Chapter

Nanotechnological Interventions and Mechanistic Insights into Wound-Healing Events

Sourabh Soni, Rubbel Singla and Yogendra S. Padwad

Abstract

Wound-healing cascade is highly dynamic and composed of four continuous but overlapping phases that are precisely programmed. Successful healing occurs when these phases occur sequentially in a specific time frame and thus require multipotent wound-healing material. Nanotechnology has revolutionized the field of wound dressings by the development of various types of nanotechnology-based drug delivery systems and materials to treat hard-to-heal wounds. In this chapter, the advantages and the limitations associated with nanoparticle-based wound-healing materials as well as recent trends and applications of nanotechnology-based approaches in advanced wound therapy for healing of cutaneous, chronic, and burn wounds will be detailed along with the molecular interventions involved. Efforts are targeted herein to address the most significant factors affecting cutaneous wound healing and the molecular mechanisms involved. This chapter describes several nanoparticle (NP)-based drug delivery systems to improve the healing potential of antimicrobial, antifungal, growth factors, and other bioactive agents. While much remains to be learned, a better understanding of the factors influencing wound repair and nanotechnological interventions therein may lead to therapeutics that improve the healing process.

Keywords: nanomaterials, wound-healing process, therapeutics, delivery systems, wound dressings, molecular mechanisms, wound-healing events

1. Introduction

Wound occurs mainly because of an injury, burn, surgery, infectious disease, or a pathological condition that leads to a compromise in the overall integrity of the tissue. Wounds are considered as major healthcare challenge affecting several million people globally due to the underlying complications resulting from infections and comorbidities such as diabetes. Lifestyle disorders enhance the risk of complications and lead to improper/delayed wound-healing processes. Despite of thousands of marketed products available to treat wounds, it is still considered as a burden to the individual and to the society at large [1].

Managing wounds is not at all easy, especially due to the various steps involved in the healing process. Most of the healing methods rely on the "tried and tested"

approach, but off-late, there has been a high influx of new products in the market, as well as the latest technologies, to increase the wound repair armamentarium. A vast majority of the new products in the market are refurbished and updated versions of the older ones. Most of the newer wound management products are the result of newer fields of research and investigation. Older wound-healing products, such as plain gauze, are still being extensively used as dressing in hospitals, but better and advanced understanding and novel technologies have resulted in certain products that aid in achieving the ideal moist, protected, and warm wound-healing microenvironment. The bioactive properties such as antimicrobial action and immune modulation create a microenvironment favorable for healing. The current wound care products in the market include alginate, cellulose, chitosan, collagen, and hyaluronic acid.

Nobel laureate Richard Feynman in 1959 first predicted the emergence of a new field of study that deals with structures ranging in the nanoscale. Sixty years later, the impact that nanotechnology has in our lives these days is huge, it is playing a role in important fields such as diagnostics and therapeutics *via* its role in the development of various medical devices [2]. With the rapid growth of nanotechnology as a research field worldwide, a plethora of nanomaterials has garnered importance in the biomedical and healthcare sectors. Several nanotechnology-based products are currently being investigated to aid in wound healing. Owing to their interesting properties at both chemical and physical levels, nanomaterials have gained a lot of attention in research [3]. Nanodevices being innovative provide us with a wide range of benefits such as entry across cellular barriers, nonantigenic, anti-shear stress, and gas-exchange permeability, modulation of biocompatibility, and bioavailability of drugs as well as nanodelivery option [4–7]. Nanotherapies lead to improvement of the healthcare sector by enhancement of currently available medical prognosis and treatments for challenges such as impaired wound healing. Despite the development of potential biomaterials and nanotechnology-based applications for wound healing, this scientific knowledge is not translated into an increase of commercially available wound-healing products containing nanomaterials [8].

2. Wounds and wound types

A wound is generally described as a tissue disruption from the normal anatomic structure leading to a subsequent loss of function [9]. The classical way to define a wound is to say that it is a disruption in the anatomic and cellular continuity of a tissue. Wounds tend to rupture the skin's epithelial integrity and may occur with or without microbial infection [10]. Wound may take place on many occasions during an individual's lifetime due to chemical, physical, and microbial factors influencing it. The physiological response by an individual to an injury is termed as wound healing, which involves integrated sbiochemical and cellular events leading to the regaining of functional and structural integrity at the injury site [11]. Healing of a wound starts right from the time of injury and carries on throughout the wound repair with the duration and extent varying according to the wound. Wound healing involves the action of many cells, soluble mediators and growth factors, and cell-extracellular matrix interactions. The coordinated action of all these events leads to the healing of wound *via* the process of hemostasis, inflammation, and epithelization, followed by fibroplasia and angiogenesis, finally resulting in wound contraction and remodeling [10, 12].

Humans possess an *in situ* property of wound healing, that is, self-regeneration, which depends on a person's age, gender, living habits, environment, microbial

infection, and types of wounds [13]. This leads to various criteria for wound classification: etiology, level of microbial infection, and morphology. Firstly, based on etiology, the wounds could be classified into abrasions, burns, cuts, lacerations, and stab wounds. Based on microbial infections, wounds have been categorized mainly into three groups: aseptic, contaminated, and septic wounds. There is one more characterization: closed and open wounds. Wounds are termed as closed when the skin shows no damage, but the underlying layer is injured while in open wounds the skin injury leads to exposure of the underlying tissue [14]. Finally, the duration of healing is a prime factor in wound management that leads to other criteria of wound categorization: acute and chronic wounds, which are discussed here in detail.

2.1 Acute wounds

A wound that follows an orderly and timely process of healing is termed as acute wound. These wounds tend to repair themselves following the normal stages of healing and results in timely restoration of anatomical and functional trait. They are generally caused by traumatic injury or surgery with healing time ranging from a week to a maximum of a month [10].

2.2 Chronic wounds

The wounds that fail to follow the normal procedure of the healing process thus leading to impairment in timely repair are called chronic wounds, which include diabetic and pressure wounds as well as venous and arterial ulcers. Generally, one or more than one stages of the wound-healing cascade are prolonged due to various factors leading to incomplete and disrupted healing [10]. Ulcers tend to remain in the chronic inflammation stage in pathological conditions and are characterized by an abundant infiltration of neutrophils and reactive oxygen species that release enzymes such as collagenase and elastase, leading to the destruction of cells, connective tissue, and growth factors [15]. These nonhealing wounds have a perpetual inflammation state, and they frequently relapse due to disrupted and dis-coordinated healing events [10, 16].

Millions of people globally suffer from chronic wounds affect, accounting for massive healthcare costs with estimates suggesting an annual burden of about $30 billion in the USA alone [17]. Bacterial infection is the most frequent complication of chronic wounds, hence the search for effective treatment options that evade infections has been a continuous process over centuries with antibiotic-resistant strains emerging as a major concern [18]. The imbalances in various signaling networks coordinating cellular interactions lead to nonhealing, chronic wounds characterized by prolonged inflammation, decreased angiogenesis, impaired cellular function, and bacterial infection [19–21]. The rate of chronic wound healing differs from acute wounds and shows dependence on the patient's immunological status [22]. Despite extensive efforts to develop therapeutic strategies for the effective treatment of chronic wounds, so far, limited clinical success has been achieved [23].

3. Wound-healing cascade

Wound healing, the normal response in mammals to injury, is a complex and dynamic biological process that is evolutionarily conserved, highly coordinated, and

spatiotemporally regulated. The wound-healing cascade has three sequential yet overlapping but distinct phases: inflammation, new tissue formation, and remodeling [12, 16, 24]. These coordinated phases of healing involve the interaction of immune and nonimmune cells, as well as soluble mediators and extracellular matrix components [16, 25]. Additionally, the dynamic link between skin and the microbial population also contributes to the process outcome. Under normal circumstances, wounds heal by themselves, but depending on the extent of tissue damage, the process varies [26, 27]. Wound healing is a very interesting research field, with many mechanisms still not fully understood.

The process of wound healing initiates immediately after injury with the hemostasis phase. The fibrin clot formed here acts like a barrier and leads to moisture retention [10]. Traditional gauze-based dressings target this healing phase by retaining moisture and preventing excessive bleeding [28]. This is followed by inflammation (beginning soon after injury), which may continue for a week with the release of pro-inflammatory cytokines from injured tissue leading to the attraction of circulating leucocytes at the site [29]. Subsequently, the proliferation phase starts, which involves angiogenesis, granulation, epithelialization, and wound contraction. Finally, after around 3 weeks of injury begins the maturation phase, which might take as long as 2 years for completion [30]. The cascade of wound-healing events along with the signaling and the cellular changes involved is further discussed in detail and diagrammatically explained in **Figure 1**.

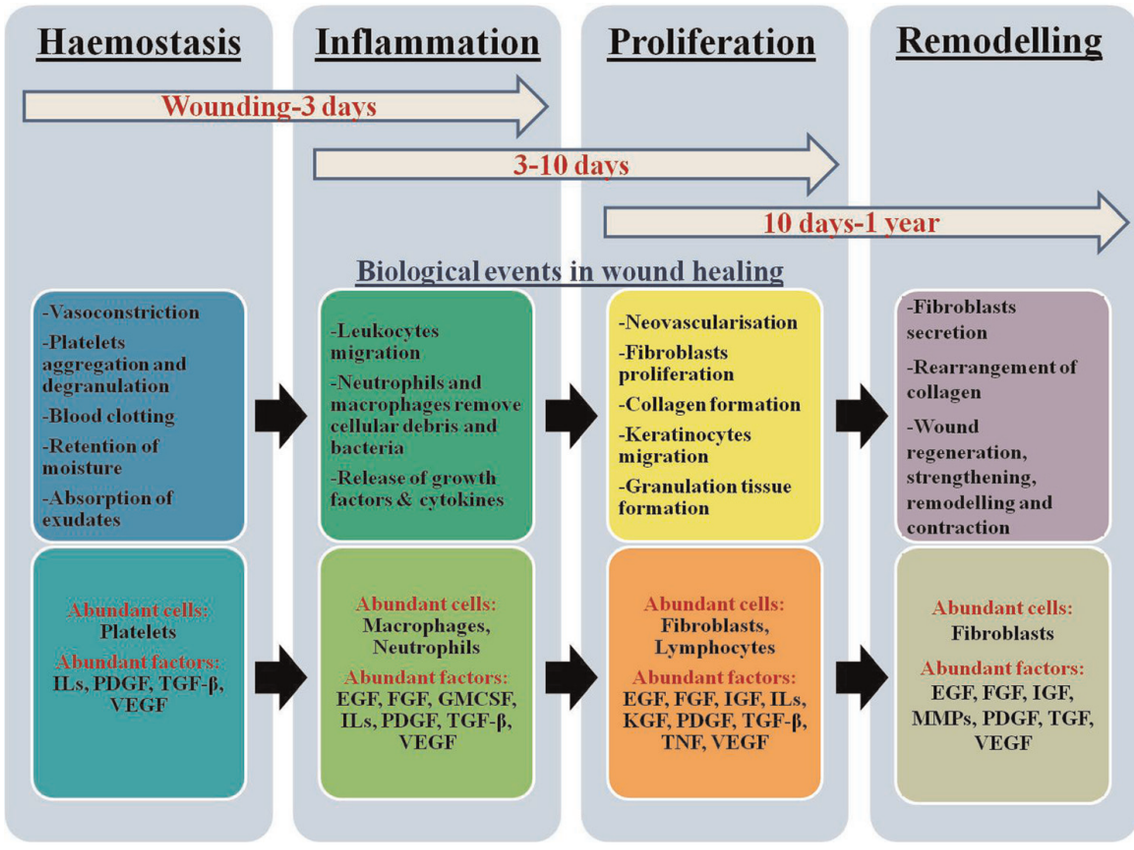

Figure 1.
Diagrammatic representation of various nanotechnology-based therapies used in different stages of wound healing. The currently employed nanostrategies for wound management are presented in this figure. The list provided here is intended to be just an illustration and is not comprehensive.

3.1 Hemostasis and inflammation phase (begins soon after injury and continues for 3 days)

Hemostasis and inflammation occur soon after tissue injury and constitute the first and the foundation phase of wound repair. It leads to the formation of a platelet plug to prevent blood loss, removal of dead tissues and to prevent infection, and employs the components of the coagulation, inflammatory, and immune cascade to accomplish these tasks (**Figure 1**) [31, 32]. Further, the fibrin matrix acts as a scaffold for the infiltrating cells that are essential for later phases of wound healing. The clot consists of collagen, fibrin molecules, fibronectin, platelets, and thrombin. The fibrin clot operates as a scaffold hence aiding the migration of neutrophils, monocytes, leukocytes, keratinocytes, fibroblasts, and endothelial cells, and simultaneously forms a matrix for concentrating cytokines and growth factors [31, 33, 34]. The soluble factors released by these initiate the inflammatory phase aimed at establishing an immune barrier against infections [10].

In response to complement system activation, the next step is the recruitment of neutrophils to the wound site [35]. Following the formation of the clot, a stress signal is transmitted with neutrophils being the first cells to respond. This causes nearby vessels to vasodilate and accumulation of inflammatory mediators to facilitate the sudden rise in cellular traffic. Neutrophils release proteolytic enzymes such as proteases, which clean up the wounded area by removing cellular debris and invading bacteria [18, 31]. Neutrophils lead to the creation of reactive oxygen species that in combination with chlorine lead to wound sterilization [31]. *In vitro* studies have indicated the possibility that neutrophils could change the cytokine expression and phenotype of macrophages, leading to an innate immune response during wound healing [36]. This phase is sometimes called as the "lag phase," where in the absence of tensile strength in the wound, the recruitment of the migrating cells and various factors must be managed for the healing process [33].

Approximately 2–3 days post-injury, the monocytes from the neighboring tissue migrate to the wound site and finally differentiate into macrophages, which are seemingly crucial for the healing cascade. They cause phagocytosis of cell debris, apoptotic cells, and pathogens and lead to the secretion of various soluble factors [37, 38]. Despite their importance, the role of neutrophils and macrophages has not been well elucidated in wound repair. Reports have suggested that deficiency of one of these cells can be compensated *via* redundancy in the inflammation phase [39], whereas when cells of both types are absent, wound repair still takes place, with a lesser scarring response [40]. Macrophages synthesize various enzymes such as collagenases, cytokines, and growth factors like epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), interleukins (ILs), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF), leading to the promotion of cell proliferation and extracellular matrix (ECM) synthesis (**Figure 1**) [31, 41]. The inflammatory phase essentially supplies growth factors and cytokines that keep the wound-healing process intact and are quite crucial in the later stages of wound repair [25, 42]. Past evidence suggests that the extent of scarring depends on the amount of inflammation. This is validated by reports that show that the hypothesis behind scarless healing of fetus wounds is the lack of intrauterine inflammation [43].

3.2 Proliferative phase (Day 3–10)

Proliferation is the second phase of wound healing, which takes place from 3 to 10 days post-injury. This stage is characterized by new tissue formation, cellular

migration, and proliferation (**Figure 1**). In this phase, the focus is on covering the injured surface, the synthesis of granulation tissue, and the reformation of the vascular network [33, 44, 45]. It indicates the start of angiogenesis and the synthesis of the ECM components. In the first step, keratinocytes migrate to the injured dermis and angiogenesis occurs. New capillaries sprout replacing the fibrin clot with granulation tissue, resulting in a new substrate for migration of keratinocytes during later stages of the healing (**Figure 1**). Capillaries help in nutrient supply during the granulation and tissue deposition phase, in absence of which a chronic wound will develop [31]. The keratinocytes proliferate and mature to restore the epithelial barrier. There is also secretion of cytokines such as ILs, TNF-α, enzymes like matrix metalloproteinase (MMPs), and growth factors like EGF, PDGF, TGF-β, and VEGF by the activated macrophages and de-granulated platelets (**Figure 1**) [46]. The concentrations of these factors vary and are dependent on the immunological state of the individual [42]. Excessive synthesis of granulation tissue and collagen causes scar formation [47]. Several pathways play an important role in restoring the normal anatomy, physiology, and functional status of the injured tissue with mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K)/Akt, TGF-β, and Wnt/β-catenin being the major ones [48].

Vascular system restoration is a complex cascade in the injured tissue involving various cellular and molecular events. The most important angiogenesis upregulators are VEGF and FGF [49]. In a study, VEGF application to diabetic animal wounds normalized the healing process [50]. Later in this phase of repair, fibroblasts differentiate into myofibroblasts upon stimulation by macrophages [51]. Myofibroblasts being contractile in nature bring the wound edges together over time. Further, in combination with myofibroblasts, they produce extracellular matrix and lead to collagen and scar formation [52]. Fibroblasts secrete IL-6 and keratinocyte growth factors (KGFs) that act as stimuli causing migration of neighboring keratinocytes to the injury site, leading to their proliferation and differentiation into the epidermis [31]. Keratinocytes present at the wound edges cause re-epithelialization of the wound [53, 54].

Proliferative phase ends with the formation of acute granulation tissue [31]. During this phase, the proliferating endothelial cells get activated by VEGF, leading to the formation of new capillaries. By this time, the remodeling phase has already been initiated. The fibrin-based wound matrix is replaced with scar tissue characterized by high-cellular density due to fibroblasts, granulocytes, and macrophages, as well as capillaries and collagen bundles, hence termed granulation tissue [25, 33, 44, 53, 55]. This tissue is highly vascular because angiogenesis is still incomplete. By the end of this phase, myofibroblasts differentiation reduces the population of maturing fibroblasts, which are further terminated *via* apoptosis [56].

3.3 Maturation and remodeling phase (Day 10–1 year)

Remodeling is the third and the last phase of wound repair that begins around 2 weeks post-injury and may last up to 1 year or longer, based on various factors [10]. During this stage, all the cellular processes activated post-injury wind down and come to an end, the new epithelium is formed, and the final scar tissue develops. The granulation tissue formation ceases *via* cellular apoptosis, leading to an acellular and avascular mature wound [57]. Majority of the cellular components exit from the wound site or undergo apoptosis, and the resulting mass consists of collagen and other ECM proteins. During wound maturation, the ECM components undergo various

changes characterized by collagen deposition in a well-arranged network form (**Figure 1**). 4–5 weeks post-injury, collagen continues to be synthesized. Stronger collagen I replace the previously predominant collagen III produced during the proliferative phase [58]. Normally, around 90% of collagen present in skin is type I, but during the granulation phase in the wounded skin type III, levels reach up to 30% [31]. In cases with excessive collagen formation, generation of hypertrophic scar occurs [31]. The regulation of skin integrity and homeostasis occurs through the epithelial–mesenchymal interactions [59].

Further, myofibroblasts lead to wound contractions thereby decreasing the scar surface [38, 60]. The angiogenesis processes now diminish causing the blood flow in the wound to decline, and the acute metabolic activity in the wound now slows down and finally diminishes. However, the injured tissue never tends to regain the properties of healthy skin. Certain skin components never recover completely such as the hair follicles and sweat glands that possess no potential to heal potential post-injury [61]. The epidermal layer of the scar formed differs from surrounding healthy skin post-healing [33]. Even years after the injury, the collagen in the scar never gets the fully organized structure seen in healthy skin. Furthermore, the wound strength never returns to 100% [31].

4. Management of wounds through nanotherapeutic approaches

As described above, wound healing is a dynamic and highly regulated process. Wound closure can be realized by either regeneration or repair. The process of healing has been described as a playing orchestra where an organized interplay of various factors, such as cells, cytokines, and growth factors, leads to tissue repair [55]. However, when this intricate balance gets disrupted, the healing process is affected with recent reports suggesting that the absence of a particular cell or a mediator gets compensated so that the tissue repair process goes on [42]. The currently used wound management strategies have certain limitations in fulfilling the needs of comprehensive care [62]. Hence, wound healing remains a challenge and a burden [63]. The treatment modalities in practice are specifically based on the type of wounds, with inadequate management leading to complications such as chronic wounds, fibrosis, compromised tissue functioning, or even amputation [64]. The best prevention technique against undesirable outcomes during healing is the effective management during the early stages of the process to prevent wounds to progress to chronic conditions.

Recent advances in interdisciplinary research have brought bioactive materials to the forefront as smart wound care materials [8, 65]. Bioactive materials can potentially be exploited to target any phase of the healing process by their direct cellular interactions or indirectly through ECM. Biopolymers are one of the most widely exploited bioactive materials used for wound care because they possess useful properties such as ability to support cell growth, biocompatibility, biodegradability, durability, and regenerative potential [66]. Dermal substitutes and human skin equivalents are the two food and drug administration (FDA)-approved biomaterial therapies promoting healthy healing *via* their interaction with the wound microenvironment [67–70]. Due to the increased understanding of the healing process, there has been an emergence of dressing-based wound-healing materials [71]. Modern dressings have been designed for successful healing by providing a beneficial wound microenvironment with control on moisture and absorbance of excess exudates. Active dressings

have been known to alter the wound microenvironment *via* targeting microbial load and excessive proteases or aiding tissue growth by alginate, chitosan, hyaluronan, and collagen matrices acting as scaffolds [72–74]. There have been plenty of reports that demonstrate the advantages of modern dressings harboring cells and recombinant growth factors, but still, the majority of the clinical modalities are based on safety evaluation rather than efficacy [75].

Chronic wounds demand very robust and efficacious therapies that address the various challenges of a deregulated healing process. Many of the novel approaches have failed in delivering specific healing outcomes, which have further made way for the introduction of several nanotechnology-based wound-healing therapies [7]. The chronic nature and associated complications of nonhealing wounds have led to the emergence of nanotechnology-based therapies for ultimately repairing the injured tissue [65]. Multitudes of nanotherapeutic approaches have been introduced to efficiently manage the wounds and remove any related possible complications (**Figures 2** and **3**) [76]. The advantage of using nanomaterials over other wound dressings is their tenability, low cytotoxicity, good biocompatibility, drug delivery ability, and versatility of physicochemical properties endowing them with many unique features [71, 76, 77]. Furthermore, nanoscale helps them in enhanced penetration to the injury site and a high interaction probability with the biological target [78]. Consequently, NPs possess the ability of controlled and sustained the release of therapeutics, resulting in accelerated wound repair [72].

5. Nanoparticles used as therapeutic materials for wound repair

A range of metallic NPs, polymeric NPs, peptide-loaded nanostructures, and carbon-based nanomaterials have been investigated for applicability in wound repair due to the astonishing physical, chemical, and biological characteristics, such as their ease of fabrication, biodegradability, and biocompatibility [79, 80]. The aim of any injury due to burns or accidents is to achieve healing and epithelialization as soon as possible without any side effects [81]. A variety of nanomaterials used for the treatment of wounds and their brief mechanistic action are given in detail in the following sections (**Figures 2** and **3**).

5.1 Metallic nanoparticles

In the past literature, different varieties of metal or metal oxide NPs were reported for their wound-healing application. Metal NPs, such as silver, gold, and zinc, are frequently used for dermal wound treatment due to their ease of use and large surface-area-to-volume ratio. These metal NPs provide a moist wound environment and possess strong antimicrobial activity [82]. Silver NPs (AgNPs) are among the ones used in most of the dressings available in the market. AgNPs as well as silver are known to show great antimicrobial action against wide spectra of microbes that include bacteria, fungi, yeast, and even viruses [83]. A nanogel prepared from biologically synthesized AgNPs from cell-free extract of *Saccharomyces boulardii* resulted in superior healing of burn wounds in rats as compared to marketed formulations [81]. AgNPs successfully interrupt the quorum sensing mechanism, resulting in decreased biofilm formation and detoxification of the bacterial toxins [84]. AgNPs carry the silver ions (Ag^+) that are solely responsible for antimicrobial activity by interfering with the respiratory chains at the cytochrome, damaging cell wall, binding with DNA,

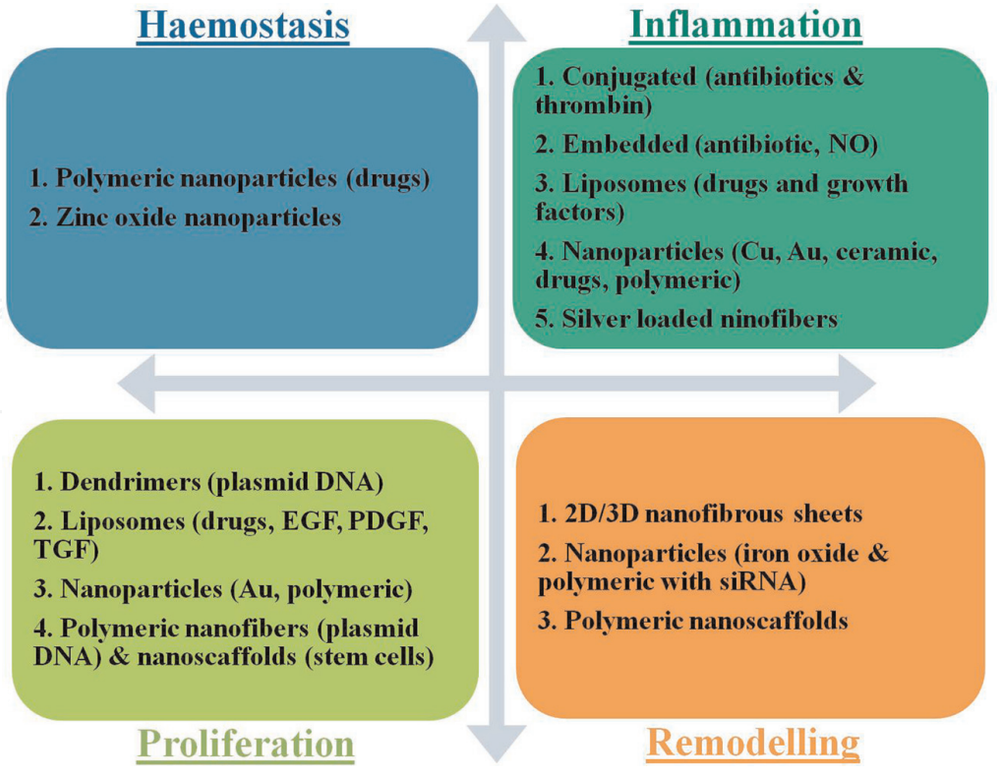

Figure 2.
Schematic representation of the main biological phases and events in the wound healing cascade along with the most abundant cells and soluble factors present in each phase, which is responsible for wound repair. The arrows at the top indicate the timeline of healing phases suggesting the overlapping nature of the wound healing cascade.

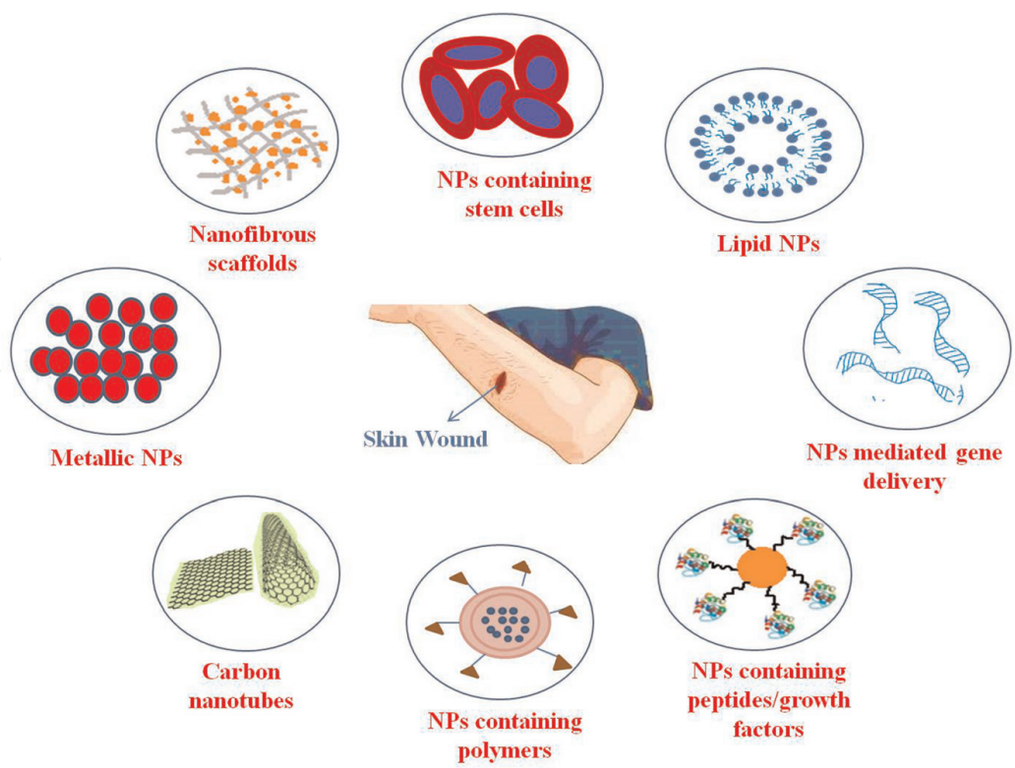

Figure 3.
Pictorial representation of the various types of nanotechnology-driven wound repair materials currently under research/available in the market.

and inhibiting its replication [85, 86]. In a recent *in vitro* study, it was demonstrated that the use of AgNPs led to a significant decrease in levels of inflammatory cytokines, oxidative stress in human keratinocytes and dermal fibroblasts that ultimately accelerated the rate of healing [87]. In a burn wound model, topical application of AgNPs tends to reduce the neutrophil count and IL-3 levels along with an increase in levels of IL-10, TGF-β, VEGF, and interferon-gamma (IFN-γ) [88].

Gold NPs (AuNPs) are way more biocompatible than other metallic NPs. It is very exciting to describe that AuNPs alone or along with other drugs have also been examined for their wound-healing efficacy [71]. The proteasome inhibitory activity, antibacterial, and antioxidant potential of AuNPs synthesized using aqueous extract of the rind of *Citrullus lanatus* may serve as potential candidates for wound healing [89]. Electrospun scaffold containing pharmaceutical intermediate-capped AuNPs provided a remedy for the treatment of full-thickness wounds infected by multi-drug resistant (MDR) bacteria [90]. The antibacterial mechanism of action of AuNPs illustrates that AuNPs alter the membrane potential and inhibit the ATP synthase enzyme that ultimately causes a collapse in the energy metabolism of the cell and cell death [91].

The inherent antibacterial nature of zinc oxide (ZnO) NPs promotes the applicability of such nanomaterials in numerous hydrogel-based wound dressings. In a study, cotton wound dressings impregnated with AgNPs, ZnO NPs, and mixed Ag/ZnO NPs resulted in high antibacterial action of wound dressings (**Figure 3**). Bandages impregnated with a liquid solution of AgNPs showed more antibacterial activity as compared to ZnO and mixed Ag/ZnO NPs [92]. ZnO-NPs successfully prepared from aqueous leaf extract of the plant *Barleria gibsoni* exhibited a remarkable wound-healing potential in rats and acted as an effective and better topical antimicrobial formulation to treat burn wounds [93]. In another study, the authors explored the healing potential of Ag-ZnO composite NPs in Wistar rats and showed comparatively faster healing in 10 days as compared to pure AgNPs and dermazin (the standard drug) [94]. Topical administration of antibacterial ZnO NPs also decreased bacterial skin infections in mice model by the induction of disintegration of the cell membrane and oxidative stress response in macrophages [95]. Iron oxide NPs were also evaluated for wound healing purposes. Fe_2O_3 NPs conjugated with thrombin significantly stimulated incisional wound healing by improving the tensile strength of the skin and reducing scar formation [96].

5.2 Nanoparticles containing polymers

Wound dressing materials are often based on polymeric nanostructures that include either synthetic or natural polymers. Mainly, poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), and polycaprolactone (PCL) are the mostly used synthetic polymers to engineer biomaterials for wound care applications [97]. Natural biodegradable polymers are chitosan, cellulose, alginates, and hyaluronic acids, which have played a well-versed role in the healing of wounds. In a study, electrospun nanofibers prepared from the blend of PLA and hyperbranched polyglycerol and loaded with curcumin showed wound-healing potential. *In vitro* scratch assay results indicated that the curcumin-loaded electrospun nanofibers were able to completely cover the wound within 36 h [98]. In another study, curcumin-PLGA nanostructures resulted in two-fold increase in wound-healing potential when compared to either PLGA or curcumin. Curcumin-loaded PLGA NPs reduced reactive oxidative species (ROS) and downregulated expression of

anti-oxidative molecules (glutathione peroxidase and NFκβ) that are responsible for reducing the inflammatory responses (**Figures 2** and **3**) [99]. A hybrid alginate hydrogel cross-linked by calcium gluconate crystals deposited in PCL-PEG-PCL was shown to promote wound regeneration in a full-thickness skin defect model of rats that suggested their great potential in skin tissue engineering [100].

Another important biopolymer, collagen is structural component of the extracellular matrix and is known for providing excellent strength to tissues [101]. Collagen nanofibers mat incorporated with AgNPs resulted in accelerated re-epithelialization, collagen production, and better wound contraction compared with plain collagen nanofibers. Due to its excellent biocompatibility and bioadhesive nature, collagen nanofibers mat promotes cell adhesion and interacts with cells and regulates cell migration, proliferation, and survival. Collagen dressings also result in accelerated fibroblast production and promote wound healing [102]. Another collagen-derived biopolymer, that is, gelatin has been used mainly in the establishment of biocompatible and biodegradable wound dressings. The porosity and interfiber distance of gelatin structure tend to promote healing. In a report, topical administration of gelatin wound scaffolds resulted in rapid wound closure and faster wound repair in a rat model [103]. Chitosan is another natural polymer that acts as an optimum wound-healing material as it bears film-forming capacity, gel-forming characteristics, positive charge, and a strong tissue adhesive trait in response to increased coagulation of blood [104]. Its analogous structure with glycosaminoglycan (main component of extracellular matrix) plays a great role in its utility in tissue engineering biomaterials [105]. In a study, chitosan-Ag-ZnO nanocomposite dressing enhanced the wound healing and promoted re-epithelialization and collagen deposition [106]. Similarly, a spongy bilayer wound dressing material composed of chitosan-Ag NPs and chitosan-*Bletilla striata* polysaccharide also showed hastened healing of skin wounds of mice, and the bilayer displayed improved mature epidermization and less inflammation on day 7 [107]. In another report, insulin-delivering chitosan NPs coated onto the electrospun PCL/collagen demonstrated nearly full wound closure when compared to the sterile gauze, which showed approximately 45% of wound closure [108].

Cellulose, another important biopolymer occurring abundantly, has been used widely in wound dressing applications due to its biodegradability, biocompatibility, and high tensile strength. Methylcellulose-containing AgNPs showed excellent antibacterial action and burn wound healing [109]. Nanocomposites containing cellulose nanocrystals (CNCs) incorporated with silver NPs have also been used for acute and diabetic wound healing in mice. The nanocellulose in these nanocomposites possessed good water-absorbing capacity and porous nature that assisted in the rapid healing of acute and diabetic wounds [110–112].

5.3 Peptide encapsulated nanostructures

Peptides of various types possess astonishing functions for wound-healing applications. But the controlled and prolonged delivery of peptides to the wound site is quite challenging. The use of NPs for encapsulation of peptides serves as a platform to provide sustained and controlled delivery of peptides and protect the peptides from degradation, thus promoting rapid healing of wounds [113]. In a study, solid lipid nanoparticle (SLN) encapsulating simultaneously LL37 and serpin A1 was used to deliver the agent at specific ratios. The developed nanostructures resulted in faster wound repair by promoting wound closure in fibroblasts and keratinocytes and increasing antibacterial action against bacteria *S. aureus* and *E. coli* as compared to

bare LL37 or serpin A1 alone. LL37 is well-known endogenous host defense peptide possessing the antimicrobial trait and takes part in the regulation of the healing process. Further, Serpin A1, an elastase inhibitor has been reported to demonstrate healing properties [114]. In another report, a recombinant fusion protein comprised of stromal cell-derived growth factor-1 (SDF1) and elastin-like peptide (ELPs) was developed, which possess the tendency to self-assemble into NPs. SDF1 is known to promote neovascularization for early re-epithelialization of cutaneous wounds in diabetic mice. ELPs are non-immunogenic, non-pyrogenic, and biologically compatible derivatives of tropoelastin. The topical application of wounds treated with SDF1-ELP NPs resulted in 95% closure of full-thickness wounds by day 21, and complete closure by day 28. On the other hand, only 80% of wound closure was achieved by treatment with free SDF1, ELP alone, or vehicle control by day 21, and the wounds took 42 days for complete closure [115]. In another study, heparin mimetic peptide nanofibers angiogenic scaffolds were developed for slow release of growth factors and protection from degradation. Heparin mimetic peptide nanofibers have the potential to bind and enhance the activity and production of major angiogenic growth factors, such as VEGF, and thus provided a therapeutic way to accelerate the healing of diabetic wounds [116]. Another similar study demonstrated the use of heparin-mimetic peptide nanofiber gel for increasing the rate of healing of burn injuries [117].

A different study reported the simple one-step cross-linking strategy for the preparation of collagen peptide with recombinant human collagen (RHC)-chitosan nanofibers for wound healing. The results showed rapid epidermalization and angiogenesis in Sprague–Dawley (SD) rat scalding model after treatment with *in situ* cross-linked nanofibers (**Figure 3**). The *in situ* cross-linked nanofibers behaved well as a scaffold showing better cell attachment and proliferation. The breakdown products of RHC played a role as chemotactic agents for the faster synthesis of granulation tissue for showing better healing performance [118]. A different type of hybrid multifunctional nanofibrous matrix composed of poly(citrate)-*ε*-poly-lysine and PCL was designed to inhibit MDR bacteria and enhance full-thickness wound healing [119].

5.4 Carbon-based nanomaterials

Carbon nanomaterials, such as graphene oxide (GO)-NPs, carbon dots, and fullerenes, possess the potential to be used as skin repair agents (**Figure 3**). The application of carbon nanotubes (CNTs) to wound healing provides enhanced functionality for dressing, delivery of antiseptics in a controlled manner, and real-time monitoring of healing events. Polyvinyl alcohol (PVA) functionalized multi-walled CNTs further conjugated with glucose oxidase enzyme showed antibacterial activity against bacterial pathogens due to the generation of hydrogen peroxide. The antibacterial activity of the developed nanomaterials opened innovative ways for the potential of such materials in wound-healing applications [120]. In a study, GO nanosheets incorporated in ultrafine biopolymer fibers were tested for skin wound-healing potential in adult male rats. From the *in vivo* studies, it was found that a large open wound ($1.5 \times 1.5\ cm^2$) was completely regenerated after 14-day of injury. Pathological studies confirmed the formation of thick dermal tissue and complete epithelialization in the presence of 1.5wt% GO nanosheets [121]. In a different study, a novel 3D collagen scaffold containing carbon-based 2D layered material, GO was characterized for periodontal healing of dogs. GO scaffold was implanted into dog class II furcation defects, and periodontal healing was examined after 4 weeks of surgery. The outcomes suggested that GO scaffold was biocompatible and possessed excellent bone and

periodontal tissue formation ability [122]. Onion-derived carbon nanodots that comprised hydrophilic group-decorated amorphous nanodots exhibited accelerated healing in a full-thickness wound model of rat model attributed to its radical scavenging action [123]. Carbon C_{60} fullerenes exhibited fascinating properties that balance several pathological mechanisms accountable for hampering the wound repair pathway [124]. In a different study, C_{60} fullerene functionalized with cationic three dimethyl pyrrolidinium groups was examined to rescue mice from fatal wound infections of Gram-negative species, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The study results successfully revealed that mice infected with *P. mirabilis* showed 82% survival due to the photodynamic therapy of fullerenes as compared to only 8% survival of mice without treatment [125].

5.5 Solid lipid nanomaterials

Various kinds of lipid nanomaterials, such as SLN, liposomes, micelles, nanostructured lipid carriers (NLC), or vesicles, have been used as therapies for wound healing (**Figure 3**). SLNPs encapsulated with morphine were reported to increase keratinocyte migration, proliferation, and differentiation responsible for accelerated wound repair [126]. In this context, liposome with silk fibroin hydrogel core was designed to stabilize bFGF. The study indicated that the skin permeability of bFGF was significantly enhanced by the developed liposomal system, and a major part of the encapsulated growth factor penetrated the skin dermis. Application of bFGF encapsulated liposomes resulted in improvement in the morphology of hair follicles at the wound site with hair regrowth shown on a deep second scald mice model. The healing action was mainly found to be associated with inhibiting scar formation and promoting vascular growth in dermis, which may serve as a potential candidate to improve wound healing [127]. In another study, liposomes were loaded with dexamethasone-phosphate. The liposomes were further surface modified with either PEG or phosphatidylserine. Both formulations resulted in decreased IL-6 and TNF-α release and increased efferocytosis activity. A faster uptake and a higher potency were induced by phosphatidylserine-modified liposomes as compared to PEG-modified liposomes. Liposomes after shell modification with phosphatidylserine promoted dexamethasone delivery to macrophages and induced a phenotype favorable for chronic wound healing [128]. The topical administration of recombinant human EGF loaded into lipid nanocarriers showed accelerated healing of full-thickness cutaneous wounds in a porcine model. The administration of 20 μg of nanoencapsulated lipid carriers twice a week increased the wound closure rate, as well as improved the wound quality in *in vivo* experiments [129].

Micelles were also proposed as suitable candidates for the delivery of hydrophobic molecules to the wound site for the healing of chronic wounds. In this respect, a biodegradable hydrogel system cutaneous wound dressing was developed containing curcumin encapsulated in micelles. Curcumin suffers from problems such as low water solubility, poor oral bioavailability, and rapid first-pass metabolism. The application of developed micelles in both incision and excision wound models showed higher collagen level, better granulation, remarkable reduction in superoxide dismutase content, and small increase in catalase activity causing an enhancement in the healing of cutaneous wounds [130]. Another study demonstrated that clarithromycin-loaded micelles were prepared *via* self-assembly of chitosan with a mixture of linoleic and oleic acids. These micelles exhibited good biocompatibility, induced cell proliferation, and showed 20-times greater clarithromycin loading

capacity in comparison to its water-saturated solution, suggesting the potential of micelles in wound-healing applications [131].

6. Nanomaterials as delivery agents carrying therapeutic molecules

Many therapeutic molecules such as nitric oxide, various antibiotics and antioxidative compounds, bioactive molecules, and vitamins possess great potential for wound healing. But each of these has few problems associated with solubility, stability, degradation by enzymes, temperature sensitivity, and so on. Because of these problems, the topical applicability of these compounds decreases to a large extent. Therefore, a need was felt to develop nanotechnology-based carriers to encapsulate these compounds for enhancing their efficacy.

6.1 Nanoparticles containing bioactive molecules for healing

Nanoencapsulated bioactive molecules also served as promising approach for skin tissue engineering. NPs encapsulated with bioactive ingredients help in slow and sustained release of the active moiety to the target site and increase the stability of molecules [132]. As an important natural molecule, curcumin suffers from limitations of poor solubility and fast degradation rate that hinder its applicability as an antioxidative and antimicrobial agent. To overcome these limitations, curcumin-loaded NPs have been synthesized, which enhanced the wound healing in the murine model as compared to pure curcumin [133]. In another report, efforts have been made to prepare curcumin-loaded chitosan NPs that were then impregnated into collagen-alginate scaffolds. Such prepared nanoscaffolds resulted in complete epithelization and granulation tissue formation for rapid healing of wounds in diabetic mice (**Figures 2** and **3**) [134]. An important study demonstrated the effect of guanidinylated chitosan NPs loaded with five different bioactive molecules (EGF, ascorbic acid, hydrocortisone, insulin, and 1,25-dihydroxyvitamin D_3). These biomolecules are beneficial for stimulating skin fibroblasts and keratinocyte proliferation but have limited applicability as they are unstable in wound dressing fabrication and storage. The prepared wound dressings resulted in stable delivery of bioactive factors and enhancement of skin wound healing in Wistar rats [135]. In a similar study, curcumin NPs were embedded in gelation microsphere hydrogels. These thermosensitive and MMP-9 responsive hydrogels induced drug release at the wound bed and resulted in acceleration of diabetic skin wound healing due to their ability to promote cell migration and antioxidant nature [136].

Another important flavonoid, quercetin, is also known for its antioxidant nature. But its topical application is limited due to low solubility, low stability, and less release after application. To overcome these limitations, quercetin was encapsulated into mesoporous silica NPs carrying a copolymer. These NPs showed thermoresponsive behavior that acts to provide the potential of such particles in dermal delivery [137]. In another study, topical application of chitosan-fibrin scaffolds impregnated with quercetin significantly accelerated the process of wound healing [138]. Similarly, baicalin-loaded nanohydrogels comprised of a dispersion of cholesterol-derivatized gellan in phosphate buffer. Baicalin (a flavone glycoside)-loaded nanohydrogels demonstrated complete skin restoration and inhibition of specific inflammatory markers causing rapid skin wound healing in *in vivo* mice [139].

6.2 Nanoparticles for nitric oxide delivery

Nitric oxide is an important diatomic molecule that is synthesized by three different isoforms of enzyme nitric oxide synthases by the conversion of amino acid L-arginine. In the skin, different forms of nitric oxide are produced and released by various cells involved, such as macrophages, keratinocytes, melanocytes, and fibroblasts. In wound healing, nitric oxide is synthesized in the early inflammatory phase by inflammatory macrophage cells, whereas many cells secrete nitric oxide in the proliferative phase during wound healing. Nitric oxide being lipophilic in nature possesses the tendency to interact with various biomolecules. It can cross various biological barriers to reach the target site and diffuse along a concentration gradient to rapidly move from cell to cell. Nitric oxide is antibacterial in nature, modulates the immune response, maintains homeostasis and regulates wound healing, and helps in collagen deposition, cell proliferation, and wound contraction [140]. In this context, topical application of nitric oxide for acute and chronic wound healing always remained a challenge. To meet this challenge, engineering NPs mediated nitric oxide release to the wound bed served as a novel approach to allow its free radicals to exert antibacterial action [141].

In this respect for nitric oxide delivery, nitric oxide released from NPs system constituted of composite of polymer/glass hydrogel was tested for its efficacy in methicillin-resistant *Staphylococcus aureus* (MRSA) abscesses in mice. The study results documented that antibacterial nitric oxide-NPs treatment of abscesses reduced the involved area and bacterial load, and ultimately improved the skin architecture [142]. In a recent study, a novel wound-healing material was formulated by combining chitosan with electrospun PCL nonwoven mat for the loading of nitric oxide. Nitric oxide was released in a sustained fashion from the developed wound dressing under the physiological conditions. *In vivo* wound healing evaluated in full-thickness cutaneous wounds of mice resulted in accelerated wound healing through re-epithelialization and granulation formation due to immunomodulation and enhanced collagen synthesis provided by the sustained release of nitric oxide [143]. Nitric oxide released from silica NPs has been demonstrated to exert a bactericidal activity against *P. aeruginosa*, which is one of the important pathogens causing wound infections in hospitals [144]. In a different report, nitric oxide donor precursor glutathione was encapsulated chitosan NPs with an encapsulation efficiency of 99.60% (**Figure 2**). Small size and positive zeta of chitosan NPs led to the delivery of nitric oxide through the skin for topical applications due to the affinity of positively charged chitosan NPs with negatively charged phospholipids that further result in changing the permeability of the skin membrane and reducing the skin barriers [145].

6.3 Antibiotics-loaded nanoparticles for wound repair

There are many types of wounds that fail to heal and turn into chronic wounds [146]. The major cause of delayed healing of such wounds is the persistence of infectious agents or microbial growth around the wound bed [147]. The major goal of any wound-healing treatment is to control the microbial infections to allow normal healing to proceed. Conventional therapies to treat microbial infections are based on either the systemic administration of antibiotics or the topical application of antibiotic formulations [146]. The systemic administration of antibiotics causes toxicity along with kidney and liver complications, but topical application of antibiotics provides high local concentration with a short residence time on the wound surface [148].

Therefore, an appropriate antimicrobial therapy of the wound to control microbial colonization is still required for optimum wound care. The delivery of antibiotic therapy *via* NPs offers great potential advantages, such as slow and sustained delivery, targeted delivery, and decrease in toxicity and improvement in antimicrobial and pharmacokinetic properties (**Figure 2**) [149].

In this respect, a novel wound dressing based on the Spanish Broom fibers impregnated with vancomycin-loaded chitosan NPs was designed, which showed an increased antibacterial action against *S. aureus* and was not toxic to HaCaT keratinocytes as compared to the fibers containing vancomycin without NPs [150]. In another study, chitosan nanofibers mat was functionalized with thiol groups, and gentamicin-loaded liposomes (17% loading efficiency) were immobilized covalently. Liposomes showed sustained and controlled release of gentamicin during 16 h, achieving a steady state at 24 h [151]. Nanofibers of small diameter exhibit unique properties such as excellent mechanical properties, flexibility in surface functionalities, and high specific surface area for wound healing [152]. Chitosan is used in such dressings because its biodegradable, cell adhesive, and possesses hemostatic activity and high mechanic strength [153]. In another study, ZnO NPs were coated with gentamicin and integrated into the chitosan matrix to yield a ZnO/gentamicin-chitosan gel (**Figure 2**). The resulting gel showed 91% of gentamicin release after 8 h and evidenced a four-fold minimum inhibitory concentration (MIC) reduction for *S. aureus* and 2-fold reduction of MIC for *P. aeruginosa*. The resulting antibacterial gels could serve as potential candidates for wound healing [154]. Few other reports well-documented the loading of antibiotics in NPs or nanofibers matrix for the sustained release of antibiotic drugs to prepare wound dressings. Ampicillin-incorporated electrospun polyurethane scaffolds [155], PVA films containing tetracycline hydrochloride-loaded quaternized chitosan NPs [156], tetracycline hydrochloride-loaded electrospun nanofibers mats based on chitosan, and PVA NPs [157] wound dressings have shown biocompatibility and strong antibacterial activity against different strains of bacteria.

7. Engineered scaffolds and nanocomposites for wound healing

One most important feature of the modern dressings used for the therapeutic purpose for skin tissue repair involves the designing of engineered biocompatible nanoscaffolds to mimic the structure of ECM (**Figures 2** and **3**). Such nanoscaffolds should possess the properties such as porous nature, biocompatibility, tendency to incorporate and release various growth factors/antibiotics in a controlled manner, and support for the attachment of cells and their proliferation [158]. Scaffold-based tissue-engineered nanocomposites have been known to accelerate chronic wound healing by improvement in angiogenesis [159]. Various nanotechnology-based methods such as electrospinning, phase separation, and self-assembly have been devised for the formation of such scaffolds [65]. Electrospinning is the most favored method among all these methods for the preparation of nanofibrous scaffolds for skin tissue engineering [160]. For promoting diabetic wound healing and increasing collagen content, nanofibrous glucophage-loaded collagen/PLGA scaffolds were fabricated by electrospinning [161]. In another study, electrospun PCL scaffolds resulted in fibroblast attachment and proliferation. Full-thickness skin wounds of guinea pigs healed within 35 days after treatment with these membranes [162]. Similarly, electrospun membrane of PCL/chitosan nanofibers/aloe vera was fabricated to mimic both the

layers of skin. Top dense layer was composed of PCL to provide mechanical strength to the wounded site, and bottom layer of the dressing consisted of chitosan nanofibers and aloe vera to provide bactericidal activity for promoting skin wound healing [163]. Electrospun chitosan-poly(ethylene oxide) (PEO) nanofibrous scaffold-incorporated PLGA NPs were studied for *in vivo* wound healing [164]. In another report, chitosan NPs and TiO_2 NPs were incorporated in polyurethane nanocomposites membranes, which showed 71.5% improvement in swelling when compared to neat polyurethane membrane and resulted in increase in tensile strength and antibacterial activity suitable for wound healing [165]. Multilayer wound dressing prepared by electrospun polyurethane nanofibers loaded with Semellil extract and other layer chitosan nanofiber resulted in 94% wound closure after 14 days [166].

Another material, that is, nanocomposites have also been proved beneficial for wound healing due to the properties possessed by its dispersed phase and reinforcing fillers. The nanocomposites serve as optimum wound dressing materials as they provide better mechanical strength due to the synergistic action of both the components. In a study, nanocomposites of collagen sponges and AuNPs showed greater tensile strength and indicated a faster wound healing [167]. In a different report, cerium nanocrystals were immobilized onto mesoporous silica NPs to develop ROS scavenging nanocomposites for wound healing. Ceria nanocrystals are known to reduce ROS and to protect the cell from oxidative damage. Silica NPs act as nanobridge between nanomaterial and tissue matrix for rapid wound closure. The nanocomposite prepared from both the components resulted in accelerated wound healing due to their synergistic action [168]. Another type of nanocomposites was prepared by using bacterial cellulose and hyaluronic acid. Bacterial cellulose has high-water-absorbing capacity and porous nature responsible for enhancing the wound-healing rate, whereas hyaluronic acid is biocompatible and possesses gel-forming capacity. The developed nanocomposites facilitated the growth of fibroblast cells and promoted the tissue repair [169]. Nanocomposites wound dressing for chronic wounds prepared from halloysite and chitosan oligosaccharides showed better skin re-epithelization and reorganization as compared to halloysite or chitosan alone [170].

8. Nanomaterials incorporated with growth factors for healing

Healing occurs as a cellular response to wounding/injury, and it involves a variety of cells such as macrophages, fibroblasts, keratinocytes, and neutrophils. In general, the wound-healing process is regulated by various factors, such as microbial infections, wound type, patient conditions, lesser growth factors, and cytokine release. Impaired or delayed wound healing is very much affected by the decreased production of different growth factors by the cells that will ultimately cause the lengthening of healing time and leads to various other complications [171]. Various growth factors such as VEGF, EGF, PDGF, FGF, and TGF-β play a great role in promoting the wound-healing process *via* decreasing inflammation, promoting cell proliferation of fibroblasts and epithelial cells, increasing angiogenesis and ultimately re-epithelialization [158]. Topical administration of growth factors as wound dressings is quite unsatisfactory due to their low biodegradability, instability of protein structure under certain physiological conditions, and enzymatic degradation [172]. In this regard, new drug delivery systems to deliver growth factors at the target wound site in a controlled fashion were developed using nanotechnology (**Figures 2** and **3**) [173].

In this context, gold NPs have been used to conjugate the keratinocyte growth factor. The gold NPs effectively promoted the proliferation of keratinocytes in contrast to unloaded gold NPs or keratinocyte growth factor. *In vivo* full-thickness wound model resulted in enhanced of healing by promoting re-epithelialization by the application of growth factor conjugated NPs. In this study, gold NPs were favored for use due to their biocompatibility and versatile nature for surface functionalization [174]. In another study, recombinant human EGF-loaded nanostructured lipid carriers (NLCs) were checked for their wound-healing efficacy in full-thickness excisional wound porcine model. *In vivo* healing experiments showed that topical application of 20 μg of recombinant human epidermal growth factor (rhEGF)-NLC enhanced the percentage rate of wound closure by day 25 as compared to administration of 75 μg of free rhEGF and NLC by migration and proliferation of fibroblast cells and deposition of collagen in the newly healed wound [129]. Human EGF was loaded into thiolated heparin and diacrylated PEG hydrogels *via* photopolymerization for wound healing. *In vivo* full-thickness wounds in mice showed accelerated wound closure as compared to EGF solution due to sustained release of EGF from biocompatible hydrogel [175]. In a similar manner, wound dressing composed of chitosan-hyaluronic acid composite sponge containing VEGF encapsulated fibrin NPs was designed for diabetic wounds. From the released studies, it was found that 64% of the encapsulated VEGF in NPs was released in 72 h with an initial burst release of 29% in 2 h, which was supposed for the initial sprouting of blood vessels. *In vitro* studies showed that endothelial cells seeded on these hydrogels showed capillary-like tube formation beneficial in wound-healing angiogenesis [176]. From all these studies, it can be inferred that NPs enhance the release of growth factors and thus accelerate the wound-healing process.

9. Gene (RNAi and siRNA) delivery by nanotherapeutic agents for wound repair

It is a well-known fact that miRNAs can be critical regulators of wound repair [177], but the miRNAs involved and their specific role in wound healing remains unclear. Recently, a new method for the identification of functional miRNAs, which get elevated during skin injury, has been described. This group has identified miR-223 as a new potential therapeutic target influencing acute inflammation in wounds that are *S. aureus* infected [178].

RNAi therapy has been used to specifically silence gene expression of overexpressed targets in chronic wounds [179]. NP-based approach has been implemented to protect the effector molecule in siRNA from degradation *via* intracellular RNases leading to targeted delivery [180]. Of late, gold NP conjugates with spherical nucleic acid (SNA) have also been employed for efficient *in vivo* siRNA delivery [181]. The importance of SNA nanotechnology lies in its ability to cross the epidermal barrier, thereby permitting its use in topical therapeutics (**Figures 2** and **3**). Nevertheless, a pertinent demand for more efficacious and refined novel RNAi-based therapeutics for tissue repair. Such products should overcome the drawbacks of presently available materials in use and provide for better retention, bioavailability, effectiveness, safety, and selective targeting [180].

A combination of gene therapy and tissue engineering commonly called as gene-activated matrix therapy has come to the fore as a method to enhance or knockdown a specific target gene playing a role in bone, cartilage, or skin regeneration [182]. The major advantage of this approach is the higher stability of DNA in comparison to the

growth factor therapy [182, 183]. However, the major flaw of this technique is the need for repeated injections of colloidal and naked DNA to the wound site and the short-term and inconsistent gene expression [183]. To overcome these issues, nucleic acids have been impregnated into electrospun nanofibrous meshes to increase tissue regeneration and to decrease scarring [183]. More recently, polyester scaffolds have been used for the management of cutaneous wounds [179, 184]. Furthermore, electrospun scaffolds having a mixture of PLA and PCL were employed for the delivery of plasmid that encodes keratinocytes' growth factor [184].

10. Nanomaterials incorporated with stem cells to prompt healing

Therapeutic approaches for wound healing that involves stem cells have been studied extensively in the past, and they have shown great promise in promoting angiogenesis and facilitating re-epithelialization [185]. Adipose-derived stromal cells (ASCs), bone marrow (BM)-derived endothelial progenitor cells, BM-derived mesenchymal stem cells (BM-MSCs), BM-derived mononuclear cells (BM-MNCs), placenta-derived SCs, and umbilical cord-derived MSCs have been shown to have a therapeutic role in wound repair [185, 186]. The probable mechanism of action is, however, not well elucidated, but the hypothesis says that SC therapy tends to provide a dynamic wound microenvironment *via* their paracrine effect, hence, hastening the healing cascade [187]. In a recent study, it was shown that when MSCs are directly delivered to the wound site, they induce cell death; this effect was then attenuated by using bioengineered delivery platform for therapies [188].

Currently, SCs are used for local administration to wounds by the way of dressings, injections, sprays, and systemic administration. Recently, nanotechnology-driven approaches were employed to synthesize nanomatrices possessing customized biophysical properties, leading to controlled differentiation of SCs. BM-MSCs attached to collagen/PLGA nanofiber scaffold showcased faster closure of wounds [189]. The BM-MSC/nanoscaffold composite approach was explored for wound healing and regeneration (**Figures 2** and **3**). Subsequently, a mixture of MSCs, growth factors, and the matrix was used in the production of nanoscaffolds to mimic human skin characteristics [187, 190]. Polymeric nanofibers having biomimetic potential can simulate the native tissue thereby forming an ideal SC niche. Despite significant advancements in this field in the past, no SC therapy for chronic wound management has yet been FDA approved [185]. It is highly likely that in the future, viable treatments will use SCs in combination with other local/systemic therapies.

11. Nanotechnology-driven targeted delivery achieves cell-type specificity

The field of targeted/site-specific delivery of nanomaterials is still in its young days in comparison to various other nanotechnologies used for wound-healing applications. Targeted delivery of therapeutics is highly recommended and significant to reduce side effects, improve efficacy, and reduce therapy costs [180]. This also overcomes the limitation of a low viable cell number homing the target tissue that has often been associated with systemic stem cell therapy. This platform is highly versatile and can be customized for the targeted delivery of cells, DNA, proteins siRNA, and small drugs, to any target tissue. This is achieved by complexing the NP with the therapeutic agent and with the aid of a molecular recognition molecule integrated within the NP.

Recently, SDF1 was used for targeted delivery to the injury site employing ROS stimulus-responsive polymeric NPs as delivery vehicles [191]. The nanocarrier-targeted delivery platform demonstrated high efficiency and biocompatibility to direct SCs to the injured tissues, resulting in enhanced angiogenesis and repair of injury with no toxicity or immunogenicity involved.

12. Advantages of nanotechnology over conventional methods for healing

Nanotechnology offers several advantages to nanomaterial dressings as compared to dressings prepared by conventional methods for wound healing applications. The various advantages of nanomaterials considered for skin tissue engineering include: (i) nano-dimensions impart proper structure to cells/tissues for their adhesion, differentiation, and proliferation, (ii) due to particular chemical composition and physical structure, nanomaterials serve as analogous structures to extracellular matrix [192], (iii) nanomaterials have high mechanical strength due to which these can act to reinforce various organic/synthetic scaffolds for tissue engineering [193], (iv) high conductivity of carbon-based nanomaterials provides electrical stimulation to scaffolds for skin tissue repair [194, 195], (v) micro/nanoencapsulation of important growth factors/bioactive agents help them to release these molecules in a slow and sustained manner at the target wound site [196, 197], (vi) NPs impart better biocompatibility, bioactivity and enhance interactions of scaffolds to cells or proteins [198], and (vii) NPs possess astonishing properties that are far better in terms of high Young's modulus, high tensile strength, and high surface-area-to-volume ratio as compared to the bulk materials from which nanomaterials are being prepared. All these advantages of NPs make them successful candidates for tissue repair and wound healing applicability.

13. Conclusions and future perspectives

Millions of people around the globe are being affected by chronic wounds, with current research showing limited success in producing FDA-approved efficacious therapeutic agents. This may be attributed to the fact that chronic wound pathology is highly complex as is the tissue repair process. Hence, researchers are in dire need to develop alternative therapeutic approaches for the management of nonhealing wounds that would be viable and efficient as per the FDA norms. The factors that are known to impede the development of therapeutics for chronic wounds include variability of patients and comorbidities, limited understanding of patient pathophysiology, complexity and costs associated with clinical trials, and the general lack of awareness in the public.

Despite a multitude of previously existing materials, healing in chronic conditions is still compromised. Hence, the future wound repair materials should possess a plethora of functions and properties such as antimicrobial, biomimetic, bioresponsive, and hemostasis to provide a suitable microenvironment for wound repair. Therefore, developing an appropriate combinational therapy that targets dysfunctional cellular processes remains the major challenge. Future advances in the understanding of the complex wound healing process will surely aid in this front. The emergence of multifunctional nanotechnologies, in the wound healing arena, showcases the high expectations toward this field. However, gaining in-depth information about their

physicochemical properties and their possible toxicity remains a huge hurdle in promoting these nanotechnologies for human use. Recent developments in the nano-field have led to the development of matrices, scaffolds, skin substitutes, embedded/loaded dressings, etc., which mimic the integrity of the skin. Soon unique phenotype–genotype characteristics will lead way to tailored therapies. This will provide a platform to create new nanotechnology-driven approaches, hence, streamlining and facilitating personalized treatment plans.

For the clinical translation of nanotechnology-based products, there is an urgent need for improved tools and better analytical methods. Comprehensive efforts are necessary to develop chronic wound care products with target and site-specificity to negate the undesirable effects of the nanosystems in humans. In due course of time and with the ever-increasing reports about exciting new nanotechnology platforms, the day is not far when the international standards on biocompatibility and toxicity of various nanotherapies are met with. In a nutshell, our current knowledge about the development of nanotherapeutics, together with our understanding of phenotype–genotype characteristics and chronic wound pathology, will be instrumental in promoting and conceptualizing next-generation wound repair nanotechnologies.

Acknowledgements

The authors acknowledge the Director, CSIR-IHBT for his valuable guidance and support. RS and SS thank UGC and CSIR, respectively, for providing research fellowships and AcSIR for Ph.D. registration. YSP thanks the financial assistance from the Council of Scientific and Industrial Research (CSIR), Government of India in the form of project MLP0204. The CSIR-IHBT publication number of this article is 4347.

List of abbreviations

Ag^+	silver ions
AgNP	silver nanoparticle(s)
ASCs	adipose-derived stromal cells
AuNP	gold nanoparticle(s)
bFGF	basic fibroblast growth factor
BM-MNCs	bone marrow-derived mononuclear cells
BM-MSCs	bone marrow-derived mesenchymal stem cells
BM	bone marrow
CNCs	cellulose nanocrystals
CNT	carbon nanotubes
ECM	extracellular matrix
EGF	epidermal growth factor
ELPF	elastin-like peptide
FDAF	food and drug administration
FGF	fibroblast growth factor
GO	graphene oxide
IFN-γ	interferon-gamma
ILs	interleukins
KGF	keratinocyte growth factor
MAPK	mitogen-activated protein kinases

MDR	multi-drug resistant
MIC	minimum inhibitory concentration
MMPs	metalloproteinase
MRSA	methicillin-resistant *S. aureus*
NLC	nanostructured lipid carriers
NP	nanoparticle(s)
PCL	polycaprolactone
PDGF	platelet-derived growth factor
PEG	poly(ethylene glycol)
PEO	poly(ethylene oxide)
PI3K	phosphoinositide 3-kinase
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PVA	poly(vinyl alcohol)
RHC	recombinant human collagen
rhEGF	recombinant human epidermal growth factor
ROS	reactive oxidative species
SC	stem cell(s)
SD	sprague dawley
SDF1	stromal cell-derived growth factor-1
SLN	solid lipid nanoparticle
SNA	spherical nucleic acid
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
ZnO	zinc oxide

Author details

Sourabh Soni[1,2$†], Rubbel Singla[2,3†] and Yogendra S. Padwad[1,2*]

1 Pharmacology and Toxicology Laboratory, Dietetics and Nutrition Technology Division, CSIR—Institute of Himalayan Bioresource Technology (CSIR-IHBT), Palampur, Himachal Pradesh, India

2 Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

3 Nanobiology Laboratory, Biotechnology Division, CSIR—Institute of Himalayan Bioresource Technology (CSIR-IHBT), Palampur, Himachal Pradesh, India

*Address all correspondence to: yogendra@ihbt.res.in

$Current address: Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, MA, USA.

† These authors contributed equally.

References

[1] Dhivya S, Padma VV, Santhini E. Wound dressings—A review. BioMedicine. 2015;**5**(4):24-28

[2] Seetharam RN. Nanomedicine—Emerging area of nanobiotechnology research. Current Science. 2006;**91**(3): 260

[3] Nandhini J, Neeraja P, Rajkumar SRJ, Umapathy V, Suresh S. Comparative studies of microwave and sol–gel-assisted combustion methods of $NiFe_2O_4$ nanostructures: synthesis, structural, morphological, opto-magnetic, and antimicrobial activity. Journal of Superconductivity and Novel Magnetism. 2017;**30**(5): 1213-1220

[4] Suri SS, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. Journal of Occupational Medicine and Toxicology. 2007;**2**(1): 16-21

[5] Medintz IL, Mattoussi H, Clapp AR. Potential clinical applications of quantum dots. International Journal of Nanomedicine. 2008;**3**(2):151

[6] Sandhiya S, Dkhar SA, Surendiran A. Emerging trends of nanomedicine—An overview. Fundamental & Clinical Pharmacology. 2009;**23**(3):263-269

[7] Shalaby MA, Anwar MM, Saeed H. Nanomaterials for application in wound healing: Current state-of-the-art and future perspectives. Journal of Polymer Research. 2022;**29**(3):1-37

[8] Blanco-Fernandez B, Castaño O, Mateos-Timoneda MÁ, Engel E, Pérez-Amodio S. Nanotechnology approaches in chronic wound healing. Advances in Wound Care. 2021;**10**(5):234-256

[9] Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Percoraro RE, Rodeheaver G, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. Wound Repair and Regeneration. 1994;**2**(3):165-170

[10] Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. Journal of International Medical Research. 2009;**37**(5):1528-1542

[11] Mittal A, Sardana S, Pandey A. Herbal boon for wounds. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;**5**:1-12

[12] Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. Physiological Reviews. 2019;**99**(1):665-706

[13] Ghosh PK, Gaba A. Phyto-extracts in wound healing. Journal of Pharmacy & Pharmaceutical Sciences. 2013;**16**(5): 760-820

[14] Chhabra S, Chhabra N, Kaur A, Gupta N. Wound healing concepts in clinical practice of OMFS. Journal of Maxillofacial and Oral Surgery. 2017; **16**(4):403-423

[15] Diegelmann RF, Evans MC. Wound healing: An overview of acute, fibrotic and delayed healing. Frontiers in Bioscience. 2004;**9**(1):283-289

[16] Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Science Translational Medicine. 2014;**6** (265):265sr6

[17] Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, et al.

An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. Value in Health. 2018;**21**(1):27-32

[18] Han G, Ceilley R. Chronic wound healing: A review of current management and treatments. Advances in Therapy. 2017;**34**(3):599-610

[19] Liang L, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, et al. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. Wound Repair and Regeneration. 2016;**24**(6):943-953

[20] Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and biomarkers for wound healing. Plastic and Reconstructive Surgery. 2016;**138**(3): 18S-28S

[21] Loesche M, Gardner SE, Kalan L, Horwinski J, Zheng Q, Hodkinson BP, et al. Temporal stability in chronic wound microbiota is associated with poor healing. Journal of Investigative Dermatology. 2017;**137**(1):237-244

[22] Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, part 1: Normal and chronic wounds: Biology, causes, and approaches to care. Advances in Skin & Wound Care. 2012; **25**(7):304-315

[23] Mahmoudi M, Gould LJ. Opportunities and challenges of the management of chronic wounds: A multidisciplinary viewpoint. Chronic Wound Care Management and Research. 2020;**7**:27

[24] Garraud O, Hozzein WN, Badr G. Wound healing: Time to look for intelligent,'natural'immunological approaches? BMC Immunology. 2017; **18**(1):23-30

[25] Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;**453**(7193): 314-321

[26] Kirsner RS, Eaglstein WH. The wound healing process. Dermatologic Clinics. 1993;**11**(4):629-640

[27] Regan MC, Barbul A. The cellular biology of wound healing. Wound Healing. 1994;**1**:3-17

[28] Sezer AD, Cevher E. Biopolymers as wound healing materials: Challenges and new strategies. In: Biomaterials Applications for Nanomedicine. London, UK: InTech; 2011

[29] Steed DL. The role of growth factors in wound healing. Surgical Clinics of North America. 1997;**77**(3):575-586

[30] Cooper DM. Optimizing wound healing. A practice within nursing's domain. The Nursing Clinics of North America. 1990;**25**(1):165-180

[31] Broughton G, Janis JE, Attinger CE. Wound healing: An overview. Plastic and Reconstructive Surgery. 2006;**117** (7eS):1eS-32eS

[32] Stoica AE, Chircov C, Grumezescu AM. Nanomaterials for wound dressings: An up-to-date overview. Molecules. 2020;**25**(11):2699

[33] Robson MC, Steed DL, Franz MG. Wound healing: Biologic features and approaches to maximize healing trajectories. Current Problems in Surgery. 2001;**38**(2):72-140

[34] Woo YC, Park SS, Subieta AR, Brennan TJ. Changes in tissue pH and

temperature after incision indicate acidosis may contribute to postoperative pain. Anesthesiology: The Journal of the American Society of Anesthesiologists. 2004;**101**(2):468-475

[35] De Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: Going forward in reverse. Nature Reviews Immunology. 2016;**16**(6):378-391

[36] Daley JM, Reichner JS, Mahoney EJ, Manfield L, Henry WL, Mastrofrancesco B, et al. Modulation of macrophage phenotype by soluble product (s) released from neutrophils. The Journal of Immunology. 2005;**174**(4):2265-2272

[37] Koh TJ, DiPietro LA. Inflammation and wound healing: The role of the macrophage. Expert Reviews in Molecular Medicine. 2011;**13**:e23-e34

[38] Profyris C, Tziotzios C, Do Vale I. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics: Part I. The molecular basis of scar formation. Journal of the American Academy of Dermatology. 2012;**66**(1):1-10

[39] DiPietro LA, Wilgus TA, Koh TJ. Macrophages in healing wounds: Paradoxes and paradigms. International Journal of Molecular Sciences. 2021; **22**(2):950

[40] Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R, et al. Wound healing in the PU. 1 Null mouse—Tissue repair is not dependent on inflammatory cells. Current Biology. 2003;**13**(13): 1122-1128

[41] DiPietro LA, Polverini PJ. Role of the macrophage in the positive and negative regulation of wound neovascularization. Behring Institute Mitteilungen. 1993; **92**:238-247

[42] Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. Journal of Investigative Dermatology. 2007;**127**(3):514-525

[43] Redd MJ, Cooper L, Wood W, Stramer B, Martin P. Wound healing and inflammation: Embryos reveal the way to perfect repair. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2004;**359** (1445):777-784

[44] Strodtbeck F. Physiology of wound healing. Newborn and Infant Nursing Reviews. 2001;**1**(1):43-52

[45] Bauer SM, Bauer RJ, Velazquez OC. Angiogenesis, vasculogenesis, and induction of healing in chronic wounds. Vascular and Endovascular Surgery. 2005;**39**(4):293-306

[46] Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. Blood Reviews. 2015;**29**(3):153-162

[47] Potekaev NN, Borzykh OB, Medvedev GV, Pushkin DV, Petrova MM, Petrov AV, et al. The role of extracellular matrix in skin wound healing. Journal of Clinical Medicine. 2021;**10**(24):5947

[48] Bielefeld KA, Amini-Nik S, Alman BA. Cutaneous wound healing: Recruiting developmental pathways for regeneration. Cellular and Molecular Life Sciences. 2013;**70**(12):2059-2081

[49] Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB. Therapeutic strategies for enhancing angiogenesis in wound healing. Advanced Drug Delivery Reviews. 2019;**146**:97-125

[50] Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, et al.

Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. The American Journal of Pathology. 2004;**164**(6): 1935-1947

[51] Opalenik SR, Davidson JM. Fibroblast differentiation of bone marrow-derived cells during wound repair. The FASEB Journal. 2005;**19**(11): 1561-1563

[52] Werner S, Krieg T, Smola H. Keratinocyte–fibroblast interactions in wound healing. Journal of Investigative Dermatology. 2007;**127**(5):998-1008

[53] Martin P. Wound healing—Aiming for perfect skin regeneration. Science. 1997;**276**(5309):75-81

[54] Lau K, Paus R, Tiede S, Day P, Bayat A. Exploring the role of stem cells in cutaneous wound healing. Experimental Dermatology. 2009;**18**(11):921-933

[55] Krafts KP. Tissue repair: The hidden drama. Organogenesis. 2010;**6**(4): 225-233

[56] Darby IA, Desmoulière A. Scar formation: Cellular mechanisms. In: Textbook on Scar Management. Cham: Springer; 2020. pp. 19-26

[57] Lux CN. Wound healing in animals: A review of physiology and clinical evaluation. Veterinary Dermatology. 2022;**33**(1):91-e27

[58] Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. Bioengineering. 2021;**8**(5):63

[59] Szabowski A, Maas-Szabowski N, Andrecht S, Kolbus A, Schorpp-Kistner M, Fusenig NE, et al. c-Jun and JunB antagonistically control cytokine-regulated mesenchymal–epidermal interaction in skin. Cell. 2000;**103**(5): 745-755

[60] Gurtner GC, Evans GR. Advances in head and neck reconstruction. Plastic and Reconstructive Surgery. 2000; **106**(3):672-682

[61] Levenson SM, Geever EF, Crowley LV, Oates JF III, Berard CW, Rosen H. Healing of rat skin wounds. Annals of Surgery. 1965;**161**(2):293-308

[62] Sarabahi S. Recent advances in topical wound care. Indian Journal of Plastic Surgery: Official Publication of the Association of Plastic Surgeons of India. 2012;**45**(2):379-387

[63] Jarbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Systematic Reviews. 2017;**6**(1):15-21

[64] Sen CK. Human wound and its burden: Updated 2020 compendium of estimates. Advances in Wound Care. 2021;**10**(5):281-292

[65] Hamdan S, Pastar I, Drakulich S, Dikici E, Tomic-Canic M, Deo S, et al. Nanotechnology-driven therapeutic interventions in wound healing: Potential uses and applications. ACS Central Science. 2017;**3**(3):163-175

[66] Baranwal J, Barse B, Fais A, Delogu GL, Kumar A. Biopolymer: A sustainable material for food and medical applications. Polymers. 2022;**14**(5):983

[67] Veves A, Falanga V, Armstrong DG, Sabolinski ML. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: A prospective

randomized multicenter clinical trial. Diabetes Care. 2001;**24**(2): 290-295

[68] Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of dermagraft in improving the healing of chronic diabetic foot ulcers: Results of a prospective randomized trial. Diabetes Care. 2003;**26**(6):1701-1705

[69] Driver VR, Lavery LA, Reyzelman AM, Dutra TG, Dove CR, Kotsis SV, et al. A clinical trial of Integra Template for diabetic foot ulcer treatment. Wound Repair and Regeneration. 2015;**23**(6): 891-900

[70] Wei EX, Kirsner RS, Eaglstein WH. End points in dermatologic clinical trials: A review for clinicians. Journal of the American Academy of Dermatology. 2016;**75**(1):203-209

[71] Kalashnikova I, Das S, Seal S. Nanomaterials for wound healing: Scope and advancement. Nanomedicine. 2015; **10**(16):2593-2612

[72] Parani M, Lokhande G, Singh A, Gaharwar AK. Engineered nanomaterials for infection control and healing acute and chronic wounds. ACS Applied Materials & Interfaces. 2016;**8**(16): 10049-10069

[73] Tamer TM, Valachová K, Hassan MA, Omer AM, El-Shafeey M, Eldin MSM, et al. Chitosan/hyaluronan/ edaravone membranes for anti-inflammatory wound dressing: In vitro and in vivo evaluation studies. Materials Science and Engineering: C. 2018;**90**: 227-235

[74] Zeng HY, Huang YC. Basic fibroblast growth factor released from fucoidan-modified chitosan/alginate scaffolds for promoting fibroblasts migration. Journal of Polymer Research. 2018;**25**(3):1-9

[75] Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: A comprehensive review. Advances in Wound Care. 2014;**3**(7):445-464

[76] Korrapati PS, Karthikeyan K, Satish A, Krishnaswamy VR, Venugopal JR, Ramakrishna S. Recent advancements in nanotechnological strategies in selection, design and delivery of biomolecules for skin regeneration. Materials Science and Engineering: C. 2016;**67**:747-765

[77] Wang F, Zhang W, Li H, Chen X, Feng S, Mei Z. How effective are nano-based dressings in diabetic wound healing? A comprehensive review of literature. International Journal of Nanomedicine. 2022;**17**:2097

[78] Mordorski B, Rosen J, Friedman A. Nanotechnology as an innovative approach for accelerating wound healing in diabetes. Diabetes Management. 2015; **5**(5):329-332

[79] Gaharwar AK, Peppas NA, Khademhosseini A. Nanocomposite hydrogels for biomedical applications. Biotechnology and Bioengineering. 2014; **111**(3):441-453

[80] Kumar N, Chamoli P, Misra M, Manoj MK, Sharma A. Advanced metal and carbon nanostructures for medical, drug delivery and bio-imaging applications. Nanoscale. 2022;**14**: 3987-4017

[81] Kaler A, Mittal AK, Katariya M, Harde H, Agrawal AK, Jain S, et al. An investigation of in vivo wound healing activity of biologically synthesized silver nanoparticles. Journal of Nanoparticle Research. 2014;**16**(9):2605-2614

[82] Mihai MM, Dima MB, Dima B, Holban AM. Nanomaterials for wound

healing and infection control. Materials. 2019;**12**(13):2176

[83] Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. Journal of Nanobiotechnology. 2011; **9**(1):30-37

[84] Singh BR, Singh BN, Singh A, Khan W, Naqvi AH, Singh HB. Mycofabricated biosilver nanoparticles interrupt *Pseudomonas aeruginosa* quorum sensing systems. Scientific Reports. 2015;**5**:13719

[85] Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli*. Canadian Journal of Microbiology. 1974;**20**(6):883-889

[86] Xiu ZM, Zhang QB, Puppala HL, Colvin VL, Alvarez PJ. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Letters. 2012; **12**(8):4271-4275

[87] Frankova J, Pivodova V, Vágnerová H, Juráňová J, Ulrichová J. Effects of silver nanoparticles on primary cell cultures of fibroblasts and keratinocytes in a wound-healing model. Journal of Applied Biomaterials & Functional Materials. 2016;**14**(2):137-142

[88] Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM, et al. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem: Chemistry Enabling Drug Discovery. 2007;**2**(1): 129-136

[89] Patra JK, Baek KH. Novel green synthesis of gold nanoparticles using Citrullus lanatus rind and investigation of proteasome inhibitory activity, antibacterial, and antioxidant potential. International journal of nanomedicine. 2015;**10**:7253-7264

[90] Yang X, Yang J, Wang L, Ran B, Jia Y, Zhang L, et al. Pharmaceutical intermediate-modified gold nanoparticles: Against multidrug-resistant bacteria and wound-healing application via an electrospun scaffold. ACS Nano. 2017;**11**(6):5737-5745

[91] Gobin AM, O'Neal DP, Watkins DM, Halas NJ, Drezek RA, West JL. Near infrared laser-tissue welding using nanoshells as an exogenous absorber. Lasers in Surgery and Medicine. 2005;**37** (2):123-129

[92] Khatami M, Varma RS, Zafarnia N, Yaghoobi H, Sarani M, Kumar VG. Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages. Sustainable Chemistry and Pharmacy. 2018;**10**:9-15

[93] Shao F, Yang A, Yu DM, Wang J, Gong X, Tian HX. Bio-synthesis of *Barleria gibsoni* leaf extract mediated zinc oxide nanoparticles and their formulation gel for wound therapy in nursing care of infants and children. Journal of Photochemistry and Photobiology B: Biology. 2018;**189**: 267-273

[94] Kantipudi S, Sunkara JR, Rallabhandi M, Thonangi CV, Cholla RD, Kollu P, et al. Enhanced wound healing activity of Ag–ZnO composite NPs in Wistar Albino rats. IET Nanobiotechnology. 2017;**12**(4):473-478

[95] Pati R, Mehta RK, Mohanty S, Padhi A, Sengupta M, Vaseeharan B, et al. Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine:

Nanotechnology, Biology and Medicine. 2014;**10**(6):1195-1208

[96] Ziv-Polat O, Topaz M, Brosh T, Margel S. Enhancement of incisional wound healing by thrombin conjugated iron oxide nanoparticles. Biomaterials. 2010;**31**(4):741-747

[97] Mir M, Ali MN, Barakullah A, Gulzar A, Arshad M, Fatima S, et al. Synthetic polymeric biomaterials for wound healing: A review. Progress in Biomaterials. 2018;**7**(1):1-21

[98] Perumal G, Pappuru S, Chakraborty D, Nandkumar AM, Chand DK, Doble M. Synthesis and characterization of curcumin loaded PLA—Hyperbranched polyglycerol electrospun blend for wound dressing applications. Materials Science and Engineering: C. 2017;**76**: 1196-1204

[99] Chereddy KK, Coco R, Memvanga PB, Ucakar B, des Rieux A, Vandermeulen G, et al. Combined effect of PLGA and curcumin on wound healing activity. Journal of Controlled Release. 2013;**171**(2):208-215

[100] Liao J, Jia Y, Wang B, Shi K, Qian Z. Injectable hybrid poly (ε-caprolactone)-b-poly (ethylene glycol)-b-poly (ε-caprolactone) porous microspheres/alginate hydrogel cross-linked by calcium gluconate crystals deposited in the pores of microspheres improved skin wound healing. ACS Biomaterials Science & Engineering. 2018;**4**(3): 1029-1036

[101] Chen FM, Liu X. Advancing biomaterials of human origin for tissue engineering. Progress in Polymer Science. 2016;**53**:86-168

[102] Rath G, Hussain T, Chauhan G, Garg T, Goyal AK. Collagen nanofiber containing silver nanoparticles for improved wound-healing applications. Journal of Drug Targeting. 2016;**24**(6): 520-529

[103] Bilgic HAYATİ, Demiriz M, Ozler M, Ide T, Dogan N, Gumus S, et al. Gelatin based scaffolds and effect of EGF dose on wound healing. Journal of Biomaterials and Tissue Engineering. 2013;**3**(2):205-211

[104] Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Effects of chitin and chitosan on blood coagulation. Carbohydrate Polymers. 2003;**53**(3):337-342

[105] Xu J, Strandman S, Zhu JX, Barralet J, Cerruti M. Genipin-crosslinked catechol-chitosan mucoadhesive hydrogels for buccal drug delivery. Biomaterials. 2015;**37**:395-404

[106] Lu Z, Gao J, He Q, Wu J, Liang D, Yang H, et al. Enhanced antibacterial and wound healing activities of microporous chitosan-Ag/ZnO composite dressing. Carbohydrate Polymers. 2017;**156**:460-469

[107] Ding L, Shan X, Zhao X, Zha H, Chen X, Wang J, et al. Spongy bilayer dressing composed of chitosan–Ag nanoparticles and chitosan–*Bletilla striata* polysaccharide for wound healing applications. Carbohydrate Polymers. 2017;**157**:1538-1547

[108] Ehterami A, Salehi M, Farzamfar S, Vaez A, Samadian H, Sahrapeyma H, et al. In vitro and in vivo study of PCL/collagen wound dressing loaded with insulin-chitosan nanoparticles on cutaneous wound healing in rats model. International Journal of Biological Macromolecules. 2018;**117**:601-609

[109] Kim MH, Park H, Nam HC, Park SR, Jung JY, Park WH. Injectable methylcellulose hydrogel containing

silver oxide nanoparticles for burn wound healing. Carbohydrate Polymers. 2018;**181**:579-586

[110] Singla R, Soni S, Kulurkar PM, Kumari A, Mahesh S, Patial V, et al. In situ functionalized nanobiocomposites dressings of bamboo cellulose nanocrystals and silver nanoparticles for accelerated wound healing. Carbohydrate Polymers. 2017a;**155**: 152-162

[111] Singla R, Soni S, Patial V, Kulurkar PM, Kumari A, Mahesh S, et al. In vivo diabetic wound healing potential of nanobiocomposites containing bamboo cellulose nanocrystals impregnated with silver nanoparticles. International Journal of Biological Macromolecules. 2017b;**105**:45-55

[112] Singla R, Soni S, Patial V, Kulurkar PM, Kumari A, Padwad YS, et al. Cytocompatible anti-microbial dressings of *Syzygium cumini* cellulose nanocrystals decorated with silver nanoparticles accelerate acute and diabetic wound healing. Scientific Reports. 2017c; **7**(1):1-13

[113] Sun T, Zhan B, Zhang W, Qin D, Xia G, Zhang H, et al. Carboxymethyl chitosan nanoparticles loaded with bioactive peptide OH-CATH30 benefit nonscar wound healing. International Journal of Nanomedicine. 2018;**13**:5771

[114] Fumakia M, Ho EA. Nanoparticles encapsulated with LL37 and serpin A1 promotes wound healing and synergistically enhances antibacterial activity. Molecular Pharmaceutics. 2016; **13**(7):2318-2331

[115] Yeboah A, Cohen RI, Faulknor R, Schloss R, Yarmush ML, Berthiaume F. The development and characterization of SDF1α-elastin-like-peptide nanoparticles for wound healing. Journal of Controlled Release. 2016;**232**:238-247

[116] Senturk B, Mercan S, Delibasi T, Guler MO, Tekinay AB. Angiogenic peptide nanofibers improve wound healing in STZ-induced diabetic rats. ACS Biomaterials Science & Engineering. 2016;**2**(7):1180-1189

[117] Yergoz F, Hastar N, Cimenci CE, Ozkan AD, Tekinay T, Guler MO, et al. Heparin mimetic peptide nanofiber gel promotes regeneration of full thickness burn injury. Biomaterials. 2017;**134**: 117-127

[118] Deng A, Yang Y, Du S, Yang S. Electrospinning of in situ crosslinked recombinant human collagen peptide/ chitosan nanofibers for wound healing. Biomaterials Science. 2018;**6**(8): 2197-2208

[119] Xi Y, Ge J, Guo Y, Lei B, Ma PX. Biomimetic elastomeric polypeptide-based nanofibrous matrix for overcoming multidrug-resistant bacteria and enhancing full-thickness wound healing/skin regeneration. ACS Nano. 2018;**12**:10772-10784

[120] Santos JCC, Mansur AA, Ciminelli VS, Mansur HS. Nanocomposites of poly (vinyl alcohol)/functionalized-multiwall carbon nanotubes conjugated with glucose oxidase for potential application as scaffolds in skin wound healing. International Journal of Polymeric Materials and Polymeric Biomaterials. 2014;**63**(4):185-196

[121] Mahmoudi N, Eslahi N, Mehdipour A, Mohammadi M, Akbari M, Samadikuchaksaraei A, et al. Temporary skin grafts based on hybrid graphene oxide-natural biopolymer nanofibers as effective wound healing substitutes: Pre-clinical and pathological studies in animal models. Journal of Materials

Science: Materials in Medicine. 2017; **28**(5):73-85

[122] Kawamoto K, Miyaji H, Nishida E, Miyata S, Kato A, Tateyama A, et al. Characterization and evaluation of graphene oxide scaffold for periodontal wound healing of class II furcation defects in dog. International Journal of Nanomedicine. 2018;**13**:2365-2376

[123] Bankoti K, Rameshbabu AP, Datta S, Das B, Mitra A, Dhara S. Onion derived carbon nanodots for live cell imaging and accelerated skin wound healing. Journal of Materials Chemistry B. 2017;**5**(32):6579-6592

[124] Zhou X, Wang H, Zhang J, Li X, Wu Y, Wei Y, et al. Functional poly (ε-caprolactone)/chitosan dressings with nitric oxide-releasing property improve wound healing. Acta Biomaterialia. 2017; **54**:128-137

[125] Lu Z, Dai T, Huang L, Kurup DB, Tegos GP, Jahnke A, et al. Photodynamic therapy with a cationic functionalized fullerene rescues mice from fatal wound infections. Nanomedicine. 2010;**5**(10): 1525-1533

[126] Küchler S, Wolf NB, Heilmann S, Weindl G, Helfmann J, Yahya MM, et al. 3D-wound healing model: Influence of morphine and solid lipid nanoparticles. Journal of Biotechnology. 2010;**148**(1): 24-30

[127] Xu HL, Chen PP, Wang LF, Tong MQ, Ou ZH, Zhao YZ, et al. Skin-permeable liposome improved stability and permeability of bFGF against skin of mice with deep second degree scald to promote hair follicle neogenesis through inhibition of scar formation. Colloids and Surfaces B: Biointerfaces. 2018;**172**:573-585

[128] Gauthier A, Fisch A, Seuwen K, Baumgarten B, Ruffner H, Aebi A, et al. Glucocorticoid-loaded liposomes induce a pro-resolution phenotype in human primary macrophages to support chronic wound healing. Biomaterials. 2018;**178**: 481-495

[129] Gainza G, Bonafonte DC, Moreno B, Aguirre JJ, Gutierrez FB, Villullas S, et al. The topical administration of rhEGF-loaded nanostructured lipid carriers (rhEGF-NLC) improves healing in a porcine full-thickness excisional wound model. Journal of Controlled Release. 2015;**197**:41-47

[130] Gong C, Wu Q, Wang Y, Zhang D, Luo F, Zhao X, et al. A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing. Biomaterials. 2013;**34** (27):6377-6387

[131] Bonferoni MC, Sandri G, Dellera E, Rossi S, Ferrari F, Mori M, et al. Ionic polymeric micelles based on chitosan and fatty acids and intended for wound healing. Comparison of linoleic and oleic acid. European Journal of Pharmaceutics and Biopharmaceutics. 2014;**87**(1): 101-106

[132] Cardoso AM, de Oliveira EG, Coradini K, Bruinsmann FA, Aguirre T, Lorenzoni R, et al. Chitosan hydrogels containing nanoencapsulated phenytoin for cutaneous use: Skin permeation/ penetration and efficacy in wound healing. Materials Science and Engineering: C. 2019;**96**:205-217

[133] Krausz AE, Adler BL, Cabral V, Navati M, Doerner J, Charafeddine RA, et al. Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. Nanomedicine: Nanotechnology, Biology and Medicine. 2015;**11**(1):195-206

[134] Karri VVSR, Kuppusamy G, Talluri SV, Mannemala SS, Kollipara R,

Wadhwani AD, et al. Curcumin loaded chitosan nanoparticles impregnated into collagen-alginate scaffolds for diabetic wound healing. International Journal of Biological Macromolecules. 2016;**93**: 1519-1529

[135] Li L, Xing R, Li J, Li P. Fabrication of guanidinylated chitosan nanoparticles loaded with bioactive factors for facilitating wound healing. International Journal of Polymeric Materials and Polymeric Biomaterials. 2022;**71**(3): 173-179

[136] Liu J, Chen Z, Wang J, Li R, Li T, Chang M, et al. Encapsulation of curcumin nanoparticles with MMP9-responsive and thermos-sensitive hydrogel improves diabetic wound healing. ACS Applied Materials & Interfaces. 2018;**10**(19):16315-16326

[137] Ugazio E, Gastaldi L, Brunella V, Scalarone D, Jadhav SA, Oliaro-Bosso S, et al. Thermoresponsive mesoporous silica nanoparticles as a carrier for skin delivery of quercetin. International Journal of Pharmaceutics. 2016;**511**(1): 446-454

[138] Vedakumari WS, Ayaz N, Karthick AS, Senthil R, Sastry TP. Quercetin impregnated chitosan–fibrin composite scaffolds as potential wound dressing materials—Fabrication, characterization and in vivo analysis. European Journal of Pharmaceutical Sciences. 2017;**97**:106-112

[139] Manconi M, Manca ML, Caddeo C, Cencetti C, di Meo C, Zoratto N, et al. Preparation of gellan-cholesterol nanohydrogels embedding baicalin and evaluation of their wound healing activity. European Journal of Pharmaceutics and Biopharmaceutics. 2018;**127**:244-249

[140] Schaffer MR, Efron PA, Thornton FJ, Klingel K, Gross SS, Barbul A. Nitric oxide, an autocrine regulator of wound fibroblast synthetic function. The Journal of Immunology. 1997;**158**(5): 2375-2381

[141] Englander L, Friedman A. Nitric oxide nanoparticle technology: A novel antimicrobial agent in the context of current treatment of skin and soft tissue infection. The Journal of Clinical and Aesthetic Dermatology. 2010;**3**(6):45

[142] Han G, Martinez LR, Mihu MR, Friedman AJ, Friedman JM, Nosanchuk JD. Nitric oxide releasing nanoparticles are therapeutic for *Staphylococcus aureus* abscesses in a murine model of infection. PloS ONE. 2009;**4**(11):e7804

[143] Zhou Z, Joslin S, Dellinger A, Ehrich M, Brooks B, Ren Q, et al. A novel class of compounds with cutaneous wound healing properties. Journal of Biomedical Nanotechnology. 2010;**6**(5):605-611

[144] Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, et al. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. Journal of Bacteriology. 2009;**191**(23):7333-7342

[145] Pelegrino MT, Weller RB, Chen X, Bernardes JS, Seabra AB. Chitosan nanoparticles for nitric oxide delivery in human skin. MedChemComm. 2017; **8**(4):713-719

[146] Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences. 2008;**97**(8):2892-2923

[147] Scales BS, Huffnagle GB. The microbiome in wound repair and tissue

fibrosis. The Journal of Pathology. 2013; **229**(2):323-331

[148] Hurler J, Berg OA, Skar M, Conradi AH, Johnsen PJ, Škalko-Basnet N. Improved burns therapy: Liposomes-in-hydrogel delivery system for mupirocin. Journal of Pharmaceutical Sciences. 2012;**101**(10):3906-3915

[149] Sharma A, Kumar Arya D, Dua M, Chhatwal GS, Johri AK. Nano-technology for targeted drug delivery to combat antibiotic resistance. Expert Opinion on Drug Delivery. 2012;**9**(11): 1325-1332

[150] Cerchiara T, Abruzzo A, Palomino RAÑ, Vitali B, De Rose R, Chidichimo G, et al. Spanish Broom (*Spartium junceum* L.) fibers impregnated with vancomycin-loaded chitosan nanoparticles as new antibacterial wound dressing: Preparation, characterization and antibacterial activity. European Journal of Pharmaceutical Sciences. 2017;**99**: 105-112

[151] Monteiro N, Martins M, Martins A, Fonseca NA, Moreira JN, Reis RL, et al. Antibacterial activity of chitosan nanofiber meshes with liposomes immobilized releasing gentamicin. Acta Biomaterialia. 2015;**18**:196-205

[152] Leung V, Ko F. Biomedical applications of nanofibers. Polymers for Advanced Technologies. 2011;**22**(3): 350-365

[153] Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: General approaches and a review of recent developments. Journal of the Royal Society Interface. 2011;**8** (55):153-170

[154] Vasile BS, Oprea O, Voicu G, Ficai A, Andronescu E, Teodorescu A, et al. Synthesis and characterization of a novel controlled release zinc oxide/ gentamicin–chitosan composite with potential applications in wounds care. International Journal of Pharmaceutics. 2014;**463**(2):161-169

[155] Sabitha M, Rajiv S. Preparation and characterization of ampicillin-incorporated electrospun polyurethane scaffolds for wound healing and infection control. Polymer Engineering & Science. 2015;**55**(3):541-548

[156] Niamlang P, Tongrain T, Ekabutr P, Chuysinuan P, Supaphol P. Preparation, characterization and biocompatibility of poly (vinyl alcohol) films containing tetracycline hydrochloride-loaded quaternized chitosan nanoparticles. Journal of Drug Delivery Science and Technology. 2017;**38**:36-44

[157] Alavarse AC, de Oliveira Silva FW, Colque JT, da Silva VM, Prieto T, Venancio EC, et al. Tetracycline hydrochloride-loaded electrospun nanofibers mats based on PVA and chitosan for wound dressing. Materials Science and Engineering: C. 2017;**77**: 271-281

[158] Tocco I, Zavan B, Bassetto F, Vindigni V. Nanotechnology-based therapies for skin wound regeneration. Journal of Nanomaterials. 2012;**2012**:4

[159] Nosrati H, Aramideh Khouy R, Nosrati A, Khodaei M, Banitalebi-Dehkordi M, Ashrafi-Dehkordi K, et al. Nanocomposite scaffolds for accelerating chronic wound healing by enhancing angiogenesis. Journal of Nanobiotechnology. 2021;**19**(1):1-21

[160] Bigliardi PL, Tobin DJ, Gaveriaux-Ruff C, Bigliardi-Qi M. Opioids and the skin—Where do we stand? Experimental Dermatology. 2009;**18**(5):424-430

[161] Lee CH, Chang SH, Chen WJ, Hung KC, Lin YH, Liu SJ, et al. Augmentation of diabetic wound healing and enhancement of collagen content using nanofibrous glucophage-loaded collagen/PLGA scaffold membranes. Journal of Colloid and Interface Science. 2015;**439**:88-97

[162] Augustine R, Dominic EA, Reju I, Kaimal B, Kalarikkal N, Thomas S. Electrospun poly (ε-caprolactone)-based skin substitutes: In vivo evaluation of wound healing and the mechanism of cell proliferation. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2015;**103**(7): 1445-1454

[163] Miguel SP, Ribeiro MP, Coutinho P, Correia IJ. Electrospun polycaprolactone/Aloe vera_chitosan nanofibrous asymmetric membranes aimed for wound healing applications. Polymers. 2017;**9**(5):183

[164] Xie Z, Paras CB, Weng H, Punnakitikashem P, Su LC, Vu K, et al. Dual growth factor releasing multi-functional nanofibers for wound healing. Acta Biomaterialia. 2013;**9**(12):9351-9359

[165] Jafari A, Hassanajili S, Karimi MB, Emami A, Ghaffari F, Azarpira N. Effect of organic/inorganic nanoparticles on performance of polyurethane nanocomposites for potential wound dressing applications. Journal of the Mechanical Behavior of Biomedical Materials. 2018;**88**:395-405

[166] Amanzadi B, Mirzaei E, Hassanzadeh G, Mahdaviani P, Boroumand S, Abdollahi M, et al. Chitosan-based layered nanofibers loaded with herbal extract as wound-dressing materials on wound model studies. Biointerface Research in Applied Chemistry. 2019;**9**:3979-3986

[167] Akturk O, Kismet K, Yasti AC, Kuru S, Duymus ME, Kaya F, et al. Collagen/gold nanoparticle nanocomposites: A potential skin wound healing biomaterial. Journal of Biomaterials Applications. 2016;**31**(2):283-301

[168] Wu H, Li F, Wang S, Lu J, Li J, Du Y, et al. Ceria nanocrystals decorated mesoporous silica nanoparticle based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing. Biomaterials. 2018;**151**:66-77

[169] Li Y, Jiang H, Zheng W, Gong N, Chen L, Jiang X, et al. Bacterial cellulose–hyaluronan nanocomposite biomaterials as wound dressings for severe skin injury repair. Journal of Materials Chemistry B. 2015;**3**(17):3498-3507

[170] Sandri G, Aguzzi C, Rossi S, Bonferoni MC, Bruni G, Boselli C, et al. Halloysite and chitosan oligosaccharide nanocomposite for wound healing. Acta Biomaterialia. 2017;**57**:216-224

[171] Kota SK, Meher LK, Jammula S, Kota SK, Krishna SVS, Modi KD. Aberrant angiogenesis: The gateway to diabetic complications. Indian Journal of Endocrinology and Metabolism. 2012; **16**(6):918

[172] Singer AJ, Clark RA. Cutaneous wound healing. New England Journal of Medicine. 1999;**341**(10):738-746

[173] Garcia-Orue I, Pedraz JL, Hernandez RM, Igartua M. Nanotechnology-based delivery systems to release growth factors and other endogenous molecules for chronic wound healing. Journal of Drug Delivery Science and Technology. 2017;**42**:2-17

[174] Pan A, Zhong M, Wu H, Peng Y, Xia H, Tang Q, et al. Topical application of keratinocyte growth factor conjugated gold nanoparticles accelerate wound

healing. Nanomedicine: Nanotechnology, Biology and Medicine. 2018;**14**(5):1619-1628

[175] Goh M, Hwang Y, Tae G. Epidermal growth factor loaded heparin-based hydrogel sheet for skin wound healing. Carbohydrate Polymers. 2016;**147**: 251-260

[176] Mohandas A, Anisha BS, Chennazhi KP, Jayakumar R. Chitosan–hyaluronic acid/VEGF loaded fibrin nanoparticles composite sponges for enhancing angiogenesis in wounds. Colloids and Surfaces B: Biointerfaces. 2015;**127**: 105-113

[177] Luan A, Hu MS, Leavitt T, Brett EA, Wang KC, Longaker MT, et al. Noncoding RNAs in wound healing: A new and vast frontier. Advances in Wound Care. 2018;7(1):19-27

[178] de Kerckhove M, Tanaka K, Umehara T, Okamoto M, Kanematsu S, Hayashi H, et al. Targeting miR-223 in neutrophils enhances the clearance of *Staphylococcus aureus* in infected wounds. EMBO Molecular Medicine. 2018;**10**(10):e9024-e90444

[179] Castleberry SA, Almquist BD, Li W, Reis T, Chow J, Mayner S, et al. Self-assembled wound dressings silence MMP-9 and improve diabetic wound healing in vivo. Advanced Materials. 2016;**28**(9):1809-1817

[180] Whittam AJ, Maan ZN, Duscher D, Wong VW, Barrera JA, Januszyk M, et al. Challenges and opportunities in drug delivery for wound healing. Advances in Wound Care. 2016;**5**(2):79-88

[181] Randeria PS, Seeger MA, Wang XQ, Wilson H, Shipp D, Mirkin CA, et al. siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown. Proceedings of the National Academy of Sciences. 2015;**112** (18):5573-5578

[182] Wang C, Ma L, Gao C. Design of gene-activated matrix for the repair of skin and cartilage. Polymer Journal. 2014;**46**(8):476-482

[183] Choi JS, Kim HS, Yoo HS. Electrospinning strategies of drug-incorporated nanofibrous mats for wound recovery. Drug Delivery and Translational Research. 2015;**5**(2): 137-145

[184] Kobsa S, Kristofik NJ, Sawyer AJ, Bothwell AL, Kyriakides TR, Saltzman WM. An electrospun scaffold integrating nucleic acid delivery for treatment of full-thickness wounds. Biomaterials. 2013;**34**(15):3891-3901

[185] Ojeh N, Pastar I, Tomic-Canic M, Stojadinovic O. Stem cells in skin regeneration, wound healing, and their clinical applications. International Journal of Molecular Sciences. 2015;**16** (10):25476-25501

[186] Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Badiavas EV. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells and Development. 2015;**24**(14): 1635-1647

[187] Tartarini D, Mele E. Adult stem cell therapies for wound healing: biomaterials and computational models. Frontiers in Bioengineering and Biotechnology. 2016;**3**:206-212

[188] Li M, Zhao Y, Hao H, Han W, Fu X. Mesenchymal stem cell–based therapy for nonhealing wounds: Today and tomorrow. Wound Repair and Regeneration. 2015;**23**(4):465-482

[189] Ma K, Liao S, He L, Lu J, Ramakrishna S, Chan CK. Effects of nanofiber/stem cell composite on wound healing in acute full-thickness skin wounds. Tissue Engineering Part A. 2011;**17**(9-10):1413-1424

[190] Chueng STD, Yang L, Zhang Y, Lee KB. Multidimensional nanomaterials for the control of stem cell fate. Nano Convergence. 2016;**3**(1):23-38

[191] Tang T, Jiang H, Yu Y, He F, Ji SZ, Liu YY, et al. A new method of wound treatment: Targeted therapy of skin wounds with reactive oxygen species-responsive nanoparticles containing SDF-1α. International Journal of Nanomedicine. 2015;**10**:6571-6585

[192] Yi H, Rehman FU, Zhao C, Liu B, He N. Recent advances in nano scaffolds for bone repair. Bone Research. 2016;**4**: 16050

[193] Guo B, Lei B, Li P, Ma PX. Functionalized scaffolds to enhance tissue regeneration. Regenerative Biomaterials. 2015;**2**(1):47-57

[194] Edwards SL, Werkmeister JA, Ramshaw JA. Carbon nanotubes in scaffolds for tissue engineering. Expert Review of Medical Devices. 2009;**6**(5): 499-505

[195] Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and tissue engineering: From discovery to applications. Nano Letters. 2010;**10**(9):3223-3230

[196] van Rijt S, Habibovic P. Enhancing regenerative approaches with nanoparticles. Journal of the Royal Society Interface. 2017;**14**(129): 20170093

[197] De Witte TM, Fratila-Apachitei LE, Zadpoor AA, Peppas NA. Bone tissue engineering via growth factor delivery: From scaffolds to complex matrices. Regenerative Biomaterials. 2018;**5**(4): 197-211

[198] Walmsley GG, McArdle A, Tevlin R, Momeni A, Atashroo D, Hu MS, et al. Nanotechnology in bone tissue engineering. Nanomedicine: Nanotechnology, Biology and Medicine. 2015;**11**(5):1253-1263