Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 13-38.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

COMPLICATIONS ASSOCIATED WOUND HEALING IN DIABETIC PATIENTS: DOES NANOTECHNOLOGY HAVE ANY SUPERIOR THERAPEUTIC ADVANTAGES?

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Delayed wound healing is one of the main complications facing diabetic patients worldwide. It can be followed by serious consequences such as ulcer, gangrene, amputation unless treated correctly. Four phases of normal wound healing are compromised in diabetic patients due to complex pathophysiology; thus, wounds healing time is prolonged. Consequently, the probability of wounds infections in diabetic patients is higher than non-diabetic patient and it is much higher with immunocompromised diabetic patients. There are several strategies for treatment of diabetic wounds such as; dressings, growth factors, stem cells, antidiabetic drugs, antihypertensive drug, etc. However, some of these strategies are still under investigations to ascertain their safety, while others are clinically non-applicable due to rapid biodegradation. Nanotechnology might be a novel and a promising alternative strategy for diabetic wound healing as nanoparticles could overcome limitations of medical products currently used. Some nanoparticles such as; metal-based nanoparticles (MBNPs) demonstrated an excellent antibacterial activity including resistant strains inhabitant diabetic wound, moreover, some of MBNPs could enhance the healing of wound in diabetic patients via modulating many disturbed issues recorded in four phases of wound healing. This review explores the recent literature in this area, discusses the complications associated diabetic patients with special focus on delayed wound healing, pathophysiology of wound healing in diabetic patients, treatment approved for wound healing and controlling wound infection. In addition, shedding the light on nanotechnology application to improve wound healing, with a special concern devoted to MBNPs including their antibacterial mechanisms and barriers faced to their clinical application.

Keywords: *Diabetes, Metal-based Nanoparticles, Wound healing*

INTRODUCTION

Diabetes mellitus (DM) is defined as a group of metabolic pathologies characterized by compromised insulin production and/or function, leading to hyperglycemia[1] and in some critical cases kidneys, eyes, and legs are highly affected with a negative impact on the patient health both quality of life and/or survival expectations[2]. This could end up with a low health-related quality of life, especially regarding social and mental health perspectives[2]. In 2019, DM was considered as one of the top ten diseases causing death worldwide, especially in lower-middle-income countries[3]. The number of diabetic patients is expected to increase to 102 million by 2030[4] and to 700 million by 2045[5]. Thus, there is a high demand to have a proper therapeutic intervention to control symptoms of DM as well as novel strategies to reduce the incidence of complications in diabetic patients. To recommend proper therapeutic interventions, we need to be familiar with different types of

Received in 16/9/2022 & Accepted in 29/10/2022

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DM. DM involves the following four types; Type 1 diabetes or Insulin-dependent diabetes mellitus (T1D or IDDM), Type 2 diabetes or Insulin-independent diabetes mellitus (T2D or IIDDM), Gestational diabetes mellitus (GDM), DM developed due to external factors such as disease or drug intake. Each type will be briefly descripted below;

Type 1 diabetes or Insulin-dependent diabetes mellitus (T1D or IDDM)

T1DM is a chronic condition usually diagnosed in children & young people due to the destruction of insulin-making cells known as beta cells and it affects around 5 to 10% of diabetic patients[5]. T1D involved two types according to the cause beta islet cells destruction and they are autoimmune/immune-mediated diabetes (Type 1DA) and idiopathic diabetes (Type 1DB)[6&7]. The clinical manifestation of T1D patients is low body mass index (BMI) with a high risk of diabetic ketoacidosis. The therapeutic intervention of T1D includes administration of exogenous insulin to limit the incidence of diabetic ketoacidosis[8], the latter is due to utilization of fat for production of energy and thus over production of ketone bodies in blood, rendering the blood pH acidic. This has a negative impact on other biological and metabolic process in the human body and could lead to coma or death[9&10].

Type 2 diabetes or Insulin-independent diabetes mellitus (T2D or IIDDM)

T2D is the most common chronic type of diabetic patients and it counts for around 90% of patients diagnosed with diabetes[5]. People who are at high risk for T2D are; obese people, those who eats unhealthy food such as junk food, food enriched with carbohydrates, and they don't practice regular physical exercise. T2D might be caused by one or more of the following factors;

- Defects in insulin secretion due to damage of beta islet cells
- Defects of insulin action or in other words, low sensitivity of cells to insulin action known as insulin resistance. Thus, when those patients eat a carbohydrate enriched meal, the cells are unable to uptake glucose due to low blood insulin level or insensitivity of cells to insulin. This drives glycolysis in liver to produce glucose to compensate the underutilization of glucose by cells. Taken together, this results in massive increase of blood glucose level. Therefore, the adopted initial strategy for symptoms management of T2D patients is controlling diet, eating a healthy food with minimum intake of carbohydrates as well as practicing physical exercise. If these were not enough to control symptoms, a therapeutic intervention is essential and the patient is prescribed to take an oral hypoglycemic agent and in some non-responsive cases, insulin injections are further recommended[11].

Gestational diabetes mellitus (GDM)

This commonly identified in pregnant females with no previous history of DM at the second or third trimester of pregnancy[12]. Literature attributed the incidence of GDM to hormonal imbalance[13&14]. The placenta produces a variety of hormones to maintain the pregnancy, and some of these hormones such as estrogen, cortisol, and human placental lactogen can increase the resistance of cells to insulin and therefore, appearance of DM symptoms.

DM developed due to external factors such as disease or drug intake

Symptoms of DM might appear in patients who are suffering from other diseases or intake some medications, for example, monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)[15&16].

DM Complications

DM is accompanied by several complications, and they could be classified into acute and chronic complications, each will be briefly discussed below;

Acute metabolic complications

They commonly occur in T1D patients due to long-lasting insulin deficiency leading to hyperglycemia, and diabetic ketoacidosis[17-19].

Chronic systemic complications

They affect both T1D and T2D patients and could be divided into (a) Micro vascular complications where small blood vessels such as capillaries are affected (b) Macro vascular complications where large blood vessels such as arteries are affected[12].

Micro and macro vascular complications are the major complications in DM, and they are considered as the major cause of morbidity and mortality in diabetic patients. Micro and macro vascular complications are associated with many fetal diseases due to circulatory dysfunction (vascular endothelial dysfunction), that might lead in some cases to organ dysfunction. Vascular endothelial dysfunction occurs due to decreasing the elasticity of blood vessels, this could be due to imbalance between endothelium-derived vasoconstrictors [thromboxane A2, prostaglandin H2, endothelin 1] and endothelium-derived vasodilators [e.g. nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor].

It is worth noting that, NO is considered as the most important vasodilator in the human body as it is reported to be responsible for maintaining the hemostasis of blood vessels, suppressing platelet aggregation, prevent adhesion of platelet, leukocyte, and monocyte to endothelium and, regulates the production of chemokines and cytokines[5]. Thus, loss of NO might be associated with increasing the incidence of prothrombotic and proinflammatory effect[20].

In other words, decreasing the synthesis of vasodilators (in particular NO) and increasing the synthesis of vasoconstrictor resulting in a narrower blood vessel, and this triggers the formation of atherosclerotic vessels leading to some serious diseases such as peripheral vascular disease (PVD), atherosclerosis, cerebrovascular disease, and cardiovascular disease (CVD)[21]. Loss of blood vessels elasticity leading to narrowing the atherosclerotic blood vessels, and thus reduce the supply of blood, oxygen, and nutrients to body organs, and this might affect the functionality of the supplied organ.

All together were reported to be responsible for difficulty of wound healing in diabetic patients[21], and when the blood supply to the organ is compromised for a very long time, it was reported to result in organ dysfunction causing serious diseases such are neuropathy[22], nephropathy[23], and retinopathy[9-24].

As revealed, the complications associated diabetic patients led to serious diseases that need close monitoring by physicians. In the current review, we are interested in incidence of diabetic foot ulcers as well as different treatment strategies applied to avoid progression to amputation[25]. Diabetic foot ulcer is considered as one of the major complications facing diabetic patients that is commonly affect around 9.1 million to 26.1 million annually worldwide according to International Diabetes Federation[26]. WHO reported that about 15 to 20% of diabetic patients may develop an ulcerative lesion[27], moreover, 40 to 60 % of non-traumatic lower-limb amputations occur in diabetic patients, where 85 % of these amputations were preceded by an ulcer[28]. As discussed, neuropathy and circulatory deficiency were common in diabetic patients, both are responsible for loss of sensation with less possibility of wound healing and a high incidence of infection leading to diabetic foot ulcers. Thus, there is an essential need to have a quick and effective treatment of diabetic wound and restricting the possibility of wound infections. Moreover, all diabetic patients are required to undergo a regular foot screen to identify whether they are at low, moderate, high, or very high risk for the development of foot complications[29]. Literatures have reported that the severity of diabetic foot ulcer was classified into different grades according to the presence or absence of ulcers[26], and they are:

- **Grade 0:** shows no ulcers in the tissues affected, however there is a high chance of subsequent problems
- **Grade 1:** is indicative of a partial and/or full thickness ulcer
- **Grade 2:** refers to a deep ulcer that has reached the ligaments and muscles but has not affected the bones
- **Grade 3:** indicates a deep ulcer that has developed cellulitis or an abscess.
- **Grade 4:** is used to describe the presence of local gangrene
- **Grade 5:** refers to a severe gangrene that has spread across the entire tissue.

Identifying the grade of diabetic foot guides the clinician to the appropriate therapeutic intervention. For more information about different systems applied for classification of the diabetic foot, the reader is directed to the work published by Noha M. Abdelsalam and Colleagues[27], and Rosa Elvira Minchala Urgilés and Colleagues[28].

To develop an effective strategy for prevention and treatment of diabetic foot ulcer, we need to understand pathophysiology of wound healing in diabetic patients that will be explained below;

Pathophysiology of wound healing in diabetic patients

Wound healing is one of the biggest problems facing diabetic patients, about 20% of diabetic patients develop diabetic wounds worldwide[31]. Approximately 50%–70% of all limb amputations are due to diabetic wounds[32] and it was reported that in every 30 s, one leg is amputated due to diabetic wounds worldwide[31]. The Problem of wound healing in diabetic patients is a result of complex pathophysiology involving vascular, neuropathic, immune, and biochemical components, the latter refers to Pathways that the body proceeds in case of hyperglycemia or in case of glucose metabolism which produce a high amount of reactive oxygen species (ROS) and another components affect negatively on wound healing[33]. Understanding different types of wounds will promote physicians to recommend the best strategy for wound healing.

There are two types of wounds: (1) Acute wounds which generally heal easily[34], and (2) Chronic wounds commonly occur in diabetic patients, and these wounds do not heal in an organized set of stages and usually take more than 12 weeks for healing[35]. Wound healing takes place in four different phases as presented in Figure 1 and they are; hemostasis phase, inflammatory phase, proliferation phase, and remodeling phase.

The first stage of wound healing is hemostasis, where a constriction of the injured blood vessels occurs to minimize blood loss. In addition, the injured tissues are associated with the exposure of subendothelial collagen, which promotes the activation of platelets. Activated platelets adhere to one another to form a hemostatic plug, which activates the clotting cascade, and releases a myriad of soluble factors to promote coagulation and inflammation to cease the blood loss and retard the invasion of microbes to injured tissues[36&37]. The second phase is inflammatory phase, where the aggregated platelets in the formed clot release regulators to stimulate the release of several cytokines and growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor β (TGFβ)[1]. These regulators attract immune cells such as; neutrophils and monocytes, whose passage favored by concomitant vasodilation. The monocytes convert into macrophages, which engulf debris and pathogens along with growth factors and other cytokines and destroy any remaining neutrophils[1]. These macrophages have two phenotypes: M1 (pro-inflammatory) and M2 (pro-healing). Initially, macrophages are polarized to the pro-inflammatory phenotype M1 to help the host defense against pathogens. Then, macrophages of the M2 phenotype are polarized to form an anti-inflammatory response to go into the proliferation phase and repair damaged tissue[38]. Keratinocytes migrate to the injured tissues to promote epithelization while local fibroblasts proliferate for the formation of early granulation tissue[39]. This granulation tissue is composed of procollagen, elastin, proteoglycans, and hyaluronic acid (HA)[39]. The first stage of wound healing starts immediately, while the second stage starts a few hours after injury and proceeds fast, commonly reported by 48 to 72 hrs[1].

In diabetic patients, due to elevated blood glucose levels, the hemostasis phase is disrupted. Diabetes is commonly associated with a prothrombotic state which characterized by platelets hypersensitivity and an abnormality in the coagulation system[40], thus make diabetic patients at high risk for thrombosis and stroke[41]. So, using antiplatelets or anticoagulant agents is urgent for diabetic patients for protection from thrombosis. This inhibition of coagulation will impair hemostasis, leading to a delay in clog formation.

Moreover, the elevation in glucose blood level and production of a high amount of ROS negatively affect the inflammatory phase of wound healing[42]. As a different phenotypic macrophage with a lower engulfment capacity

is secreted instead, and this impairs the removal of debris and pathogens from injured tissues, all together, enhances the microbial contamination of injured tissues and this negatively impacted the wound healing process in diabetic patients[43]. In addition, the different phenotypic macrophages retard the conversion of M1 macrophage, a pro-inflammatory cell to M2 macrophage, a pro-healing cell (that normally occurs in non-diabetic patients and is necessary to move to the proliferation phase, the third phase of wound healing). Moreover, the accumulation of different phenotypic macrophages identified in the injured wound of diabetic patients is associated with a higher level of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) than that detected in non-diabetic patients, and this again prolongs the inflammatory phase compared to non-diabetic patients and thus retard moving to the proliferation phase of wound healing[38-44&45]. Furthermore, an increase of pro-inflammatory cytokines (IL-1β [interleukin-1β] and TNF-α) and matrix metalloproteinase-9 (MMP-9) with decreased anti-inflammatory signals (CD206 [mannose receptor c type 1], IGF-1 [insulin-like growth factor 1], TGF-β [transforming growth factor beta] and IL-10 [interleukin-10]) will lead to abnormal apoptosis of fibroblasts and keratinocytes, together with decreased angiogenesis and again this delays the occurrence of proliferation phase of wound healing[46].

The third phase of wound healing is the proliferative phase, where, in normal non-diabetic injured patients, macrophages (M2), a pro-healing cell releases growth factors such as vascular endothelial growth factor (VEGF) to promote the activation of fibroblasts to migrate and proliferate to the injured tissues in order to release collagen at the injured tissue to form extracellular matrix (ECM) and rebuild the injured tissues[47] as well as promoting the angiogenesis[35&36]. In the proliferative phase, keratinocytes also migrate to promote reepithelization[50]. In diabetic patients, the function of both fibroblasts and keratinocytes is compromised as in literature, different phenotypic cells of fibroblast and keratinocytes are reported[51] and this retards the reepithelization and angiogenesis in the proliferative phase[47]. In addition, due to persistently high levels of pro-inflammatory cytokines, there is a higher expression of proteases such as matrix metalloproteinase (MMP) and lower expression of antiproteases such as tissue inhibitor of metalloproteinase (TIMP). The imbalance between MMP and TIMP leads to abnormal ECM deposition and degradation[46].

The remodeling phase is the final phase of wound healing, and it is essential to remodel the repaired tissue. In nondiabetic patients, fibroblasts in this phase differentiate into myofibroblasts which decrease the size of the wound, causing wound contraction[47]. Collagen type III, which was synthesized at high levels and deposited randomly to form ECM in the previous phase, is replaced by type I. ECM is matured with higher tensile strength (the load capacity per unit area) as the tensile strength of collagen type I is much higher than collagen type III. Moreover, blood supply to the damaged area reduces and goes back to normal, and the wound repair process proceeds toward restoration of the physiologic structure of the skin[1-31]. But in diabetic patients, fibroblasts do not properly differentiate into myofibroblasts, leading to reduced mechanical tension of ECM. The imbalance between MMPs, responsible for degrading the disorganized collagen and tissue inhibitor of metalloproteinases (TIMPs) ,as mentioned previously, leads to abnormal ECM maturation[46]. At the end of the remodeling phase, acute wounds are completely healed, while the diabetic wounds are still present and not healed. So, treatments for delayed diabetic wound healing are necessary to provide the factors and conditions that are deficient in the diabetic environment and cause disruption in the flow of healing process. The next section will display the most recent strategies available for treatment of diabetic wounds.

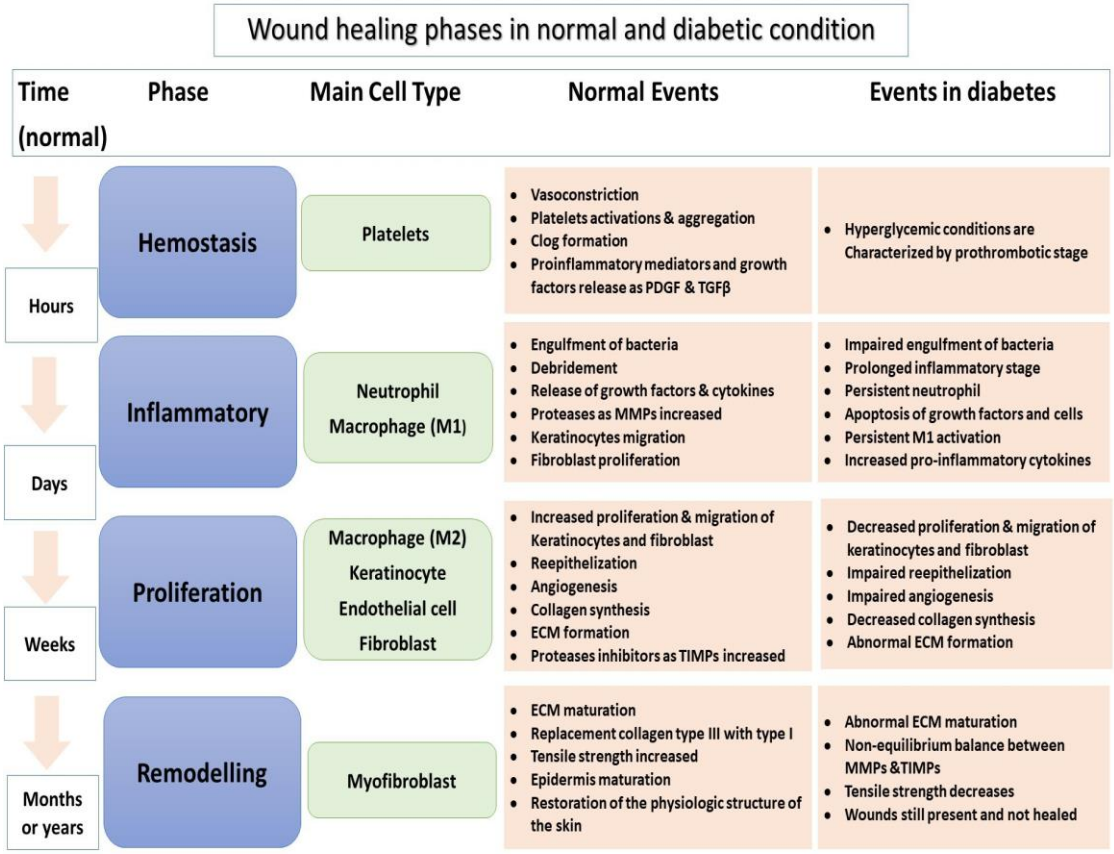

Fig.1: Diagrammatic presentation of wound healing phases in non-diabetic versus diabetic patients.

Wound Healing Treatments

The standard wound-care practice for impaired wound healing includes control of the infection together with debridement, off-loading to relieve pressure, and maintenance of a moist wound bed[29]. Diabetic patients, as discussed previously, are suffering from the incidence of chronic wounds with a high susceptibility to microbial infection due to loss of the innate skin barrier and a decrease in the immunity of diabetic patients[52].

Debridement

Debridement is the removal of debrides and non-viable tissues that serve as a reservoir for bacteria and increase the peak plantar pressure, as in hyperproliferative and hyperkeratotic epidermis and calluses. Thus, debridement exposes the healthy tissue and allows cells to migrate and proliferate to repair the injured area. Debridement can be applied through different techniques, and they are: **Sharp/Surgical**: is considered as the most efficient technique for rapidly debriding the wound, where a scalpel or curette is used for removal of non-viable tissues resulting in complete removal of the necrotic tissue and prevention of its spreading out.

Mechanical: involves usage of wet-to-dry gauze dressings, wound irrigation, and pulsative lavage of necrotic tissue. However, wet-to-dry gauze dressings are painful and thus are not recommended.

Autolytic: is performed by applying dressings to create a moist environment to allow the body's natural enzymes to break down necrotic tissue. Occlusive or semi-occlusive dressings such as hydrocolloids, hydrogels, and films are commonly used for the purpose of retaining the moist in the skin and this decreases the loss of moisture from the skin and activates the catalytic activity of enzymes to lysis the callus[29].

Dressings

Wound dressings are considered one of the well-established and efficient strategies for improving wound healing especially in diabetic patients[53]. Although there are many types of

dressings, the ideal wound dressings should have the following criteria;

- Permit gaseous exchange, thermal insulation, make it easier to remove drainage and debris to improve the tissue healing processes
- be harmless and not cause an adverse or immunological reaction
- Prevent the development of infections, which could slow down the healing of wounds.
- Encourage patient compliance, by enabling self-application and removal
- Assist in the development of granulation tissue and epithelialization
- Promote the moist environment necessary for optimal healing.

However, there is no single dressing which can efficiently meet all the needs for a particular wound and healing phase but there are dressings that have distinct characteristics to be applied for wound healing depending on the phase of wound healing previously discussed[54-56].

Types of wound dressings are presented in Figure 2 and they involve:

Passive dressings: are non-occlusive dressings, and they are used only for the purpose of wound coverage facilitating wound healing. They are non-adherent dressings as they almost impregnated with a non-petroleum-based substance.

Interactive dressings: act as a barrier against the access of bacteria and thus are able to protect the wound against further microbial infections. They can be occlusive (should be avoided for infected wounds) or semi-occlusive and made of different materials which may be adherent or non-adherent such as; films, foams, alginates, hydrogels, and hydrocolloids[57].

Foam and alginate dressings are examples of non-adhesive dressings, and they are characterized by being highly absorbent and thus are very effective for heavily exuding wounds (heavily exudating ulcers require frequent change to reduce maceration of surrounding skin). Hydrogels facilitate autolysis and may be useful in management of ulcers containing necrotic tissues[58&59]. Recently, a more complicated and efficient wound dressing was developed such as composite dressing, it is an expensive and special type of interactive dressing that composed of multiple layers, commonly three layers and each layer is physiologically distinct[60]. The outer most layer protect the wound from infection, middle layer usually composed of absorptive material which maintains moisture environment to assist autolytic debridement, bottom layer composed of non-adherent material which prevents sticking to young granulating tissues[56].

Bioactive dressings: are interactive dressings incorporated with active substances or biomolecules to deliver them in wounds[61]. Examples of these substances are antimicrobial agents [e.g. silver and antiseptic iodine], growth factors, nitric oxide, and enzymes to restrict or inhibit the microbial growth and facilitate proliferation of cells, growing of new tissues and enzymes are included to facilitate the debridement of non-viable tissues.

Bioengineered dressings could be bioactive dressing as they are able to release growth factors and cytokines incorporated in dressings to accelerate wound healing process. Apligraf® was the first bioengineered living cell therapy approved by the FDA, it consists of keratinocytes and fibroblast on collagen matrix[35-56].

It is worth noting that type of dressing is selected according to the phase of wound healing as discussed earlier as well as the requirements of the patient, and the costs[58&59].

Nanotechnology, a recent approach that were used to develop novel dressing incorporating nano-materials are also available to be used for promotion of wound healing in diabetic patients and this will be discussed later in details[30]. Briefly, nanomaterials that facilitate wound healing process could be added into different types of wound dressing previously discussed in order to enhance the efficiency of these dressings.

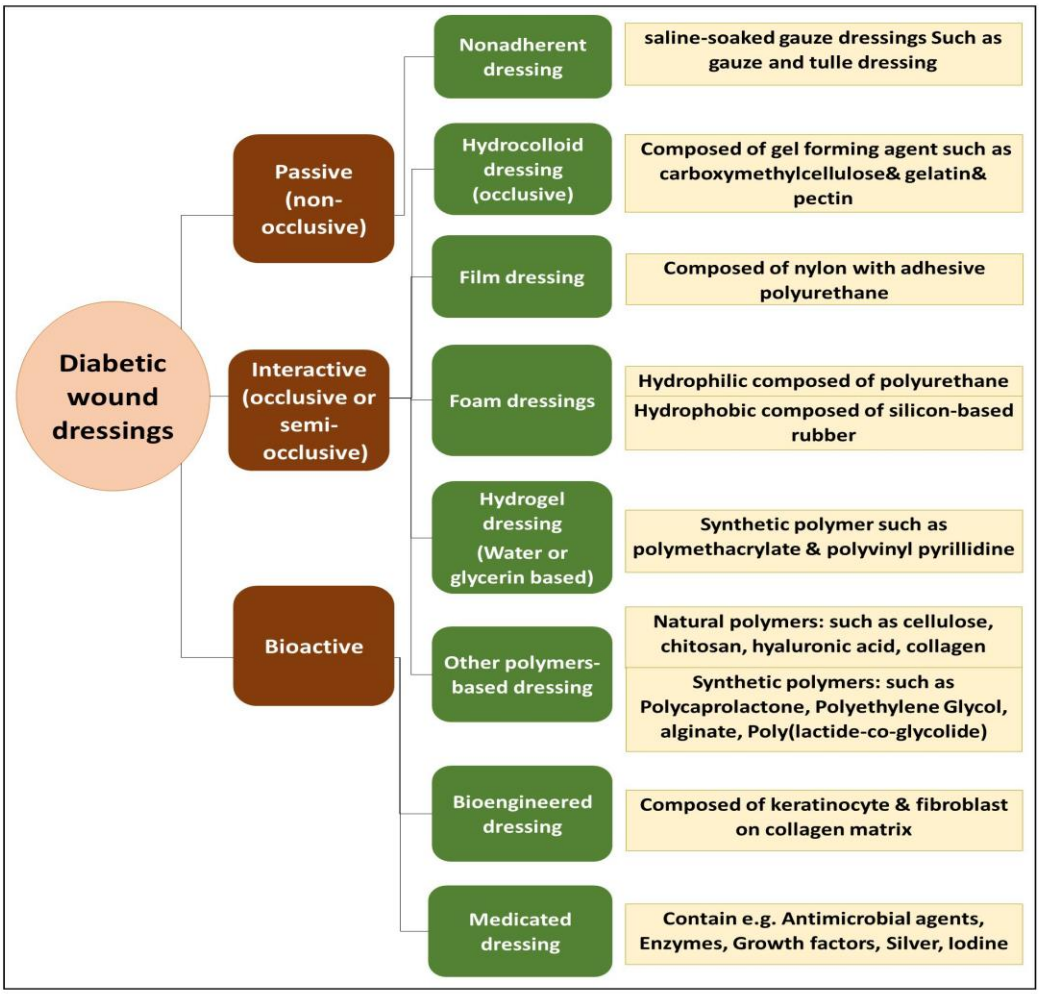

Fig. 2: Different types of diabetic wound dressings.

Growth factors

Several growth factors are reported to be involved at various stages of the healing process, where they are responsible for activating a variety of cellular and molecular responses[62]. For example, they are reported to stimulate the formation of granulation tissue, modulate the inflammatory response, promote angiogenesis, stimulate the formation of ECM, remodeling, and re-epithelization[63]. There is only one (FDA) approved growth factor in the market for the treatment of diabetic foot ulcer (DFU), Regranex®, which is a recombinant platelet-derived growth factor (PDGF)[64&65]. Some of the other growth factors such as; vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor beta-1 (TGFβ1), were tested in clinical trials with a special focus on treating diabetic foot ulcers. However, those clinical studies have some drawbacks where the results revealed a high risk of bias as well as safety issues were not well-addressed[1-63]. Several limitations were reported for the application of growth factors as a treatment to facilitate wound healing in diabetic patients[1-66&67], and they are; high doses of growth factors are needed to be applied repeatedly to achieve therapeutic effects[66], Protease enzymes in cells enhance rabid degradation of growth factors and thus restrict their therapeutic action. Thus, there is a high demand for a delivery system to protect growth factors against degradation and maintain their therapeutic activity, as well as sustain and control their release at the wound site. Nanoparticles, hydrogels, and nanofibers are delivery systems that have been reported as efficient delivery systems for growth factors that overcome their limitations and recommended to be used in diabetic patients[68-72].

Nissen et al reported that VEGF-A is the primary pro-angiogenic factor in normal healing wounds[62]. The results of clinical trials showed that single-dose topical application of VEGF to wounds alone has limited success due to its short half-life. In order to overcome the disadvantages of short half-life and repeated delivery of VEGF, it was hypothesized that encapsulation of VEGF into a delivery system might be beneficial. A previous study investigated this hypothesis where porous hyaluronic acid hydrogel encapsulated proangiogenic (pVEGF) plasmids for local gene therapy in diabetic wound healing. It was demonstrated that the hyaluronic acid hydrogel matrix provided a mechanical barrier to wound healing as well as minimized the degradation of VEGF. However, levels of pVEGF transfection do not appear to be high enough to promote angiogenesis by increasing the density or size of blood vessels[73].

Stem Cells

Stem cells were reported to be an effective therapy for diabetic foot ulcers[74]. They are currently used as an option for re-vascularization and thus could be an alternative to amputation for some patients. Adult mesenchymal stem cells (MSCs) have shown efficacy in several clinical trials and are included in commercially available topical products[75&76]. Stem cells transplanted in the wounded area were reported to release cytokines and growth factors that are able to promote cell recruitment, angiogenesis, ECM remodeling, and exert an immunomodulatory action[77]. The morphology, size, and surface phenotype of MSCs derived from different tissues such as bone marrow - derived mesenchymal stem cells (BM-MSCs), adipose-derived mesenchymal stem cells (AMSCs), umbilical cord-derived mesenchymal stem cells (UC-MSCs), placenta-derived mesenchymal stem cells (PMSCs), and human amniotic fluid-derived stem cells (AF-MSCs), and they were reported to have no significant differences[78]. Stem cells have some limitations that may be related to issues of safety, efficacy, immunological incompatibility, potential legal, and ethical issues such as the use of embryonic stem cells is ethically controversial, as well as strict donor screening is needed to avoid disease transmission. Additionally, the amounts of stem cells obtained from participants before the treatment are usually small and to enrich cell concentration, cell culture and induced differentiation in vitro are necessary. That requires a relatively high-level laboratory technique. Besides, some stem cells may lose function or mutate due to impropriate induced process[74,79-81]. BM-MSCs are firstly discovered and deeply studied in many clinical trials with satisfactory clinical efficacy. Although other types of stem cells have been evaluated for diabetic wound healing at preclinical trials, BM-MSCs were reported to have no immunologic restriction and do not stimulate alloreactivity because they have capability of escaping lysis[78]. BM-MSCs can accelerate wound repair by enhancing the migration, angiogenesis, and re-epithelialization. In a study on diabetic rats, it was reported that BM-MSCs could promote angiogenesis and thicken granulation tissue by increasing the expression of VEGF[82]. Another study on diabetic and non-diabetic Albino rats, AMSCs were combined with platelet-rich plasma as a suggested therapy to enhance wound healing. This study was evaluated by assessing wound closure rate, epidermal thickness, dermal collagen, and angiogenesis, better wound healing after 7 and 14 days was reported for combined therapy compared to their individual. The combined therapy enhanced the re-epithelialization and formation of granulation tissue, moreover, a marked increase in percentage of collagen production, epidermal thickness, and angiogenesis was observed[83].

A study reported by Yang and his colleagues investigated the wound healing capacity of human mesenchymal stem cells (BM-MSCs) encapsulated into scaffolds in the diabetic mouse wound model, different densities of cells/ cm^2(0.83, 2.5, and 7.5×10^5) were assessed[84]. Their topical application was associated with a significant improvement of wound epithelialization, and it was 90%, 108%, and 77% for 0.83, 2.5, and 7.5×10^5 cells/cm^2, respectively.

Estrogen

Estrogen is a hormone that has been reported to accelerate wound healing of chronic wounds when applied topically[1-85], and its mechanism of action is reported to be through: formation of capillary-like structures in

endothelial cells, stimulating release of PDGF by macrophages, formation of granulation tissue, and enhancing collagen deposition[86&87]. The wound healing capability of estrogen was assessed in diabetic mice[87], and it was found that estrogen enhanced wound healing by increasing the number of both epithelial precursor cells and mesenchymal stem cells, where they were contributing in neo-angiogenesis and tissue regeneration, respectively. Moreover, it has been reported that the migratory promoting effect of keratinocytes responsible for re-epithelization involved interaction with estrogen receptor β (ERβ)[88].

Antidiabetic Drugs

Medicines used for therapeutic management for T1DM and T2DM, such as oral administration of metformin, topical application of insulin, some sulfonylureas (such as glyburide), thiazolidinediones, and diphenyl peptidase 4 (DPP-4)inhibitors, were reported to have a broad range of different effects that may be useful in the treatment of chronic wounds when they are applied orally or topically[89]. Some of these effects involved a reduction of the pro-inflammatory macrophages phenotype, an increase of anti-inflammatory phenotype of macrophage, a decrease of MMPs levels, an increase of the numbers of keratinocytes and fibroblasts, induction of angiogenesis, accelerating rate of wound closure, and supporting granulation tissue development.

Antihypertensive drugs

Despite controversial results are available regarding the use of systemic propranolol in the treatment of chronic wounds, this β-blocker has been tested since activation of β2-adrenergic receptor is known to inhibit keratinocyte migration and delay re-epithelialization. Interesting data were provided by a study in which a 1% propranolol cream was applied to chronic wounds in diabetic mice. Topical propranolol was effective in inducing re-epithelialization, functional angiogenesis, and increased ECM turnover confirming its potential in the treatment of wound healing[90].

Timolol is another β-blocker, was administrated in a study daily topically at a concentration of 2.9 mM for 7 days as a combination product with MSC seeded at a density of 2.5×10^5 cells/cm^2 on an Integra matrix wound. This combination product was investigated as a wound enhancer in a diabetic mice model. This treatment resulted in optimal wound epithelialization, lowered the pro-inflammatory cytokines IL-1B and IL6 levels, decreased neutrophils by 44.8%, and shifted the macrophage ratio of M2/M1 to 1.9 in the wound. Systemic absorption of timolol was below the HPLC limit of quantification, suggesting that with the 7-day treatment, accumulative steady-state timolol concentration is minimal[84]. NB (NB), a beta-1 receptor blocker with a vasodilator effect, was loaded into microsponge gel where microsponges slowly released the entrapped drug on the wound surface from porous structure, and gel provided a moist environment for wound management in later stages. Thus, NB-Loaded microsponge Gel l achieved a significant acceleration of diabetic wound healing in rats following topical administration (once daily), as revealed by the presence of a higher number of fibroblasts, neutrophils, and acceleration of collagen synthesis in the treated group compared to the untreated group. Moreover, presence of drug at the wound site on day 7 promotes an earlier proliferation phase. All together resulted in minimizing wound area and facilitating wound healing[91]. Thus, NB -Loaded microsponge Gel could be considered as a breakthrough for diabetic wound therapeutic management.

The angiotensin-converting enzyme (ACE) inhibitor, captopril, has shown the ability to increase wound healing scores in diabetic rats and its recognized activity as a reactive species scavenger has prompted its inclusion in novel wound dressings made of Chitosan and hyaluronic acid. This combination results in better wound reparative activity compared to the biomaterial applied alone such as chitosan or hyaluronic acid[92].

In another study, diabetic and control mice were treated with the angiotensin II inhibitor Losartan for 14 days. Losartan was obtained as pills, crushed, dissolved in PBS, and administered by oral gavage to control diabetic mice daily at a concentration of 10 mg/Kg. Treatment with Losartan for the duration of the wound healing process was sufficient to induce

a normal-like wound healing response in diabetic mice[93].

Amlodipine and enalapril significantly increased re-epithelialization in excision wound model after 11 days of orally treatment. 3 mg/kg of amlodipine significantly improved incision wound tensile strength, and 15 mg/kg of enalapril increased granulation tissue formation on diabetic wound animal model[94].

Topical application of 1% valsartan gel (ACE inhibitor) significantly accelerated closure time and increased tensile strength of healing skin in diabetic mice. It was also exhibited higher mitochondrial content and collagen deposition[95].

Statins

Statins are HMG-CoA (5-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors and are primarily used to lower cholesterol levels in blood. In addition, statins have been found to protect against ischemic injury and stimulate angiogenesis in normocholesterolemic animals[96]. Keratinocytes are capable of de novo cortisol synthesis as well as 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) reductase-mediated cholesterol synthesis. Statins promote keratinocyte migration by antagonizing HMG CoA reductase, thereby inhibiting formation of farnesyl pyrophosphate (FPP), an intermediate in the cholesterol synthesis pathway that acts on keratinocyte glucocorticoid receptors (GR) to inhibit epithelialization. Statins have also been associated with cholesterol-independent effects, modulate the immune response, decrease oxidative stress, and stimulate fracture healing and wound healing. enhancing vascular endothelial growth factor and nitric oxide synthase up-regulation.

Topical 1 and 5% atorvastatin improved wound healing in streptozotocin-induced diabetic rats, while oral pravastatin improved streptozotocin-induced diabetic rat wound healing via increased endothelial nitric oxide synthase expression and NO production. In a diabetic mouse model, intraperitoneal simvastatin increased vascular endothelial growth factor (VEGF), a crucial factor for angiogenesis and wound healing, and improved wound healing. There has been one small randomized open label pilot trial in humans investigating the efficacy of statins for DFUs. Investigators found that 80 mg of oral atorvastatin promoted healing and reduced recurrence and new ulcer formation over 6 months of follow-up[97].

Topical application of simvastatin in diabetic animal model significantly increased the number of infiltrating macrophages in granulation tissue, and most of these macrophages produced VEGF-C. Also, Simvastatin induced angiogenesis and recovery of lymphangiogenic function on primary human lymphatic endothelial cells (LECs)[96].

Nanotechnology in diabetic wound healing:

Although there are many treatments available for accelerating the healing of chronic wounds, as discussed previously, some of them have limitations either due to incomplete safety studies e.g. application of stem cells, or limited therapeutic action due to rapid degradation of medication such as; growth factors as previously discussed in section 4.3. This necessitates finding alternative approaches or delivery systems to overcome these drawbacks.

Nanotechnology has greatly impacted the field of pharmaceuticals and drug delivery. Nanomedicine is the branch of medicine that uses particles sized from 1 to 1,000 nm for either therapeutic or diagnostic purposes[98-101]. Nanomedicine as a drug delivery system was used to target the drug to a specific site and consequently overcome drug accumulation at off-target tissues and side effects associated with the drug administration[102-107]. This made nanomedicines able to overcome the limitations of conventional therapy[108], such as high frequency of drug administration[103], improve the delivery of hydrophilic drug into cells[109], improve bioavailability of poorly soluble drug, and control/sustain drug release[110-112]. Nanoparticles (NPs) have been previously applied to treat many diseases such as viral infections with promising results[101,113-115], and also showed a good antibacterial activity against multidrug resistant bacteria[100&115-117], wound healing[118&119], and inflammation[120], anticancer[121] aid crossing the blood-brain barrier[122&123] and has a potential to be used for diagnostic purposes[124]. It is worth mentioning that biological activity of nanomaterials is highly affected by their physiochemical properties (e.g. size and surface charge), as they could influence delivery of active

substances, their interactions with other biomolecules, their biodistribution, cell penetrability, receptor binding capacity, and their stability[125-127]. For instance, high surface to volume ratio, resulted from small size of NPs, enables them to penetrate deeper into the wound site and afford better association with biological components, leading to accelerated wound healing[119-128&129]. In addition, Nanoparticles with smaller size have shown higher antibacterial activity[130]. Also, surface charge of NPs greatly affects their antibacterial activity, where positively charged MBNPs interact electrostatically with negatively charged bacteria, leading to accumulation of NPs inside the bacteria and, this accompanied with exposure of bacteria to a higher amount of ROS resulting in bacterial death[130]. Furthermore, surface charge could affect stability of nanoparticles by applying electrostatic repulsion, that prevent aggregation of nanoparticles and maintain their homogenous distribution[131].

Nanomaterials used to accelerate diabetic wound healing can be classified into; inorganic nanoparticles such as metal-based nanoparticles (MBNPs), that have intrinsic antibacterial activity and wound healing activity, and organic nanoparticles such as polymeric nanomaterials, that could be used as carriers for therapeutic agents, as previously discussed. The later could be formulated into hydrogels, sponges, nanofibers, and scaffolds[127]. MBNPs were reported to be more effective when compared to organic nanomaterials due to their intrinsic physicochemical properties[128].

MBNPs were reported[105-108] to have a broad-antimicrobial activity. Literature (Table S2) reported the antibacterial activity against both Gram-positive and Gram-negative bacteria including resistant strains such as nanoparticles formulated based on the following metals; copper, cerium, gold, zinc, and silver. Moreover, MBNPs could be used as a carrier for antibiotics and biomaterials (insulin, growth factors and genes, biomaterials (hyaluronic acid or niacinamide, and antibiotics), protect them against biodegradation, and thus potentiate their actions. This enhanced their delivery into the injured site and consequently facilitate wound healing in diabetic patients[109&110]. MBNPs were reported to heal either infected/ uninfected wound, both in vitro and in vivo through prevention of persistent inflammation phase and enhancing proliferation phase that are compromised in diabetic patients, as previously discussed in section 3. So, in the current review, we are exploring recent studies performed to investigate different MBNPs reported to eradicate the bacterial strains contaminating wounds, as well as their potential to enhance the healing of infected and un-infected diabetic wounds (Table S2).

MBNPs potentiate in vitro wound healing activity

Migration, proliferation (is the ability of cells to divide after exposure to a specific stimulus), and angiogenesis are important properties of wound healing. MBNPs can be applied in vitro on fibroblast, keratinocyte, and endothelial cells to determine their ability to enhance wound healing (Table S1). Scratch assay is one of the most important in vitro wound healing tests. It shows how much MBNPs affect the migration rate of scratched cells. The more migration rate increases, the higher the ability of MBNPs to enhance wound healing, and the shorter time needed for wound contraction. Migration occurs first in the early phase of wound healing. Then cells start proliferation to provide additional cells for migration on the second and third post wound days[135]. Angiogenic activity of MONPs also should be evaluated. As it determines the ability of MBNPs to angiogenesis by forming new blood vessels in the injured area. The capability of MBNPs to angiogenesis is inferred by the increased number of capillary branches and capillary junctions formed, indicating the formation of new vascular networks. CAM assay, Matrigel assay, and tube formation assay are examples of tests used to measure the angiogenic potential of materials.

As shown in Table S1, cerium oxide (CeO_2) nano-formulae can increase migration, proliferation, and angiogenesis[112-115]. Additionally, Cu-based NPs increased the migration rate of cells and numbers of tubule junctions formed which indicated to enhancement of angiogenesis[116&117]. Also, Cu_2S significantly increased proliferation rates of HDF after 5 days[142]. A study of in vitro migration assay was applied using silver (Ag)-

pyridoxine nanoparticles (NPs) on 3T3- L1 and HaCaT cells in inflammation condition to simulate condition of diabetic wounds and the migration speed of cells was calculated[135]. The study revealed that the migration speed (is defined as the time required for cells to fill the total length of the wound area) of HaCaT cells treated with NPs increased to 0.30 µm/min compared to 0.28 µm/min for control cells, and the migration speed of 3T3-L1 cells increased to 0.46 µm/min compared to 0.17 µm/min for control cells. These results demonstrate that these NPs can promote wound healing in keratinocyte and fibroblast cells under the condition of inflammation[135]. In addition, all tested concentrations of Ag-pyridoxine NPs up to 5 µM increased proliferation of HaCaT cells, but at 8µM the number of cells were decreased compared to control due to cytotoxicity[135]. This assure as will be shown in Table S2, increasing MBNPs concentrations, will increase its wound healing activity by enhancing migration, proliferation, cell viability, and angiogenesis but at a concentration less than cytotoxic concentration.

Cytotoxicity of MBNPs

In order to use MBNPs as a topical preparation to facilitate wound healing in diabetic patients, their safety profile should be addressed. However, there are a very limited studies addressing the biosafety of MBNPs (Table S2), and only MBNPs were approved by FDA such as iron oxide nanoparticles (IONPs) for treatment of anemia[101], and calcium phosphate nanocrystal for bone graft substitute[143]. In addition, There are a few numbers of MBNPs formulations approved by FDA[144] and high percentage of them were withdrawn from market due to their cytotoxicity. There are many challenges facing nanomedicines and need to be regulated to transfer to clinical studies[145]. Some of these challenges are quality, efficacy, stability , safety, and scale up and manufacture process of nano pharmaceuticals[145]. But on the other hand, Feraheme® is an IONPs-based product that has been approved for the treatment of iron deficiency in adults with chronic kidney disease by FDA since 2009 to date[144]. In addition, ($Ca_3(PO_4)_2$) NP was reported to be effective in enhancing wound healing in diabetic mice[146], and it is one of the MBNPs approved by FDA[143]. These NPs could be repurposed and investigated for treatment of wound healing in clinical trials. This might encourage researchers to do further investigation and terminate preclinical studies to enter into clinical studies and apply them to humans.

Biocompatibility of all MBNPs is essential to be addressed in order to be applied clinically as mentioned before. Table S2 summarizes some of reported cytotoxicity studies performed for MBNPs. Literature reported that cytotoxicity of MBNPs is a concentration and time dependent, however, luckily most tested MBNPs were safe at their therapeutic concentrations (Table S2). However, Sabarinathan and his colleagues synthesized AgNPs that have showed cytotoxic and anti-proliferative effect on keratinocyte and fibroblast cells at all tested concentrations[135].

MBNPs were reported (Table S2) to be safer when incorporated into a hydrogel, wound dressing or coating its surface with another material due to controlling/ sustaining the release of cations from MBNPs at a slow rate that is enough to avoid any harm to cells. For instance, in two studies, Jisheng and Colleagues synthesized Cu metal organic framework NPs (CuFNP) using trimesic acid. These framework NPs were embedded within citrate-based hydrogel[140], and at another occasion they were coated and stabilized with folic acid[141]. Cytotoxicity was assessed for these NPs on human epithelial keratinocytes (HEKa) and human dermal fibroblasts (HDF) cells. It was found that entrapment of Cu NPs within hydrogel or coating with folic acid showed a lower cytotoxicity than CuOFN. This might be attributed to the slower release of Cu cations recorded for hydrogel and those coated with folic acid compared to CuFNP. Thus, they were more biocompatible and had a superior wound healing activity.

Inherent wound healing activity of MBNPs on diabetic animal model

Inherent wound healing activity of MBNPs may be due to having MBNPs one or more properties that modulate disordered conditions in diabetic wounds. For instance, inherent wound healing activity of MBNPs may be attributed to:

Angiogenic and antioxidant activity; cerium oxide nanoparticles (CeO_2 NPs) have a good free radical scavenging activity and angiogenic properties, when incorporated into a biodegradable gelatin methacryloyl hydrogel and tested for its healing activity on wounds of diabetic rats, the wounds covered with hydrogel provided faster healing rate and a higher number in blood vessels. A relatively thick epidermis was observed in wounds covered with hydrogel, and it is an evident to re-epithelialization, while untreated group showed a relatively thin epidermis[137]. In another study, CeO_2 NPs incorporated into Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) electro-spun membranes developed thick granulation tissue, that composed of several macrophages, fibroblasts, and new capillaries, when applied to a diabetic mice[138].

Angiogenesis induction; Cu based NPs significantly induced angiogenesis in vivo mouse model by increasing blood vessels number within the granulation tissues about four and five-fold higher than that of the control group[133,155]. Calcium phosphate nanoparticles ($Ca_3(PO_4)_2$) NPs were also reported to be effective in enhancing wound healing in diabetic mice by angiogenesis[146]. $Ca_3(PO_4)_2$ NPs were loaded into poly lactic acid (PLA) electro-spun mats fibers and applied to a pressure ulcer model in diabetic mice. On 3rd day post treatment, histological examination of CD31+ marker was performed to evaluate vascular angiogenesis. wounds treated with nanocomposite showed significantly higher vessel density than control. In addition, an increase in cellularity was also observed at the wound site indicating the presence of more granulation tissue. On 8th day post-treatment, vessel density regressed and reached similar values to control, and this normally occurs in remodeling phase of wound healing as previously discussed in section 3.

Antibacterial and antioxidant activity; MBNPs were reported in literature to have broad-spectrum antibacterial activity (Table S2). Additionally, they were proven to have a promising effect to enhance wound healing in a diabetic animal model. For instances, Gold nanoparticles (AuNPs) were synthesized by Pandi Boomi et al[147]. AuNPs were incorporated into a cotton fabric. AuNPs-coated cotton fabric showed an antioxidant activity and was evaluated for its antibacterial activity against *S. epidermidis* and *E. coli* bacterial strains which revealed a remarkable inhibition.Zone of inhibition of 31 and 26 mm diameter was against *S. epidermidis* and *E. coli*, respectively. This was further confirmed in vivo in a diabetic animal model, where the wound area is completely re-epithelialized, and neovascularization was improved compared with the control group.

Inherent antibacterial activity and over-expression of genes; Natarajan Krishnan and colleagues have synthesized silver nanoparticles (AgNPs) that have shown an excellent antibacterial activity against clinically isolated resistant bacteria strains of *P. aurginosa* and *S. aureus*[148]. In addition, wound healing activity of AgNPs was assessed on diabetic mice. AgNPs treated group significantly decreased overexpression of MMP-2 and MMP-9 (they are overexpressed in diabetic wounds, and this is responsible for prolonging the inflammation as well as enhancing degradation of ECM), and showed complete epithelialization along with high fibroblast and collagen deposition.

Biocatalytic efficiency in wound healing;

Tessy Lopez-Goerne and Colleagues reported, platinum nanoparticles (PtNPs) were incorporated into SiO2-TiO2 matrix during the sol–gel process. It was found that this nano-biocatalyst enhanced the healing processes in diabetic male rats. Their wound healing activity might be attributed to inhibition of bacterial growth, anti-inflammatory and antioxidation effect, and regulation of angiogenesis that permits efficient wound healing[149].

MBNPs was able to overcome bacterial resistance toward antibiotic

MBNPs are considered a good way for facing the resistance of antibiotics. It was reported that a combination of MBNPs and antibiotics not only can overcome resistance of some bacteria but also can enhance antibacterial activity against non-resistant bacteria and withstand the bacterial resistance[150]. It was reported that a nanohybrid

complex of Au nanoclusters capped with lysozyme and conjugated to ampicillin overcome the MRSA resistance towards ampicillin and showed a significant increase (50–89% fold increase) in antibacterial activity (Table S2). This was attributed to enhancing the accumulation of ampicillin intracellularly to exceed the saturation level required for β-lactamase and thus, the excess concentration was free to attach the cell wall of bacteria leading to bacterial death. This is associated with a lower possibility of bacterial genetic mutation developing resistant bacteria[150]. This was further confirmed invivo on a diabetic mice model where the wound infected with MRSA was successfully healed after treatment with the nanohybrid complex. Moreover, this nano-complex showed an immunomodulatory effect, and this was demonstrated in vivo by normalizing the abnormal level of inflammatory cytokines.

RezaGolmohammadi and Colleagues reported that the antibacterial activity of selenium nanoparticles (SeNPs) reduced the growth of MRSA by one-fold, and a synergistic antibacterial activity (three-fold decrease of MRSA growth) was observed when SeNPs conjugated to mupirocin, followed by its incorporation into chitosan-cetyltrimethylammonium bromide (CTAB) - based hydrogel[151].

Ag nano-cubes loaded with gentamicin is another example of combinations of MBNPs and antibiotic, resulted in antibacterial synergetic effect against MRSA[152]. Gentamicin was unable to inhibit the growth of MRSA contaminating wounds of mice. However, by conjugating gentamicin to Ag nano-cubes, a synergistic antibacterial activity was observed, and the growth of MRSA was completely inhibited, resulting in a successful wound healing. In another study performed by Lin Meia and Colleagues[153], AgNPs were synthesized in presence of monomer 2-(dimethylamino) ethyl methacrylate (DMAEMA) containing tertiary amino group. AgNPs conjugated to levofloxacin demonstrated to inhibit the development of resistant bacterial strains observed when levofloxacin was administrated only. After 30 passages, The MIC values of AgNPs against *P. aeruginosa* and *S. aureus* were the same for all passages, indicating absence of resistant bacterial strains. Contrary to a dramatic increase of MIC values recorded for levofloxacin, where MIC increased from 3.2 to 156 μg/mL and from 0.64 to 78 μg/mL for *P. aeruginosa*, and *S. aureus*, respectively, after 30 times of bacterial passaging, due to the emergence of bacterial resistant strain to levofloxacin.

MBNPs used as a nano-carrier to biomaterials

MBNPs were used as a carrier for biomaterials for many purposes such as; overcome degradation[154], synergistic effect[42], and increase selectivity and sensitivity of conventional therapy[133]. And we classified theses composites below according to how they could enhance diabetic wound healing.

ROS scavenging and decrease inflammatory response

MicroRNA-146a (miR-146a) is an anti-inflammatory molecule and influences gene expressions for many proteins regulating the process of wound healing. It was found that miR-146a was dysregulated in diabetic wounds. Decreased expression of miR-146a in diabetic wounds has been linked to increases in gene expression of pro-inflammatory molecules[155] which prevent macrophage's transition from a pro-inflammatory (M1) phenotype to a pro-healing (M2) phenotype[156]. CeO_2 NPs have an excellent antioxidant activity, rendering them able to scavenge ROS and reduce the oxidative stress at the site of diabetic wound to ensure a suitable microenvironment for cell proliferation[104]. Thus, a combination of CeO_2 NPs and miR-146a might be able to reduce the oxidative stress and inflammation synergistically at the wound site and enhance the healing of diabetic wounds. A suspension containing a mixture of CeO_2 NPs and miR-146a was applied to the wounds of diabetic pigs, promoted wound healing via induction of angiogenesis and increasing collagen I levels and collagen I:III ratios[42].

There are several studies of this combination have been already performed (see Table S2). miR-146a -conjugated CeO_2 NPs were incorporated into Zwitterionic cryogel made of (1:1) mole ratio of zwitterionic monomer [2- (methacryloloxy)ethyl] dimethyl-

(3-sulfopropyl) ammonium hydroxide (SBMA) and 3-[[2- (Methacryloyloxy) ethyl] dimethylammonio] propionate (CBMA)) to the non-zwitterionic monomer 2-hydroxyethyl methacrylate (HEMA), and applied to wounds of diabetic female mice. The results indicated that this conjugation significantly enhanced diabetic wound healing, increased microRNA-146a gene expression, decreased expression of proinflammatory cytokines such as IL6 and CXCL2, and increased structural type 1 collagen and thus improved tensile strength[155]. Moreover, miR-146a -conjugated CeO_2 NPs when mixed with 7% nano-silk solution and applied to wounds of diabetic female mice, Wounds treated with nanocomposite had significantly higher collagen levels than control and lower pro-inflammatory gene expression of IL-6 and IL-8[156].

Down-regulation or up-regulation of gene expression

Ganglioside-monosialic acid 3 synthase (GM3S) is a known target that is overexpressed in diabetic mice and is responsible for causing insulin resistance, decreasing migration, and proliferation, thus impeding wound healing. By conjugation of AuNPs with a spherical nucleic acid able to down-regulate GM3S, it resulted in the efficient delivery of nucleic acid with a marked down-regulation of GM3S expression, and increased insulin-like growth factor-1 (IGF1) receptor activation, keratinocyte migration, and proliferation and significantly increased angiogenesis by increasing the vascularity marker (CD31+) two-fold compared to control group[157].

Another nanocomposite was used to foster the healing of diabetic wounds. AuNPs were mixed with the antioxidants epigallocatechin gallate (EGCG) and α-lipoic acid (ALA), and this mixture significantly decreased the CD68 expression which is a marker of monocyte infiltration and inflammation from day 3 to day 7 after cutaneous injury in diabetic mice and decreased receptor of advanced glycation end-products (RAGE) expression in the wound of diabetic mice. The formation of advanced glycation end-products (AGE) is increased in hyperglycemic conditions, and it has been recognized as an important pathophysiological mechanism in the development of diabetic ulcers; the binding of circulatory AGE to RAGE on different cell types leads to oxidative stress and impaired function of growth factors, triggering an inflammatory response, and compromised collagen production. This leads to impaired diabetic wound healing[158].

Modulation of cytokine secretion and remodelling disturbed in diabetic condition

Insulin loaded AgNPs (IAgNPs) in carbopol-based gel significantly improved the wound healing process in non-diabetic and diabetic rats[133]. The reported underlying mechanism was a regulation of the balance between pro-inflammatory (IL-6, TNFα) and anti-inflammatory cytokines (IL-10) at the wound site. This promoted wound remodeling. IAgNPs treated groups showed a significantly higher wound closure percentage in both non-diabetic and diabetic rats, 73.33% and 60.0%, respectively, compared to untreated non-diabetic and diabetic rats, 40% and 33.33%, respectively.

Incorporation of MBNPs into biomaterials with antibacterial activity

Media, in which MBNPs were incorporated, have much effect on biological and physiochemical properties of MBNPs. Such that it can have a synergistic effect on MBNPs activity or increase the stability of MBNPs.

CeO_2 NPs are an example of MBNPs, and when incorporated into nanosheets of polyethylene glycol (PEG) modified molybdenum disulfide, was reported to show a photo-thermal antibacterial activity against *S. aureus* and *E. coli* after exposing to 808 nm (near-infrared radiation) NIR with a power density of 1.0 W cm^{-2} for 5 minutes[136]. The antibacterial activity of this nanocomposite is attributed to the photothermal antibacterial activity of molybdenum disulfide and the inherent antibacterial activity of CeO_2 NPs. In addition, nanocomposite showed good wound healing activity in diabetic rats in presence and absence of NIR irradiation, due to the antioxidants activity of CeO_2 NPs which decreased inflammation, and favored moving from the inflammatory phase to the proliferation phase[102-104].

AgNPsimpregnated chitosan-PEG hydrogel was successfully synthesized by Nosheen Masood and colleagues[120]. AgNPs impregnated chitosan-PEG hydrogel showed

strong antimicrobial potential against *E. coli* and *S. aureus*. The potential killing capability of this formula might be attributed to the synergistic effect of AgNPs and chitosan as the positive charge on chitosan enhanced interaction with the negatively charged cell wall of bacteria. Moreover, the application of AgNPs impregnated chitosan-PEG hydrogel on diabetic rabbits wound was associated with a significant wound contraction and re-epithelialization with a significant keratinocyte's migration compared to untreated animal.

In addition, AgNPs, when incorporated into a chitosan-dextran based antifouling hydrogel, have been reported to exhibit broad-spectrum antibacterial activity rather than free AgNPs[159]. Besides the antibacterial activity of chitosan, this hybrid hydrogel increased the stability of NPs and prevented their aggregation and thus enhanced the antibacterial activity of AgNPs. The antifouling hydrogel also enhanced the healing of wounds contaminated with *S. aureus* and *P. aeruginosa* in diabetic male rats by modulating therapeutic immune response due to upregulation of specific markers expression of macrophages and T lymphocytes, CD68+ and CD3+, respectively. The activation of these immunocytes boosts the host immune system to eradicate invading bacteria and accelerate wound healing[159].

Composite drugs to provide optimal environment for diabetic wound healing

As mentioned before, phases of wound healing in diabetic patients are compromised, and there are many recorded disturbed issues. Some studies would prefer to use multiple therapeutic agents to help restore the regularity of the healing process. For instance, a biodegradable chitosan-based hydrogel contains calcium alginate NPs (Ca-AlgNps) as a hemostatic agent and AgNps as an antibacterial agent mixed with fresh blood of the same animal as a vital material containing various circulatory fibrocytes, growth factors, cytokines, platelets, and macrophages which enhance wound healing, was used for scar-free healing of diabetic wounds in diabetic rats[160]. Ca-AlgNps have gained great attention in chronic wound healing as they possess a hemostatic property. When applied to wound site, the Ca ions released from alginate lead to the activation of platelets and accelerate hemostasis. The wounds healed scar-free perfectly, especially when mixed at the application site with fresh blood. In another study, a nanocomposite sponge composed of AgNPs as a broad-spectrum antimicrobial agent, hyaluronic acid to provide a moist environment, and chitosan as a hemostatic agent showed a potent antimicrobial property against *E. coli, S. aureus,* MRSA, *P. aeruginosa,* and *K. pneumonia*[134]. These nanocomposite sponges can be used as a potential wound-dressing material for DFU infected with antibiotic-resistant bacteria.

Supplementary material

Supplementary material are available upon request from authors.

Conclusion

MBNPs have been showed an excellent enhancement of diabetic wounds healing. They work on modulating many processes disrupted in diabetic wounds environment. Preclinical in vitro and *in vivo* evaluations of MBNPs demonstrated migration and proliferation of keratinocytes and fibroblasts, angiogenesis, and immune response. In addition, MBNPs balanced the level of inflammatory cells and anti-inflammatory cells and this promoted harmony in wound healing. Furthermore, MBNPs demonstrated a significant reduction of bacteria including resistant strains contaminating diabetic wounds in treatment of infected diabetic animal wounds. Therefore, MBNPs / drug loaded MBNPs are highly recommended to be repurposed in the future as a promising, alternative strategy for management of infected/uninfected diabetic wound. However, several studies are still required to be performed to ascertain their safety profiles for clinical translation.

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Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 13-38.

نشرة العلوم الصيدلية
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

المضاعفات المصاحبة لالتئام الجروح لدى مرضى السكري: هل تتمتع تقنية النانو بأي مزايا علاجية فائقة؟

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يعد تأخير التئام الجروح أحد المضاعفات الرئيسية التي تواجه مرضى السكري في جميع أنحاء العالم. ومن الممكن أن يتبعها عواقب وخيمة مثل القرحة والغرغرينا والبتر ما لم يتم علاجها بشكل صحيح. ان اضطراب مراحل التئام الجروح الاربعة لدى مرضى السكري سببه خلل مرضى بالوظائف الحيوية لأعضاء الجسم؛ وبالتالي، يزداد الوقت اللازم لالتئام الجروح. وبناء عليه، تزداد احتمالية إصابة الجروح بالعدوى الجرثومية لدى مرضى السكري وخاصة الذين يعانون من نقص المناعة مقارنة بالأشخاص الغير مصابين بالمرض. هناك العديد من الاستراتيجيات لعلاج الجروح لدى مرضى السكري مثل: الضمادات، عوامل النمو، الخلايا الجذعية، الأدوية المضادة لمرض السكر والأدوية الخافضة للضغط وما إلى ذلك. ومع ذلك، لا تزال بعض هذه الاستراتيجيات قيد التحقيق للتأكد من سلامتها، في حين أن البعض الآخر يتحلل بيولوجيا بسرعة مما يحد من استخدامه. قد تكون تقنية النانو استراتيجية بديلة جديدة وواعدة لشفاء الجروح لدى مرضى السكري حيث ان الجسيمات النانومترية يمكنها التغلب على عيوب وقيود المستحضرات الطبية المستخدمة حاليًا. بعض الجسيمات النانومترية مثل الجسيمات النانومترية المعدنية (MBNPs) أظهرت نشاطًا ممتازًا مضادً للبكتيريا بما في ذلك السلالات المقاومة والمستوطنة بجروح مرضى السكري، علاوة على ذلك، يمكن لبعض الجسيمات النانومترية المعدنية (MBNPs) تعزيز التئام الجروح بمرضى السكري عن طريق تعديل العديد من الاضطرابات المسجلة بالمراحل الاربعة الخاصة بالتئام الجروح بمرضى السكري. يعرض هذا البحث المرجعي احدث المراجع في هذا المجال ، يناقش المضاعفات المصاحبة لمرضى السكري مع التركيز بشكل خاص على تأخر التئام الجروح، الاعتلال الفسيولوجي لالتئام الجروح لدى مرضى السكري، العلاج المعتمد لالتئام الجروح و السيطرة على عدوى الجروح. بالإضافة إلى ذلك، إلقاء الضوء على تطبيق تقنية النانو لتحسين التئام الجروح، مع الاهتمام بشكل خاص بالجسيمات النانومترية المعدنية (MBNPs) متضمنة آلياتها المضادة للبكتيريا والحواجز التي تمنع تطبيقها اكلينيكيا.