaceutics, 2023. **15**(3). DOI: 10.3390/pharmaceutics15030970.

136. Ko, W.-C., et al., *Pharmacological Role of Functionalized Gold Nanoparticles in Disease Applications.* Molecules, 2022. **27**(5). DOI: 10.3390/molecules27051551.

137. Lorenzo-Anota, H.Y., et al., *Chitosan-Coated Gold Nanoparticles Induce Low Cytotoxicity and Low ROS Production in Primary Leucocytes, Independent of Their Proliferative Status.* Pharmaceutics, 2021. **13**(7). DOI: 10.3390/pharmaceutics13070942.

138. Fuster, M.G., et al., *Antibacterial Effect of Chitosan–Gold Nanoparticles and Computational Modeling of the Interaction between Chitosan and a Lipid Bilayer Model.* Nanomaterials, 2020. **10**(12). DOI: 10.3390/nano10122340.

139. Volkova, N., et al., *Application of Cryopreserved Fibroblast Culture with Au Nanoparticles to Treat Burns.* Nanoscale Research Letters, 2016. **11**(1): p. 22. DOI: 10.1186/s11671-016-1242-y.

140. Kang, J.P., et al., *Biosynthesis of gold and silver chloride nanoparticles mediated by Crataegus pinnatifida fruit extract: in vitro study of anti-inflammatory activities.* Artificial Cells, Nanomedicine, and Biotechnology, 2018. **46**(8): p. 1530-1540. DOI: 10.1080/21691401.2017.1376674.

141. Dam, P., et al., *Wound healing strategies based on nanoparticles incorporated in hydrogel wound patches.* RSC Advances, 2023. **13**(31): p. 21345-21364. DOI: 10.1039/D3RA03477A.

142. Paladini, F. and M. Pollini, *Antimicrobial Silver Nanoparticles for Wound Healing Application: Progress and Future Trends.* Materials (Basel), 2019. **12**(16). DOI: 10.3390/ma12162540.

143. Mensah, R.A., et al., *A Sustainable, Green-Processed, Ag-Nanoparticle-Incorporated Eggshell-Derived Biomaterial for Wound-Healing Applications.* Journal of Functional Biomaterials, 2023. **14**(9): p. 450.

144. Seisenbaeva, G.A., et al., *Dispersion of TiO2 nanoparticles improves burn wound healing and tissue regeneration through specific interaction with blood serum proteins.* Scientific Reports, 2017. **7**(1): p. 15448. DOI: 10.1038/s41598-017-15792-w.

145. Salvo, J. and C. Sandoval, *Role of copper nanoparticles in wound healing for chronic wounds: literature review.* Burns Trauma, 2022. **10**: p. tkab047. DOI: 10.1093/burnst/tkab047.

146. Alizadeh, S., et al., *Enhancing Diabetic Wound Healing Through Improved Angiogenesis: The Role of Emulsion-Based Core-Shell Micro/Nanofibrous Scaffold with Sustained CuO Nanoparticle Delivery.* Small, 2024: p. 2309164. DOI: https://doi.org/10.1002/smll.202309164.

147. Quignard, S., et al., *Silica nanoparticles as sources of silicic acid favoring wound healing in vitro.* Colloids and Surfaces B: Biointerfaces, 2017. **155**: p. 530-537. DOI: https://doi.org/10.1016/j.colsurfb.2017.04.049.

148. Lu, Z., et al., *Iron Nanoparticles Open Up New Directions for Promoting Healing in Chronic Wounds in the Context of Bacterial Infection.* Pharmaceutics, 2023. **15**(9). DOI: 10.3390/pharmaceutics15092327.

149. Shershakova, N.N., et al., *Wound healing activity of aqueous dispersion of fullerene C60 produced by "green technology".* Nanomedicine: Nanotechnology, Biology and Medicine, 2023. **47**: p. 102619. DOI: https://doi.org/10.1016/j.nano.2022.102619.

150. Chen, C., Y. Xi, and Y. Weng, *Progress in the Development of Graphene-Based Biomaterials for Tissue Engineering and Regeneration.* Materials, 2022. **15**(6). DOI: 10.3390/ma15062164.

151. He, H., et al., *Carbon nanotubes: applications in pharmacy and medicine.* Biomed Res Int, 2013. **2013**: p. 578290. DOI: 10.1155/2013/578290.

152. Scott, L., et al., *Carbon Nanotube-Based Scaffolds for Cardiac Tissue Engineering—Systematic Review and Narrative Synthesis.* Bioengineering, 2021. **8**(6). DOI: 10.3390/bioengineering8060080.

153. Nandhini, S.N., et al., *Recent advances in green synthesized nanoparticles for bactericidal and wound healing applications.* Heliyon, 2023. **9**(2): p. e13128. DOI: 10.1016/j.heliyon.2023.e13128.

154. Vijayaram, S., et al., *Applications of Green Synthesized Metal Nanoparticles - a Review.* Biol Trace Elem Res, 2023: p. 1-27. DOI: 10.1007/s12011-023-03645-9.

155. Mendes, C., et al., *The Use of Metallic Nanoparticles in Wound Healing: New Perspectives.* Int J Mol Sci, 2022. **23**(23). DOI: 10.3390/ijms232315376.

156. Blanco-Fernandez, B., et al., *Nanotechnology Approaches in Chronic Wound Healing.* Adv Wound Care (New Rochelle), 2021. **10**(5): p. 234-256. DOI: 10.1089/wound.2019.1094.

157. Ehtesabi, H. and R. Nasri, *Carbon dot-based materials for wound healing applications.* Advances in Natural Sciences: Nanoscience and Nanotechnology, 2021. **12**(2): p. 025006. DOI: 10.1088/2043-6262/abffc9.

158. Shariati, A., et al., *Graphene-Based Materials for Inhibition of Wound Infection and Accelerating Wound Healing.* Biomed Pharmacother, 2023. **158**: p. 114184. DOI: 10.1016/j.biopha.2022.114184.

159. Yusuf, A., et al., *Nanoparticles as Drug Delivery Systems: A Review of the Implication of Nanoparticles’ Physicochemical Properties on Responses in Biological Systems.* Polymers, 2023. **15**(7). DOI: 10.3390/polym15071596.

160. Zhang, Y., et al., *PLGA@IL-8 nanoparticles-loaded acellular dermal matrix as a delivery system for exogenous MSCs in diabetic wound healing.* Int J Biol Macromol, 2023. **224**: p. 688-698. DOI: 10.1016/j.ijbiomac.2022.10.157.

161. Chantaburanan, T., et al., *Enhanced stability and skin permeation of ibuprofen-loaded solid lipid nanoparticles based binary solid lipid matrix: Effect of surfactant and lipid compositions.* Int J Pharm X, 2023. **6**: p. 100205. DOI: 10.1016/j.ijpx.2023.100205.

162. Chawla, V., S. Sharma, and Y. Singh, *Yttrium Oxide Nanoparticle-Loaded, Self-Assembled Peptide Gel with Antibacterial, Anti-Inflammatory, and Proangiogenic*

Properties for Wound Healing. ACS Biomater Sci Eng, 2023. **9**(5): p. 2647-2662. DOI: 10.1021/acsbiomaterials.3c00134.

163. Madawi, E.A., et al., *Polymeric Nanoparticles as Tunable Nanocarriers for Targeted Delivery of Drugs to Skin Tissues for Treatment of Topical Skin Diseases.* Pharmaceutics, 2023. **15**(2). DOI: 10.3390/pharmaceutics15020657.

164. Wang, S. and E.E. Dormidontova, *Selectivity of ligand-receptor interactions between nanoparticle and cell surfaces.* Phys Rev Lett, 2012. **109**(23): p. 238102. DOI: 10.1103/PhysRevLett.109.238102.

165. Kushwaha, A., L. Goswami, and B.S. Kim, *Nanomaterial-Based Therapy for Wound Healing.* Nanomaterials (Basel), 2022. **12**(4). DOI: 10.3390/nano12040618.

166. Hu, Y., et al., *Stimuli-responsive therapeutic systems for the treatment of diabetic infected wounds.* Nanoscale, 2022. **14**(36): p. 12967-12983. DOI: 10.1039/d2nr03756d.

167. Martin, W.J., *Tissue regeneration without scarring achieved by enhancing the alternative cellular energy (ACE) pathway.* Journal of Cosmetics, Dermatological Sciences and Applications, 2017. **7**(1): p. 82-98. DOI: 10.4236/jcdsa.2017.71009.

168. Metwally, A.A., et al., *Wound healing properties of green (using Lawsonia inermis leaf extract) and chemically synthesized ZnO nanoparticles in albino rats.* Environmental Science and Pollution Research, 2022. **29**(16): p. 23975-23987. DOI: 10.1007/s11356-021-17670-5.

169. Talank, N., et al., *Bioengineering of green-synthesized silver nanoparticles: In vitro physicochemical, antibacterial, biofilm inhibitory, anticoagulant, and antioxidant performance.* Talanta, 2022. **243**: p. 123374. DOI: https://doi.org/10.1016/j.talanta.2022.123374.

170. Lokare, P., et al., *Applications of plant-based nanomedicines for wound healing – An emerging paradigm for effective therapy.* Materials Today: Proceedings, 2023. **73**: p. 494-501. DOI: 10.1016/j.matpr.2022.10.088.

171. Fleming, E. and Y. Luo, *Co-delivery of synergistic antioxidants from food sources for the prevention of oxidative stress.* Journal of Agriculture and Food Research, 2021. **3**: p. 100107. DOI: 10.1016/j.jafr.2021.100107.

172. Deshmukh, R., et al., *Novel Delivery Approches of Co-trimoxazole for Recreating its Potential Use- A Review.* International Journal of Applied Pharmaceutics, 2021. **13**(1): p. 36-42. DOI: 10.22159/ijap.2021v13i1.39623.

173. Sharma, R., K.S. Sharma, and D. Kumar, *Introduction to Nanotechnology*, in *Nanomaterials in Clinical Therapeutics*. 2022. p. 1-31. DOI: 10.1002/9781119857747.ch1.

174. Ko, N.R., et al., *Reductively-sheddable cationic nanocarriers for dual chemotherapy and gene therapy with enhanced release.* Colloids and Surfaces B: Biointerfaces, 2015. **126**: p. 178-187. DOI: 10.1016/j.colsurfb.2014.12.010.

175. Pal, R., et al., *Noble metal nanoparticle-induced oxidative stress modulates tumor associated macrophages (TAMs) from an M2 to M1 phenotype: An in vitro approach.* International Immunopharmacology, 2016. **38**: p. 332-341. DOI: 10.1016/j.intimp.2016.06.006.

176. Rather, H.A., et al., *Antioxidative study of Cerium Oxide nanoparticle functionalised PCL-Gelatin electrospun fibers for wound healing application.* Bioactive Materials, 2018. **3**(2): p. 201-211. DOI: 10.1016/j.bioactmat.2017.09.006.

177. Pastar, I., et al., *Epithelialization in Wound Healing: A Comprehensive Review.* Advances in Wound Care, 2014. **3**(7): p. 445-464. DOI: 10.1089/wound.2013.0473.

178. Miguel, S.P., et al., *An overview of electrospun membranes loaded with bioactive molecules for improving the wound healing process.* European Journal of Pharmaceutics and Biopharmaceutics, 2019. **139**: p. 1-22. DOI: 10.1016/j.ejpb.2019.03.010.

179. Li, D., et al., *Fabrication of curcumin-loaded mesoporous silica incorporated polyvinyl pyrrolidone nanofibers for rapid hemostasis and antibacterial treatment.* RSC Advances, 2017. **7**(13): p. 7973-7982. DOI: 10.1039/C6RA27319J.

180. Nqakala, Z.B., et al., *Advances in Nanotechnology towards Development of Silver Nanoparticle-Based Wound-Healing Agents.* International Journal of Molecular Sciences, 2021. **22**(20): p. 11272. DOI: doi:10.3390/ijms222011272.

181. Naskar, A. and K.-s. Kim, *Recent Advances in Nanomaterial-Based Wound-Healing Therapeutics.* Pharmaceutics, 2020. **12**(6). DOI: 10.3390/pharmaceutics12060499.

182. Cui, L., et al., *Nanomaterials for Angiogenesis in Skin Tissue Engineering.* Tissue Engineering Part B: Reviews, 2020. **26**(3): p. 203-216. DOI: 10.1089/ten.teb.2019.0337.

183. V. Singh, A., et al., *Nanomaterials: New Generation Therapeutics in Wound Healing and Tissue Repair.* Current Nanoscience, 2010. **6**(6): p. 577-586. DOI: http://dx.doi.org/10.2174/157341310793348632.

184. Jr. da Costa Fernandes, C., et al., *Layered Double Hydroxides Are Promising Nanomaterials for Tissue Bioengineering Application.* Advanced Biosystems, 2019. **3**(7): p. 1800238. DOI: 10.1002/adbi.201800238.

185. Zhang, J., et al., *Nanomaterials for diabetic wound healing: Visualization and bibliometric analysis from 2011 to 2021.* Frontiers in Endocrinology, 2023. **14**. DOI: 10.3389/fendo.2023.1124027.

186. Shi, Y., et al., *Matrix metalloproteinase inhibitors (MMPIs) as attractive therapeutic targets: Recent progress and current challenges.* NanoImpact, 2021. **21**: p. 100293. DOI: https://doi.org/10.1016/j.impact.2021.100293.

187. Manzano, M. and M. Vallet-Regí, *Mesoporous Silica Nanoparticles for Drug Delivery.* Advanced Functional Materials, 2020. **30**(2): p. 1902634. DOI: https://doi.org/10.1002/adfm.201902634.

188. Wang, Z., et al., *Tailoring gas-releasing nanoplatforms for wound treatment: An emerging approach.* Chemical Engineering Journal, 2023. **452**: p. 139297. DOI: 10.1016/j.cej.2022.139297.

189. Ding, J., et al., *Advances in Gas Therapeutics for Wound Healing: Mechanisms, Delivery Materials, and Prospects.* Small Structures, 2023. **n/a**(n/a): p. 2300151. DOI: 10.1002/sstr.202300151.

190. Yip, W.L., *Influence of oxygen on wound healing.* Int Wound J, 2015. **12**(6): p. 620-4. DOI: 10.1111/iwj.12324.

191. Teguh, D.N., et al., *Hyperbaric oxygen therapy for nonhealing wounds: Treatment results of a single center.* Wound Repair Regen, 2021. **29**(2): p. 254-260. DOI: 10.1111/wrr.12884.

192. Malone-Povolny, M.J., S.E. Maloney, and M.H. Schoenfisch, *Nitric Oxide Therapy for Diabetic Wound Healing.* Adv Healthc Mater, 2019. **8**(12): p. e1801210. DOI: 10.1002/adhm.201801210.

193. Wang, Z., et al., *Advanced Nitric Oxide Generating Nanomedicine for Therapeutic Applications.* ACS Nano, 2023. **17**(10): p. 8935-8965. DOI: 10.1021/acsnano.3c02303.

194. Gowda, B.H.J., et al., *Nanoparticle-based therapeutic approaches for wound healing: a review of the state-of-the-art.* Materials Today Chemistry, 2023. **27**: p. 101319. DOI: 10.1016/j.mtchem.2022.101319.

195. Xie, F., et al., *Therapeutic Potential of Molecular Hydrogen in Metabolic Diseases from Bench to Bedside.* Pharmaceuticals (Basel), 2023. **16**(4): p. 541. DOI: 10.3390/ph16040541.

196. Zhao, P., et al., *Molecular hydrogen promotes wound healing by inducing early epidermal stem cell proliferation and extracellular matrix deposition.* Inflamm Regen, 2023. **43**(1): p. 22. DOI: 10.1186/s41232-023-00271-9.

197. Ruksiriwanich, W., et al., *Wound Healing Effect of Supercritical Carbon Dioxide Datura metel L. Leaves Extracts: An In Vitro Study of Anti-Inflammation, Cell Migration, MMP-2 Inhibition, and the Modulation of the Sonic Hedgehog Pathway in Human Fibroblasts.* Plants, 2023. **12**(13). DOI: 10.3390/plants12132546.

198. Ning, J., et al., *Argon Mitigates Impaired Wound Healing Process and Enhances Wound Healing In Vitro and In Vivo.* Theranostics, 2019. **9**(2): p. 477-490. DOI: 10.7150/thno.29361.

199. Bisht, D., et al., *Effect of helium-neon laser on wound healing.* Indian J Exp Biol, 1999. **37**(2): p. 187-9.

200. Fitzpatrick, E., O.J. Holland, and J.J. Vanderlelie, *Ozone therapy for the treatment of chronic wounds: A systematic review.* Int Wound J, 2018. **15**(4): p. 633-644. DOI: 10.1111/iwj.12907.

201. Nastuta, A.V., et al., *Stimulation of wound healing by helium atmospheric pressure plasma treatment.* Journal of Physics D: Applied Physics, 2011. **44**(10): p. 105204. DOI: 10.1088/0022-3727/44/10/105204.

202. Georgii, J.L., et al., *Topical S-nitrosoglutathione-releasing hydrogel improves healing of rat ischaemic wounds.* J Tissue Eng Regen Med, 2011. **5**(8): p. 612-9. DOI: 10.1002/term.353.

203. Förstermann, U. and W.C. Sessa, *Nitric oxide synthases: regulation and function.* Eur Heart J, 2012. **33**(7): p. 829-37, 837a-837d. DOI: 10.1093/eurheartj/ehr304.

204. Pedraza, E., et al., *Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials.* Proc Natl Acad Sci U S A, 2012. **109**(11): p. 4245-50. DOI: 10.1073/pnas.1113560109.

205. Xing, Q., et al., *Nitric oxide regulates cell behavior on an interactive cell-derived extracellular matrix scaffold.* J Biomed Mater Res A, 2015. **103**(12): p. 3807-14. DOI: 10.1002/jbm.a.35524.

206. Schairer, D.O., et al., *The potential of nitric oxide releasing therapies as antimicrobial agents.* Virulence, 2012. **3**(3): p. 271-279. DOI: 10.4161/viru.20328.

207. Ahmed, R., et al., *Nitric oxide-releasing biomaterials for promoting wound healing in impaired diabetic wounds: State of the art and recent trends.* Biomedicine & Pharmacotherapy, 2022. **149**: p. 112707. DOI: https://doi.org/10.1016/j.biopha.2022.112707.

208. Abdallah, H.M., et al., *Nitric-Oxide-Mediated Vasodilation of Bioactive Compounds Isolated from Hypericum revolutum in Rat Aorta.* Biology, 2021. **10**(6). DOI: https://doi.org/10.3390/biology10060541.

209. Kobayashi, Y., *The regulatory role of nitric oxide in proinflammatory cytokine expression during the induction and resolution of inflammation.* Journal of Leukocyte Biology, 2010. **88**(6): p. 1157-1162. DOI: https://doi.org/10.1189/jlb.0310149.

210. Cooke, J.P. and D.W. Losordo, *Nitric oxide and angiogenesis.* Circulation, 2002. **105**(18): p. 2133-5. DOI: 10.1161/01.cir.0000014928.45119.73.

211. Witte, M.B., et al., *Enhancement of fibroblast collagen synthesis by nitric oxide.* Nitric Oxide, 2000. **4**(6): p. 572-82. DOI: 10.1006/niox.2000.0307.

212. Simmers, P., et al., *Nitric oxide stimulates matrix synthesis and deposition by adult human aortic smooth muscle cells within three-dimensional cocultures.* Tissue Eng Part A, 2015. **21**(7-8): p. 1455-70. DOI: 10.1089/ten.TEA.2014.0363.

213. De Groote, M.A. and F.C. Fang, *NO inhibitions: antimicrobial properties of nitric oxide.* Clin Infect Dis, 1995. **21 Suppl 2**: p. S162-5. DOI: 10.1093/clinids/21.supplement_2.s162.

214. Zhan, R., et al., *Nitric Oxide Enhances Keratinocyte Cell Migration by Regulating Rho GTPase via cGMP-PKG Signalling.* PLOS ONE, 2015. **10**(3): p. e0121551. DOI: 10.1371/journal.pone.0121551.

215. Ridnour, L.A., et al., *Nitric oxide regulates matrix metalloproteinase-9 activity by guanylyl-cyclase-dependent and -independent pathways.* Proc Natl Acad Sci U S A, 2007. **104**(43): p. 16898-903. DOI: 10.1073/pnas.0702761104.

216. Esplugues, J.V., *NO as a signalling molecule in the nervous system.* Br J Pharmacol, 2002. **135**(5): p. 1079-95. DOI: 10.1038/sj.bjp.0704569.

217. Wang, Y., F. Hong, and S. Yang, *Roles of Nitric Oxide in Brain Ischemia and Reperfusion.* Int J Mol Sci, 2022. **23**(8). DOI: 10.3390/ijms23084243.

218. Weinberger, B., et al., *The Toxicology of Inhaled Nitric Oxide.* Toxicological Sciences, 2001. **59**(1): p. 5-16. DOI: 10.1093/toxsci/59.1.5.

219. Shabani, M., et al., *Enhancement of wound repair with a topically applied nitric oxide-releasing polymer.* Wound Repair Regen, 1996. **4**(3): p. 353-62. DOI: 10.1046/j.1524-475X.1996.40312.x.

220. Morioka, N., et al., *Nitric oxide synergistically potentiates interleukin-1β-induced increase of cyclooxygenase-2 mRNA levels, resulting in the facilitation of*

substance P release from primary afferent neurons: involvement of cGMP-independent mechanisms. Neuropharmacology, 2002. **43**(5): p. 868-876. DOI: https://doi.org/10.1016/S0028-3908(02)00143-0.

221. Arora, D., et al., *Mechanisms of nitric oxide crosstalk with reactive oxygen species scavenging enzymes during abiotic stress tolerance in plants.* Free Radic Res, 2016. **50**(3): p. 291-303. DOI: https://doi.org/10.3109/10715762.2015.1118473.

222. Cano Sanchez, M., et al., *Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review.* Antioxidants (Basel), 2018. **7**(8). DOI: 10.3390/antiox7080098.

223. Meyer, U.T., *Rinsing Chronic Wounds with a Nitric Oxide Containing Solution – A Safe Treatment Approach to Support Wound Healing.* Current Bioactive Compounds, 2020. **16**(4): p. 394-406. DOI: http://dx.doi.org/10.2174/1573407214666181119120027.

224. Midgley, A.C., et al., *Nitric-Oxide-Releasing Biomaterial Regulation of the Stem Cell Microenvironment in Regenerative Medicine.* Advanced Materials, 2020. **32**(3): p. 1805818. DOI: https://doi.org/10.1002/adma.201805818.

225. Sandra, J.M., et al., *Study protocol: a pilot clinical trial of topical glyceryl trinitrate for chronic venous leg ulcer healing.* Wound Practice and Research, 2019. **27**(3). DOI: https://doi.org/10.33235/wpr.27.3.131-134.

226. OhioHealth. *Safety and Efficacy of the Noxsano Wound Care Bandage*. [Clinical trial] 2021; Available from: https://classic.clinicaltrials.gov/show/NCT04123093.

227. Centre, B.A.R. *Nitrogen Oxides Releasing Wound Dressing*. Available from: https://www.barc.gov.in/technologies/noxd/index.html.

228. Wu, Y.K., N.C. Cheng, and C.M. Cheng, *Biofilms in Chronic Wounds: Pathogenesis and Diagnosis.* Trends Biotechnol, 2019. **37**(5): p. 505-517. DOI: 10.1016/j.tibtech.2018.10.011.

229. Jiang, Z., et al., *Nanofiber Scaffolds as Drug Delivery Systems Promoting Wound Healing.* Pharmaceutics, 2023. **15**(7). DOI: 10.3390/pharmaceutics15071829.

230. Leavitt, T., et al., *Scarless wound healing: finding the right cells and signals.* Cell Tissue Res, 2016. **365**(3): p. 483-93. DOI: 10.1007/s00441-016-2424-8.

231. Wan, Z., et al., *Injectable adhesive self-healing biocompatible hydrogel for haemostasis, wound healing, and postoperative tissue adhesion prevention in nephron-sparing surgery.* Acta Biomaterialia, 2022. **152**: p. 157-170. DOI: https://doi.org/10.1016/j.actbio.2022.09.006.

232. Huang, Q., et al., *Sprayable chitosan nanogel with nitric oxide to accelerate diabetic wound healing through bacteria inhibition, biofilm eradication and macrophage polarization.* International Journal of Biological Macromolecules, 2024. **254**: p. 127806. DOI: https://doi.org/10.1016/j.ijbiomac.2023.127806.

233. Peng, W., et al., *Photothermal synergistic nitric oxide controlled release injectable self-healing adhesive hydrogel for biofilm eradication and wound healing.* Journal of Materials Chemistry B, 2024. **12**(1): p. 158-175. DOI: 10.1039/D3TB02040A.

234. Ali, I.H., et al., *Exogenous and endogenous nitric oxide eluting polylactic acid-based nanofibrous scaffolds for enhancing angiogenesis of diabetic wounds.*

International Journal of Biological Macromolecules, 2024. **261**: p. 129736. DOI: https://doi.org/10.1016/j.ijbiomac.2024.129736.

235. Yamamoto, Y., et al., *Current Status, Issues and Future Prospects of Personalized Medicine for Each Disease.* J Pers Med, 2022. **12**(3). DOI: 10.3390/jpm12030444.

236. Eming, S.A., T. Krieg, and J.M. Davidson, *Inflammation in wound repair: molecular and cellular mechanisms.* J Invest Dermatol, 2007. **127**(3): p. 514-25. DOI: 10.1038/sj.jid.5700701.

237. Zhuo, S., et al., *pH-Sensitive Biomaterials for Drug Delivery.* Molecules, 2020. **25**(23). DOI: 10.3390/molecules25235649.

238. Singer, A.J. and R.A. Clark, *Cutaneous wound healing.* N Engl J Med, 1999. **341**(10): p. 738-46. DOI: 10.1056/NEJM199909023411006.

239. Wilgus, T.A., *Regenerative healing in fetal skin: a review of the literature.* Ostomy Wound Manage, 2007. **53**(6): p. 16-31; quiz 32-3.

240. Shariatinia, Z., *Carboxymethyl chitosan: Properties and biomedical applications.* Int J Biol Macromol, 2018. **120**(Pt B): p. 1406-1419. DOI: 10.1016/j.ijbiomac.2018.09.131.

241. Pinelli, F., et al., *beta-Cyclodextrin functionalized agarose-based hydrogels for multiple controlled drug delivery of ibuprofen.* Int J Biol Macromol, 2023. **252**: p. 126284. DOI: 10.1016/j.ijbiomac.2023.126284.