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REVIEW ARTICLE

Nanotechnology and Diabetic Wound Healing: A Review

Dinesh Kumar Chellappan[1,*], Yap Yenese[2], Chew Chian Wei[2] and Gaurav Gupta[3,4,*]

[1]*School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia 57000;* [2]*School of Health Sciences, International Medical University, Kuala Lumpur, Malaysia 57000;* [3]*School of Pharmacy, Jaipur National University, Jagatpura 302017, Jaipur, India;* [4]*School of Medicine and Public Health, University of Newcastle, Newcastle, NSW 2308, Australia*

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Abstract: ***Background and Objective*:** The incidence of diabetes has been on the rise and the rate of rise since the turn of this century has been phenomenal. One of the various battling issues faced by diabetics all over the globe is the management of diabetic wounds. Currently, there are several management strategies to deal with the treatment of diabetic wounds. The conventional methods have several limitations. One of the major limitations is the rate and progression of healing of a diabetic wound when adopting a conventional diabetic wound management therapy. Lately, several nano techniques and nano products have emerged in the market that offer promising results for such patients. The treatment outcomes are achieved more efficiently with such nanomedical products.

***Methods*:** This review attempts to consider the currently available nanotechnological applications in the management of diabetic wounds. We take a deeper look into the available nanotherapeutic agents and the different nanocarriers that could be used in the management of diabetic wound healing. Lately, researchers around the globe have started providing evidences on the effective use of such nanoparticles in various fields of Medicine extending from genetics to various other branches of medicine. This also includes the management of diabetic wounds.

***Conclusion*:** This paper discusses the challenges faced with these nanotherapies and nanoparticles with regard to the treatment of diabetic wounds.

Keywords: Nanotechnology, diabetes, diabetic wound healing, nanotherapeutic agents, nanocarriers, WHO.

1. INTRODUCTION

Diabetes is currently on the rise as stated by the World Health Organization (WHO). It is caused primarily by obesity and unhealthy lifestyle habits. It is estimated that currently there are 382 million people globally with diabetes and is expected to hit 592 million people by 2035 [1]. According to the WHO, prevalence rates of diabetes in 1980 and 2014 were 108 million and 422 million people respectively [2]. The mortality rate in 2012 due to diabetes, as estimated by WHO, was 1.5 million deaths and mortality rate due to high blood glucose was 2.2 million deaths [2]. Prevalence of diabetes is increasing hastily, especially in middle to low-income nations [2]. Two types of diabetes, Type 1 and Type 2 are commonly known. Type 2 diabetes remains the majority, accounting for more than 85% of total diabetics, and is characterized by the body's ineffectiveness to use insulin. Type 1 diabetes is an autoimmune disorder which attacks pancreatic β-cells, reducing or impairing insulin production [1, 2]. Multisystem complications due to diabetes include diabetic retinopathy, diabetic nephropathy, diabetic cardiomyopathy, stroke and diabetic foot ulcer [2]. Diabetic foot ulcer, estimated with an annual incidence of 6% by Margolis *et al*., is the major complication among all complications and is the major cause of hospitalization [3]. The decreased blood flow, along with neuropathy, increases the risk of developing foot ulcers, infections and ultimately amputation of the lower limbs in 84% of diabetic patients [2, 3]. Impaired wound healing in diabetes has drawn much interest and has led to the development of various approaches, including nanotechnology, primarily to facilitate the acceleration of wound healing.

The current treatment options for diabetic wounds include primarily protecting the wound from further deteriorating. The wound covering methods are employed currently to achieve sufficient survival of the tissue in concern. Hydrocolloid dressings are used when a moist surrounding is required. Calcium alginate absorptive dressings and Hydrofiber dressings are currently used for excessively wet wounds. Usually, sulfa drugs or Neosporin ointment is used if there is an infection. More recently platelet-derived growth factors and enzymatic debridement using collagenase are also employed. Although there are a number of strategies to deal with diabetic wounds, there are still a number of challenges when dealing with chronic wound management, especially when treating deep exudative wounds. A number of potent

*Address correspondence to these authors at the School of Pharmacy, International Medical University, No 126, Jln Jalil Perkasa 19, Bukit Jalil, Kuala Lumpur, Malaysia 57000; Tel: +6-012-6361308;
E-mail: dinesh_kumar@imu.edu.my and School of Medicine and Public Health, University of Newcastle, Newcastle, NSW 2308, Australia;
Tel: +61 0410295637; E-mail: gauravpharma25@gmail.com

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substances are rendered ineffective, for *e.g*., curcumin, because they are hydrolyzed and degraded before they exert their action. Both at the molecular level and at a surface level, there are several complications that need to be addressed. Thus, there is a need for newer and more advanced treatment options which can deal with the existing problems. Nanotechnology and nanoparticles offer relatively newer treatment options in various areas of healthcare management including diabetic wound healing.

In general terms, nanotechnology is the study and utilization of matter in nanometer scale, in other words, a billionth of a meter, and is the study of regulating matter at the atomic and molecular scale [4]. A nanoparticle (NP) is a fundamental constituent in a defined nanostructure. These particles are investigated due to their unique size and characteristics [4]. An NP is defined as a particle having a diameter of 1 nm to 100 nm by the International Organization for Standardization (ISO), whereas, British Standards Institution (BSI) defines NPs to be having a nanoscale range at all fields or diameters of the NP [4]. Ever since the study on NPS began, these have been greatly used in various fields including industrial, environmental, food, agricultural and biomedical fields [5]. The use of NPs has increased significantly in the fields of biology and medicine due to their nanoscale size, as very small probes could allow us to understand biological processes at the cellular level [6]. Applications of NPs in Biology and Medicine include drug and gene delivery [7], tissue engineering [8] and fluorescent biological labels [9, 10].

In this review article, we have attempted to discuss the use of NPs in diabetic wound healing, emphasizing on the various types of NPs designed as nanocarriers or nanotherapeutic agents. We discuss the properties, advantages, and disadvantages of the various types of such particles and the suitability of nanocarriers used in relation to nanotherapeutic agents in diabetic wound healing. In simple terms, a nanocarrier is a nanoparticle which could be used as a transport material for a drug or any other substance of interest. Furthermore, we also discuss on nanotherapeutic agents which could be delivered to the site of action to improve and accelerate diabetic wound healing, which often leads to complications such as ulceration at lower limbs eventually leading to amputation. We have also reviewed the existing challenges faced in utilizing NPs in diabetic wound healing as well as the future perspectives of the utilization of NPs in diabetic wound healing.

2. LITERATURE REVIEW

2.1. Wound Healing

2.1.1. Normal Wound Healing

Wound healing involves several phases beginning with hemostasis, inflammation, re-epithelization, tissue granulation and tissue remodeling [11]. These phases, though have distinct functions, overlaps in several stages resulting in a complex, systematic physiological process [11, 12].

2.1.1.1. Hemostasis

Immediately after injury, hemostasis occurs; together an impermanent wound matrix is formed. In this phase, inflammation phase is initiated [13]. Various cells and signaling factors involve in the healing process and are recruited in this phase [13]. Thrombocytes come into play by causing vasoconstriction, reducing blood loss and forming blood clots, consisting of cytokines and growth factors (GFs) to fill the injured site [14]. The blood clot is a reservoir of GFs which are essential for the formation of the impermanent wound matrix, as a scaffold for keratinocytes, endothelial cells, leukocytes and fibroblast migration occurs [15]. Chemokines released by the thrombocytes cause leukocyte infiltration [16]. Both thrombocytes and leukocytes then release signaling factors such as cytokines and growth factors to activate inflammation, synthesize collagen and initiate angiogenesis. These are also released as a support for re-epithelization [16].

2.1.1.2. Inflammation

In the inflammation phase, recruitment of neutrophils occurs initially when macrophages come into play [17]. Neutrophils phagocytize and secrete proteases at the injury site to kill bacteria and then degrade the necrotic tissues [17]. Pro-inflammatory cytokines released by neutrophils intensifies inflammation and activate vascular endothelial growth factor (VEGF) for sufficient healing [17]. The entry of macrophages further undergo phagocytosis of bacteria and cell debris; and is responsible for the release of GFs, chemokines, and cytokines that are necessary for the re-epithelization phase, extracellular matrix (ECM) synthesis, angiogenesis and tissue granulation phase [17-19].

2.1.1.3. Re-Epithelization and Tissue Granulation

Re-epithelization is the process of covering the surface of the wound followed by tissue granulation and initiation of angiogenesis. These processes are facilitated by local fibroblasts, thus activating angiogenesis which is regulated by cytokines followed by synthesis of collagen [13, 20]. These events are balanced between ECM synthesis and degradation, aiding in wound closure and restoring wound mechanical strength [21]. The release of certain cytokines and GFs by epithelial and non-epithelial cells at injury site activates local keratinocytes for re-epithelization [16]. Certain GFs bind to receptors of existing blood vessels to initiate angiogenesis, by activating endothelial cells, to proliferate and migrate to injury site [22]. Matrix metalloproteinases (MMPs) lyses the surrounding tissues to enable endothelial cell proliferation [22]. Remodeling phase overlaps with the tissue granulation phase [13]. The impermanent wound matrix is eventually replaced by high density and high amount of cellular compounds, macrophages, fibroblasts, granulocytes, capillaries and collagen III [23, 24]. Fibroblasts play a major role in collagen and ECM synthesis which aids in providing a scaffold for cellular adhesion, growth, movement and differentiation of cells [23, 24]. Fibroblasts differentiate into myofibroblast which is eventually terminated by apoptosis [25].

2.1.1.4. Remodeling

Through apoptosis, tissue granulation halts [26]. Collagen III is replaced by collagen I which is stronger, and myofibroblasts cause the injury site to contract [18, 19]. Upon wound healing, angiogenesis terminates, blood flow at wound reduces, metabolic activities at wound site reduces and lastly stops [13].

2.1.2. Wound Healing in Diabetes

All phases of wound healing are affected in diabetic wounds, where chronic inflammation persists for longer than three months and the systematic process is disrupted, eventually, deviating away from the standard time course [27]. The edge of injury site shows epidermal hyperproliferation, creating an ulcer base, and accumulation of exudate which is burdened with necrotic debris [27]. Tissue granulation which is expected is absent and fibrin cuffs are observed surrounding the vessels. Little angiogenesis is observed and little or no myofibroblasts are seen. Moreover, heavy infiltration of inflammatory cells, especially neutrophils are observed [27]. The proliferation of partially activated keratinocytes, as well as reduced levels of transforming growth factor-beta (TGF-β) receptors, due to reduced migration of fibroblasts and unresponsiveness of fibroblast towards GF signals, is observed in diabetic wound healing [28, 29]. It is also thought that increased degradation of MMPs may affect the release of GFs [30]. The addition of microbes at wound site play a role in chronic wound healing, as functions of immune cells to phagocytize and perform bactericidal activities are reduced, also prolonging inflammation through heavy infiltration and accumulation of immune cells at the wound site, thereby inhibiting the healing process [31]. However, it is thought that certain immune cells present in greater amounts may help in diabetic wound healing especially Langerhans cells [32]. Debridement of the wound can help to restart re-epithelization process [33].

2.2. Currently Available Nano-Therapeutic Agents

There are currently several nanoparticles that are employed in modern medicine to treat various conditions including diabetic wound healing. These include quantum dots, gold nanoparticles, chitosan nanoparticles, superparamagnetic iron oxide nanoparticles, poly-lactic-co-glycolic acid (PLGA) nanoparticles, silver nanoparticles, polymer and liposome-based particles. These are used in several areas of healthcare management. Several medications like growth factors, antimicrobials, and other therapeutic molecules are delivered effectively to target organs and tissues through such nanoparticles. Here we discuss some of the strategies employed with respect to diabetic wound healing. Some of the primary applications of nanoparticles are diagrammatically shown in Fig. (**1**).

2.2.1. Growth Factors (GF)

Currently, growth factors are delivered through hydrogels, bio-conductive scaffolds and as injectable substances. In line with these substances, nanoparticles or nanocarriers also play a major role in delivering such substances.

Wound healing is regulated by various GFs, enabling intercellular communication [16, 34], including platelet-derived growth factors (PDGF), transforming growth factor-β (TGF- β), vascular endothelial growth factor (VEGF), endothelial growth factor (EGF) and fibroblast growth factor (bFGF) [35, 36]. The importance or functions of several important GFs in wound healing are summarized in Table **1** below. GFs incorporated in NPs deliver and release GFs to wound site directly in a sustained and controlled [37, 38] manner, maintaining the bioactivity, enhancing biological effects as well as protecting nanotherapeutic agents from enzymatic degradation [38]. Application of free forms of nanotherapeutic agents may present rapid degradation and rapid leakage, reducing bioavailability and biological effects respectively [38]. The use of single nanotherapeutic agent

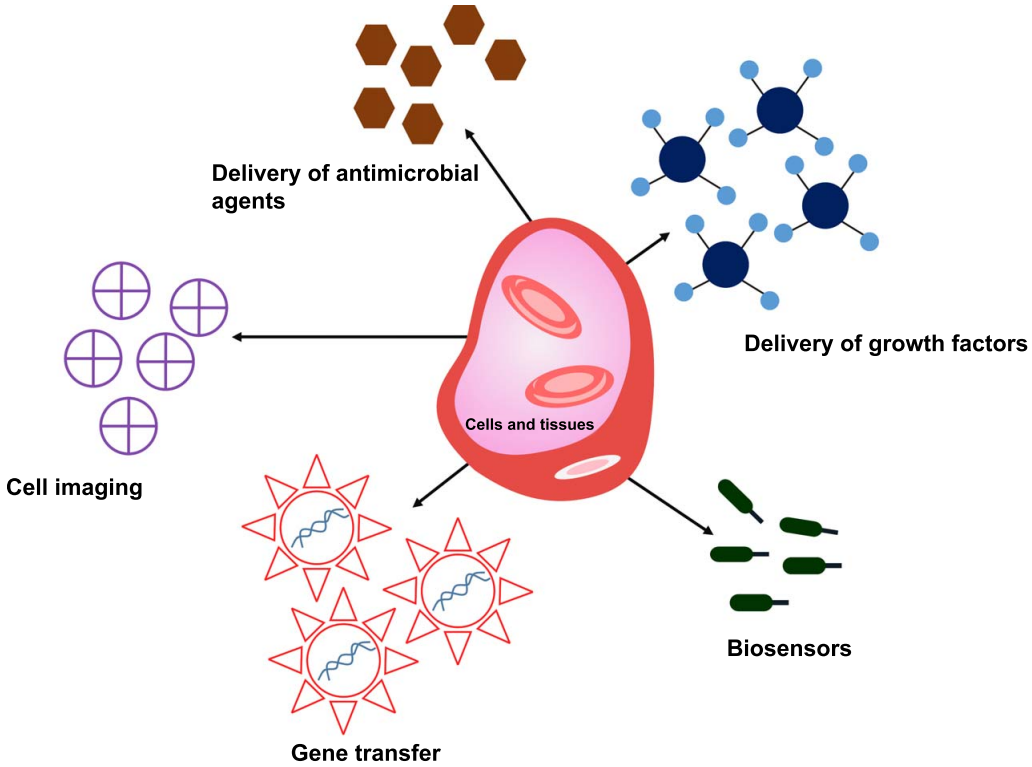

Fig. (1). Applications of nanoparticles in medicine.

Table 1. Functions of different growth factors in wound healing.

Growth Factors	Function	Refs.
Platelet-derived growth factor (PDGF)	- Angiogenic properties - Fibroblasts and keratinocytes proliferation - Re-epithelization - Collagen deposition - Inflammation - Tissue granulation - Remodelling	[37]
Transforming growth factor-beta (TGF-β)	- Cell growth - Cell proliferation - Cell differentiation - Apoptosis	[35, 36]
Vascular endothelial growth factor (VEGF)	- Angiogenic property - Endothelial cell recruitment - Tissue granulation	[37]
Endothelial growth factor (EGF)	- Re-epithelization - Vasculature sprouting	[37]
Basic fibroblast growth factor (bFGF)	- Angiogenic properties - Endothelial cell recruitment - Re-epithelization - Fibroblasts and keratinocytes proliferation - Collagen deposition	[37, 38]

treatment is less effective compared to a dual nanotherapeutic agent treatment. A study conducted by Xie *et al*,. in 2013 showed that the treatment was more effective when two nano-embedded growth factors were delivered in a single nanofiber system [39]. Nanodelivery of two GFs [37, 38], VEGF and FGF has shown to accelerate wound healing in diabetic rats and mice [37, 38]. Xie *et al*., used dual GF, encapsulated PDGF-BB in NP, embedded into VEGF nanofiber and released in a controlled and sustained manner. This promoted a much more effective wound healing process, accelerating tissue regeneration and remodeling. It also promoted angiogenesis, increasing re-epithelization and controlled tissue granulation. In later phases of healing, full tissue regeneration was achieved as observed in several *in vitro* studies as well as in rats [39]. Nanofibers have proved to support fibroblast growth while the NP releases nanotherapeutic agents in a related manner [37, 39]. Lai *et al*., used a particle-in-fiber structure as a bio-construct, providing integration with surrounding tissues, which is similar to human native skin, releasing multiple GF in a stage-wise manner. In the above-mentioned study, four GFs (VEGF, PDGF, bFGF and EGF) were incorporated into a nanocarrier, which induced vascularization and demonstrated acceleration of wound healing, along with increased collagen deposition and enhanced maturation vessels in diabetic rats (Table **1**) [37].

2.2.2. Anti-Microbial Nanoparticles

Traditionally, anti-microbial drugs are delivered through oral, injectable and topical routes as tablets, parenteral solutions or as creams, ointments, and gels respectively. However, these routes deliver a high concentration of potent substances and result in serious side effects and potential toxicity. The oral route of drug administration often requires higher doses as they undergo first pass metabolism. Although parenteral route offers maximum bioavailability, there is a high risk of adverse reactions due to the drug. Topical applications are relatively considered safer than other routes. However, these are very uncomfortable to the patient as they are greasy and oily when applied and require additional care that the drug is not lost due to accidental wiping or cleaning of the part. On the other hand, nanoparticles and nanotechnology enable an accurately targeted delivery of therapeutics or antimicrobials. They can achieve a high local concentration of the drug with relatively lesser side effects compared to the traditional delivery systems. The cost of production of nanoparticles or nanocarriers might vary depending on the nanotechnology employed. Some of these methods, though require a higher production cost, are highly effective than the traditional treatment options.

Curcumin has anti-microbial property, which has been used widely as a wound healing agent. It also enhances wound repair, especially in diabetic foot ulcer and also has antineoplastic, anti-inflammatory and anti-oxidant effects. However, curcumin has poor solubility in water and is unstable, thus undergoing rapid degradation *via* hydrolysis, reducing its bioactivity and bioavailability before reaching its target site [40-42]. Therefore, the encapsulation of curcumin in NPs could overcome the shortcomings, whereby, prolonging the bioavailability of curcumin at wound site as the NPs releases curcumin in a slow, sustained, controlled and continuous manner [43, 44]. Additionally, curcumin is proven to induce production of TGF-β1 endogenously which plays a role in wound healing [43]. Krausz *et al*., studied the inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* using curcumin NPs and have shown to enhance wound healing in an *in vivo* murine model [45]. Other antimicrobials that have been discussed includes copper [46] and silver [47-50].

2.3. Currently Available Nanocarriers

2.3.1. Gold Nanoparticles (AuNPs) as Nanocarriers

Gold nanoparticles (AuNPs) exhibit antioxidant effects and are used extensively in a number of applications including gene transfer, drug delivery, as biosensors and in cancer cell imaging [51-54], which aid in diagnosis and treatment of several ailments, especially in cutaneous wound healing directed towards fibroblasts and keratinocytes, which are predominant cells in the mammalian skin. There are several studies that have shown that gold nanoparticles enhance angiogenesis. The mechanism of action of this activity is believed through accelerated wound healing due to enhanced epithelialization, collagen deposition, and fast vascularization. However, at the same time, there are studies that report the antiangiogenic properties of gold nanoparticles. This means that the dose of this compound decides the dynamics of the action as proved by Leu *et al*. [55]. These studies were performed using human foreskin fibroblasts and human keratinocyte cells. The controls for these studies were antioxidants and other synthetic wound healing agents. Topical application of AuNPs reduces adverse effects, as involvement with the systemic circulation is avoided [55, 56]. AuNPs are capable of penetrating the skin barrier *via* nano-bio interaction with skin, as AuNPs exhibit the ability to open the stratum corneum [57], increasing the bioabsorption of AuNPs into the skin and also increasing its antioxidant effect [55, 56]. The combination of AuNP, with epigallocatechin galate (EGCG) [58] or alpha-lipoic acid (ALA) [55] or both (EA), which are anti-oxidants derived from unfermented teas and animal foods such as liver respectively, have proven to have synergistic effect in enhancing diabetic wound healing by regulating angiogenesis and anti-inflammatory effects. AuNP itself is a wound healing agent [55, 56]. Topical application of AuNP with both EGCG and ALA (AuEA) in diabetic mice increased VEGF protein expression, aiding in angiogenesis [56]. Furthermore, increased expression of RAGE (Receptor for Advanced Glycation End-products), which impairs wound healing due to increased oxidative stress [59, 60] and abnormal angiogenesis process in diabetic wounds, are suppressed by antioxidant agents [56]. In addition, Leu *et al*. have shown that topical application of AuEA has demonstrated to increase proliferation and migration of human foreskin fibroblasts (Hs68) and human keratinocyte cells (HaCaT) [55].

2.3.2. Chitosan Nanocarriers

Chitosan, a natural polymer, derived from chitin, is a component of fungal cell wall and exoskeletons of arthropods [61], which is extensively used as wound dressings to stimulate regeneration of ECM [62], as chitosan exhibits excellent properties including biodegradable, biocompatible, anti-microbial, anti-fungal, non-toxic, mucoadhesive, bioactive, non-antigenic, and with enhancing permeation properties [62, 63]. Additionally, chitosan may positively impact some biological activities which include, lowering cholesterol levels, anti-hypertension effect, the ability to interact with organic compounds, susceptibility to enzymatic hydrolysis and immune response activation [62]. However, challenges arise as chitosan exhibits poor solubility and inability to prevent loaded nano-therapeutic agents from undergoing pre-systemic metabolism, thereby reducing the bioavailability of agents, especially in organs like stomach and intestine where proteolytic enzymes are present [62]. Fortunately, chitosan could be modified chemically whereby, altering the functional group of chitosan *via* carboxylation, thiolation, and acylation or in hydrogel forms is possible to overcome the shortcomings of chitosan. This results in the formation of more stable forms of chitosan as nanocarriers for prolonged and efficient delivery of agents to wound site, without altering its properties [62]. It is well documented that chitosan successfully inhibits microorganism growth, relieves pain, and promotes hemostasis and epidermal proliferation in chronic wound healing [62, 64]. Gopal *et al*. had demonstrated the combination of chitosan-based copper nanocomposite (CCNC), where copper is a potent antimicrobial agent, and proved to accelerate wound contraction, angiogenesis, the proliferation of fibroblast and collagen deposition by up-regulating VEGF and TGF-β1, presenting synergistic effects from both copper and chitosan [46]. Li *et al*., demonstrated, in rats, enhanced re-epithelization of epidermal cells and collagen deposition through the application of nano-curcumin with N,O-carboxymethyl chitosan/oxidized alginate hydrogel (CCS-OA), which controls the release of nano-curcumin, exhibiting anti-microbial activity, and chitosan hydrogel responsible in absorbing exudate, which also immobilizes and activates GFs in exudate [43]. CCS-OA is non-cytotoxic and enhances bio absorbability [43]. Nano-curcumin/CCS-OA stimulates fibroblast proliferation, angiogenesis, and collagen deposition significantly in wound healing [43]. These combinations may potentially improve wound healing effectively. Xie *et al*., [39] and Bertoncelj *et al*., [65] electrospun chitosan and polyethylene oxide to produce a nanofibrous mesh, as nano fibrillar support, which mimics the natural ECM as well as incorporating GFs in a nanofibrous mesh, which may be a promising treatment for diabetic wound healing, as the biomimetic ECM alongside GFs encourage wound healing [39, 65].

2.3.3. Poly Lactic-Co-Glycolic Acid (PLGA) Nanocarriers

Poly-lactic-co-glycolic acid (PLGA) is a biodegradable polymeric NP, when hydrolyzed, forms lactic acid and gly-

colic acid which are metabolites, producing minimal systemic toxicity [66]. Lactate which is produced and released in a sustainable manner promotes angiogenesis thereby facilitating wound healing [67]. PLGA has gained great interest as a nanocarrier due to its excellent biocompatibility, adaption to various kinds of drugs, the capability of protecting drugs from degradation, releasing the drug in a sustained manner and possibility in modifying surface characteristics, allowing a more specific and better interaction *in vivo* [67, 68]. Furthermore, PLGA has been accepted and approved by the Food and Drug Administration (FDA) and European Medical Agency (EMA) [67, 68]. In addition, the release kinetics of nanotherapeutic agents at wound site can be manipulated easily [69]. The use of a fibrin-based scaffold, providing mechanical resistance, embedded in PLGA encapsulating nano-therapeutic agents may enhance the delivery system, as PLGA allows a controlled and sustained release system, preventing burst release of topically applied agents from leaking out of wound site rapidly, thus reducing the requirement of daily administration of agents at wound site as well as maintaining the bioactivity and bioavailability of nanotherapeutic agents [38]. This could be a potential dressing and an efficient therapeutic treatment for diabetic wound healing [38]. Chereddy *et al*., have demonstrated a significant increase in tissue granulation with increased collagen content, re-epithelization and angiogenesis with the application of GF (VEGF) incorporated into PLGA in experimental mice [70], while Losi *et al*., have demonstrated the use of a scaffold containing GFs encapsulated into PLGA, inducing tissue granulation, collagen deposition, re-epithelization and increased rate of wound closure [38]. PLGA exhibits dual roles, as a nanocarrier delivering nanotherapeutic agent to wound site and being a wound healing agent itself, releasing lactate, which promotes wound healing [38, 70].

2.3.4. Silver Nanoparticles (AgNPs) as Nanocarriers

Silver nanoparticles have several clinical applications, as silver exhibits anti-microbial activity, capable of inhibiting *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well as a number of other gram-positive and gram-negative bacteria along with anti-inflammatory effect and also displaying a good healing effect [47-50]. The use of silver has drawn much attention currently due to the rise in antibiotic-resistant bacteria. Silver exhibits broad spectrum antibacterial activity [47-49], and has multicellular targets, reducing the chances of bacteria to develop resistance towards silver and also has low systemic toxicity [49]. Silver inhibits and kills bacteria by interfering with the bacteria's respiratory chain and electron transport system as well as binding to the microbial DNA, inhibiting DNA replication and affecting the development of bacterial proteins [49]. Moreover, AgNPs can be synthesized easily in large scale, which is safe, reliable, affordable and can be fabricated in various shapes [71]. The surface of AgNPs can be easily modified, making them specific to target site due to their highly reactive negatively charged surface, benefiting AgNPs as nanocarriers [72]. The surface charge on a nanoparticle does indeed play a significant role in determining its interaction with the cell membranes. Positively charged nanoparticles penetrate straight through the membrane, embedding themselves deeply within the floating bilayer. In contrast, negatively charged nanoparticles do not penetrate the lipid membrane at all, but rather hinder membrane decomposition at given concentrations, helping it withstand extreme conditions such as elevated pH that would otherwise significantly destabilize it. Reithofer *et al*., in an *in vitro* study, studied a biomaterial, releasing silver in a sustained manner, at lower silver content and is biocompatible, to demonstrate wound healing where the biomaterial are ultra-short peptides which are capable of self-assembling when in contact with water forming hydrogels [49]. The hydrogel acts as a biomimetic platform displaying mechanical stiffness and elasticity and could be a potential wound dressing [49].

2.4. Future Perspectives

Currently, available nano-therapeutic agents and nanocarriers have been tested *in vitro* and provide potential therapeutic applications which need to be further tested in *in vivo*. GFs and cytokines differ at different phases and a better release kinetics of GFs at different phases could potentially aid wound healing. However, release kinetics are difficult to control [65]. Wounds that may present with excess proteolytic enzymes, not only delay wound healing, but also could potentially breakdown nanocarriers which will then cause a burst release of nano-therapeutics, reducing bioavailability, and leading to reduced biological effects. This could be an area of further research.

The use of several nano-therapeutic agents simultaneously in combination with nanocarriers which act as nanotherapeutics and also as a nanocarrier itself can be employed to provide a synergic effect. The development of nanotherapeutics incorporated nanocarrier could possibly provide an equilibrium and suitable environment for wound healing to occur. Substances like copper, [46] that would aid wound healing could be fabricated together with nanocarrier encapsulating nanotherapeutics, which would provide synergistic effect and accelerate wound healing. The use of nanofibers as dressings embedded with nanotherapeutics could improve wound healing [65]. Some GFs and cytokines are released at different phases. Therefore, the choice of nanocarrier which is easy to handle and easy to manipulate the release kinetics could be crucial as release kinetics for different GFs or cytokines can be manipulated. Understanding gene expression during a normal wound healing process and during an impaired wound healing process could potentially be crucial [73].

CONCLUSION

The alarming rise in the prevalence of diabetes has drawn much interest to develop an effective therapeutic approach to enhance current therapeutics and to develop future therapeutics which could accelerate impaired wound healing in diabetics. Diabetic foot ulcer, which is the major complication of diabetes as well as accounting for the major cause of hospitalization, may eventually lead to amputation of a lower limb, decreasing quality of life in diabetics. The understanding of the normal physiology of wound healing and impaired wound healing could shed some light in developing effective therapeutics to reduce the risk of amputation. An ideal therapeutic agent should provide an equilibrium environment to accelerate wound healing, keeping oxidative stress, enzy-

matic activities and hypoxia under control at the same time providing mechanical support and reducing the chances of infection, which are challenges encountered at wound site [74]. Current nanotherapeutic agents (GF, anti-microbial agents) and nanocarriers (AuNPs, chitosan, PLGA, AgNPs) have demonstrated to accelerate wound healing by regulating one or more challenges encountered at the wound site. Future directions could possibly develop nanotherapeutic agents which could possibly regulate wound healing throughout all phases as well as providing an equilibrium environment throughout the wound healing process and to reduce the chances of microvascular complications.

LIST OF ABBREVIATIONS

AgNPs	=	Silver nanoparticles
ALA	=	Alpha-lipoic acid
AuEA	=	Gold nanoparticles with epigallocatechin galate and alpha-lipoic acid
AuNPs	=	Gold nanoparticles
bFGF	=	Basic fibroblast growth factor
CCNC	=	Chitosan-based copper nanocomposite
CCS-OA	=	N, O-carboxymethyl chitosan/oxidized alginate hydrogel
EA	=	Epigallocatechin galate and alpha-lipoic acid
ECM	=	Extracellular matrix
EGCG	=	Epigallocatechin galate
EGF	=	Endothelial growth factor
EMA	=	European medical agency
FDA	=	Food and drug administration
FGF	=	Fibroblast growth factor
GF	=	Growth factor
HaCaT	=	Keratinocyte cells
Hs68	=	Human foreskin fibroblasts
ISO	=	International organization for standardization
MMPs	=	Matrix metalloproteinases
NP	=	Nanoparticle
PDGF	=	Platelet-derived growth factor
PLGA	=	Poly-lactic-co-glycolic acid
RAGE	=	Receptor for advanced glycation end products
TGF-β	=	Transforming growth factor-beta
VEGF	=	Vascular endothelial growth factor

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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