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Engineering nanoparticle therapeutics for impaired wound healing in diabetes.

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Short Review

Engineering Nanoparticle Therapeutics for Impaired Wound Healing in Diabetes

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Teaser: A review focusing on emerging nanotherapeutics capable of addressing the pathophysiology of chronic wounds in diabetes to better manage the impaired healing that current clinical treatments fail to address.

Abstract:
Diabetes mellitus is a chronic disease characterized by increased blood glucose levels, leading to damage of the nerves blood vessels, subsequently manifesting as organ failures, wounds, or ulcerations. Wounds in diabetic patients are further complicated due to reduced cytokine responses, infection, poor vascularization, and delayed healing process. Surface functionalized and bioengineered nanoparticles have recently gained attention as emerging treatment modalities for wound healing in diabetes. Here, we review the emerging therapeutic nanoparticles to treat diabetic wounds and highlight their discrete delivery mechanisms and sites of action. We further critically assess the current challenges of these nanoengineered materials for successful clinical translation and discuss their potential for growth in the clinical marketplace.

Introduction

Wound healing: pathophysiology

Diabetes mellitus is a chronic disease wherein the pancreas does not produce enough insulin, or the body cannot effectively make use of the insulin produced[1]. As a result, a common effect of diabetes is raised blood sugar, otherwise known as hyperglycaemia. Over time, hyperglycaemia may lead to serious damage to nerves and blood vessels. Nerve damage in the feet, along with reduced blood flow, increases the chances of foot ulcers, infection, and impaired wound healing. Non-healing ulceration affects 15-25% of people suffering from diabetes. Furthermore, approximately 85% of diabetes-related ulcerations lead to lower extremity amputation[2]. Diabetic ulcers are resistant to treatment due to the differences in the wound healing process in non-diabetic and diabetic patients (**Fig. 1**).

Wound healing in non-diabetic patients

Wound healing is characterized by hemostasis, followed by an inflammatory phase, proliferative phase, and a maturation phase where collagen-rich scar tissue seals the wound[3]. Hemostasis is the body's attempt to quickly stop bleeding without disrupting normal blood flow[2]. Initially, the blood vessels begin to constrict, and platelet plugs form. Subsequently, in the inflammation phase, white blood cells migrate from the bloodstream, through the tissues to the wound site, where they are able to engulf and digest contaminants[4]. Migration of white blood cells is made possible through the dilation and increased permeability of capillaries. The dilation of the capillaries allows white blood cells to cross the vessel wall to migrate to the wound. Dilated vessels also cause symptoms associated with inflammation, such as swelling and increased heat to the surrounding tissues. Monocytes mature into macrophages and continue to digest bacteria and remaining debris, as well as recruit other cell types for reconstruction.
This is followed by the proliferative phase when granulation tissue forms at the wound site[5]. Granulation tissue secretes chemicals that degrade the existing clot and has fibroblasts which are able to produce collagen to provide strength and structure to the wound. During this phase, a dense network of capillaries is built to provide oxygen and nutrients to the cells, as well as absorb or carry away any remaining debris. Re-epithelialization also occurs when the wound is covered in new tissue, and keratinocytes differentiate and proliferate to produce an epidermal layer to superficially cover the area of the wound[6]. Lastly, the maturation phase consists of replacement of the granulation tissue with type 1 collagen. As the wound continues to contract, the wound is sealed with collagen-rich scar tissue. Clinically, wounds that fail to heal within a 4-6 week timeframe may be considered chronic[7].

Wound healing in diabetic patients

Wound healing in diabetic patients undergoes hemostasis but is impaired in the inflammatory phase[8]. Inflammatory macrophages persist at the site of injury for prolonged periods of times[2]. Present macrophages produce an increased amount of pro-inflammatory cytokines and high levels of reactive oxygen species (ROS), causing persistent inflammation[2]. This results in abnormal apoptosis of fibroblasts and keratinocytes, decreased angiogenic response, reduced growth factor recruitment, and reduced collagen accumulation[9]. Common cytokine cascades normally resulting in proliferative factors is disturbed by these macrophages due to inefficient phagocytosis and apoptosis[10]. Further, a phenotypic change in the fibroblasts, causing their differentiation into myofibroblasts, leads to reduced mechanical tensions of the extracellular matrix (ECM), resulting in inefficient collagen deposition and wound closure[11]. Abnormal ECM results from decreased collagen recruitment and more disorganized collagen deposition. Increased protease activity enhances degradation of the ECM, growth factors, and collagen deposition, all of which are crucial for effective wound healing have further been demonstrated in diabetes[12].

Current clinical treatment of wounds in diabetics

According to the World Health Organizations data in 2014, up to 5% of people with diabetes in developed countries have diabetic foot ulcers resulting from a chronic wound[13]. Current methods of treatments rely on off-loading and applying dressings to the wound site[14]. However, most therapies aim to reduce infection and relieve pain, but do not improve the wound healing process. Some topical commercial applications, such as Becaplermin, include growth factors naturally produced by the body to induce cell proliferation to accelerate wound healing[15]. However, studies regarding wound healing in diabetic patients with this treatment have been limited. With the increasing prevalence of diabetes globally[13], we must address the clinical need for promoting wound healing in diabetic patients that are superior to the current clinical approaches. The favourable advantages of nanoparticles that have been demonstrated in other biomedical fields allow us to explore the possibility of using these nanoparticles to accelerate the wound healing process[16,17]. Here, we discuss various approaches to promote wound healing in diabetic models through the use of nanoparticles as either the therapeutic device or as a drug delivery vehicle. All approaches discussed in this review are pre-clinical models of wound healing and have yet to be clinically translated to human subjects. A comprehensive review of drug and drug vehicle development encompasses the difficulties and rigorous processes in the translation of the material from bench-side studies to bedside[18].

Properties of an ideal wound healing system

The success of a therapeutic treatment for wounds in diabetic patients must essentially address the pathogenesis of the wound healing process. As the wound healing process in diabetic patients is impaired in the inflammatory phase, novel therapies address the persistent inflammatory cytokines, decreased angiogenesis, and the lack of growth factor recruitment as well as impaired collagen deposition[2,9,11]. A few ways to improve the wound healing process in diabetic patients are outlined in **Fig. 2**. Briefly**,** the recruitment of anti-inflammatory cytokines that are produced by activated by M2 macrophages[19] would decrease the persistent presence of pro-inflammatory cytokines such as tumour necrosis factor (TNF-α) and interleukin 6 (IL-6)[20], ultimately normalizing the ratio of pro-inflammatory cytokines to anti-inflammatory signals, reducing apoptosis of fibroblasts and keratinocytes and potentially leading to increased angiogenesis[21]. Increased fibroblasts would also aid in increasing the amount of myofibroblasts which will help retain the mechanical tension of the ECM, and in turn, increase growth factor recruitment and collagen deposition[22].
Another way to improve the wound healing process in diabetic models can include therapies in decreasing ROS[23]. Although ROS is beneficial in the wound healing process, the excess ROS levels and ongoing oxidative stress in chronic wounds results in protein modifications and DNA damage causing apoptosis and acceleration and persistence of inflammation, both of which are detrimental to the wound healing process. Decreasing ROS levels includes increasing nitric oxide (NO) levels and decreased superoxide levels. Thrombospondin-1 (TSP-1) is an antiangiogenic adipokine that is expressed in animal models susceptible to diabetes[24]. The detrimental effect of TSP-1 on endothelial cell function is negatively correlated to NO regeneration in endothelial cells. Inhibiting ROS overproduction can result in an increase in NO bioavailability. Hence, a decrease in TSP-1, which in turn, promotes angiogenesis.

Design strategies and associated advantages of nanoparticles

There are many design challenges when considering therapies of wound healing in diabetes. These design strategies include optimizing various properties including size, surface properties, shape, surface charge, biocompatibility, biodegradability, and controlled release for the therapy using topical delivery systems to improve wound healing directly at the site. The use of nanoparticles sized between 1-100 nm is crucial for the uptake of the nanoparticles by cells. Due to their small size, cells can easily take up nanoparticles by one of the three primary endocytosis mechanisms in which they are internalized[25]. The ability of nanoparticles to penetrate the tissue system allows for the facilitation of easy uptake of drugs by cells if the nanoparticle is acting as a vehicle. It has also been shown that uptake of nanostructures by cells is much higher than that of particles sized between 1 and 10 μm[26]. Moreover, nanoparticles must be considerably lipophilic in order to be able to interact with hydrophobic cell membranes. Nanoparticles have also been shown to persist in the circulatory system for a prolonged period of time, causing fewer plasma fluctuations with reduced adverse effects before being cleared through the urinary tract due to their small size[27]. Therefore, the use of nanoparticles to provide a therapeutic effect or to act as drug vehicles is ideal for a potential wound healing strategy.

Engineered nanoparticles and nanocomposites for wound healing in diabetes

Nanoparticles can be engineered such that they themselves are the therapeutic device, or they can be engineered such that they act as drug delivery vehicles for the therapeutic molecules to accelerate the wound healing process. The following are five approaches that have been applied to promote wound healing in diabetic models (**Fig. 2**). A summary of the research articles discussed in each of the following subsections describing the five approaches is presented in **Table 1**.

Inorganic nanoparticles

Inorganic nanoparticles have been used to treat burns, chronic ulcers, and wounds in diabetic patients for their anti-infective and anti-inflammatory effects[28–31]. A common problem amongst diabetic patients with untreated and unhealed wounds is the increased risk of infection. To address the need for antimicrobial compounds, inorganic nanoparticles, including an array of metal nanoparticles, have been studied. One major advantage of silver nanoparticles (AgNPs) is their intrinsic resistance against bacteria and bacteria that produce biofilms[32]. It is important to note that bacteria that produce biofilms are commonly found in chronic wounds and they tend to enclose themselves in a self-produced extracellular polymeric substance that is resistant to conventional antibiotics[33–36]. *Kalishwaralal et al.* tested biologically synthesized AgNPs on biofilm-producing bacteria, demonstrating that AgNPs inhibited both the growth of bacteria as well as their ability to produce exopolysaccharides, the structural scaffold used to create the biofilm[37]. This intrinsic ability of AgNPs to inhibit the growth of bacteria demonstrates its ability as drug delivery vehicle for wound healing in diabetes. Another drug vehicle composed of inorganic materials is zinc oxide nanoparticles (ZnO NPs), which also show antibacterial properties[38]. Further, Zn can be used for treating diabetic ulcers due to its role in the function of more than 300 enzymes that are crucial to maintaining metabolic hemostasis in the body. To show the feasibility of ZnO NPs in the use of wound healing in diabetic patients, *Kaushik et al.* tested fibroblast growth and the antimicrobial potential of these nanoparticles and showed that upon exposure to ZnO NPs, there was a significant increase in fibroblast cells[39]. It was also shown that when exposed to pathogens, ZnO NPs displayed antimicrobial activity befitting for treatment of wounds in diabetic patients. Inorganic nanoparticles in the form of metal organic frameworks (MOFs) have also been used to deliver inorganic ions for slow and controlled release of the therapeutic ion[40,41]. *Xiao et al.* used copper-based MOFS to safely deliver copper ions in a controlled manner to avoid toxicity issues associated with high concentrations of copper **(Fig 3A)**. These copper-based MOFs were shown to release copper ions to improve angiogenesis, promote collagen deposition, and decrease wound closure times in diabetic mice models **(Fig 3B, C)**[42]

Liposome nanoparticles

Drug delivery systems consist of the administration and delivery of a pharmaceutical compound so as to achieve an enhanced therapeutic effect in a specific area of the body. Liposomes have been widely used as drug delivery vehicles across various biomedical applications[43–45]. Although the use of liposomes has its limitation in terms of batch-to-batch consistency and drug leakage, liposomes are promising nanoparticles due to their nontoxic, biodegradable, and biocompatible behaviour. Current clinical developments in treating wounds in diabetic patients involve addressing the risk of infection. Studies have shown that liposomes entrapping a bacteriophage cocktail to address multiple bacterial infections is more effective than the use of a free bacteriophage cocktail **(Fig 3D)**[46]. Both *in vitro* and *in vivo* studies suggested that liposomal entrapment of the cocktail led to better bacteriophage persistence at the wound site compared to free bacteriophage as determined by a higher phage titer. Recently, liposomes have also been developed not only to carry cargo in the treatment of wounds in diabetic patients, but also to act as antigen presenters. *Kaymakcalan et al.* developed α-gal-presenting liposomes to activate naturally occurring anti-Gal antibodies in humans[47]. It was found that when topically applying these liposomes, macrophages were recruited, pro-healing growth factors and cytokines were produced, and wound closure was accelerated both in normal wound and burn wound models *in vivo*. This application shows the potential of using antigen-presenting liposomes for topical wound healing treatments in diabetic patients.

Dendrimer nanoparticles

Dendrimers are a class of globular molecules artificially designed with a large arrangement of a variety of functional groups[48]. This gives dendrimers the potential to be promising nanocarriers for a large number of therapeutic agents due to interactions via hydrogen bonding, lipophilicity, and charge interactions.
Deng et al. took advantage of the branches of dendrimers for amplification of function, biocompatibility, and water-solubility[49]. Fibronectin-like peptides were synthesized using a dendrimer-based strategy to mimic the ability of fibronectin in facilitating the healing of skin wounds through the promotion of keratinocytes **(Fig. 3E)**. It was found that the use of a peptide sequence derived from the cell binding site of fibronectin as well as derivatives of this sequence stimulates re-epithelialization and contraction of dermal wounds *in vivo.* This approach is particularly advantageous for a diabetic wound because diabetic wounds show particularly low levels of fibronectin relative to non-diabetic wounds[21]. It was concluded that a large role in the efficacy of this treatment was linked to dendrimer branching and its ability to amplify the function of the peptide sequences.
In another study, the effect of "naked" dendrimers was studied[50]. Here, polyamidoamine (PAMAM) dendrimers were chronically administered to diabetic mice over the course of 4 weeks. After daily administration of these dendrimers, it was found that the dendrimers inhibited the epidermal growth factor receptor (EGFR)-ERK1/2-Rho kinase (ROCK) pathway, a pathway known to be critical in the development of diabetic vascular complications. Further, it has been reported that cationic PAMAM dendrimers can also act as glucose scavenging agents, meaning they scavenge excess glucose in the body—this could aid in the decreased probability of further inducing a chronic wound[51]. Taken together, dendritic nanoparticles can be used in a variety of ways to both treat and prevent chronic wounds.

Protein nanoparticles

There are a wide variety of protein nanoparticles, with each protein having distinctly different behaviours[17,52,53]. Some proteins can self-assemble into their nanoparticle form, while others must be physically or chemically conjugated to achieve such a structure. Many proteins used as nanoparticles can be modified in various ways, including both physical and chemical methods, to make them more suitable for their biomedical application[54–56]. Protein nanoparticles can be especially useful in delivering therapeutic molecules for wound healing in diabetic patients due to their biocompatibility and their ability to be easily taken up by cells. *Yeboah et al.* were able to develop a recombinant fusion protein composed of stromal cell-derived growth factor-1 (SDF1) and an elastin-like peptide[57] (**Fig 4A**). Upon expression, this protein is able to self-assemble into nanoparticles and bind to cell receptors to promote neovascularization and faster re-epithelization compared to free SDF1. *In vivo,* the SDF1-ELP nanoparticles significantly accelerated the wound healing process (fully healed by Day 28 post induced wound) compared to free SDF1, free ELP, and generic fibrin gels (fully healed by Day 42 post induced wound) (**Fig. 4B**). Similarly, *Gao et al.* also took advantage of recombinant proteins to express keratin nanoparticles with wound healing potential[58]. Keratin nanoparticles were shown to have significantly increased cell viability and migration *in vitro* and promoted epithelialization, vascularization, collagen deposition, and remodeling in *in vivo* non-diabetic wound healing models. This shows the potential of using keratin nanoparticles as a possible treatment for wound healing in diabetic patients because of their ability be taken up by cells topically at the wounded site. Although the protein nanoparticles have been widely used in biomedical applications for bioimaging and cancer therapy, they have yet to be taken advantage of in applications for wounds and ulcers in diabetic patients.

Polymeric nanoparticles

Polymeric nanoparticles have drawn increasing attention in biomedical and bioengineering fields for their various advantages including biocompatibility, biodegradability, and nontoxicity[59,60]. When conjugated with polymeric systems, drugs are protected from degradation by proteases present in a wound environment, allowing for controlled and sustained release[61]. Some of the most common polymeric nanoparticles used include poly lactic-co-glycolic acid (PLGA), alginate, gelatin, chitosan, and polyethylene glycol (PEG) as they have been designated as "generally recognized as safe" (GRAS) polymers[62].
For example, PLGA/gelatin nanofibrous mat scaffolds to sustain the release of Liraglutide, a receptor agonist that has been reported to promote angiogenesis in endothelial cells[63]. The use of PLGA allowed for a naturally degrading drug delivery vehicle and has been reported to stimulate cell proliferation which could aid in the wound healing process. Liraglutide was incorporated into the PLGA/gelatin system through a cross-linking integration method, which resulted in increased pore size, hydrophilicity, and elasticity of the nanofibrous mats. This allowed for

improved healing efficiency *in vivo* as characterized by shortened wound closure time, increased angiogenesis, and increased collagen deposition. In another approach, PLGA-polyethylenimine (PEI) nanoparticles were used to release nitric oxide at the wound site in a non-diabetic wound healing model[64] (**Fig. 4C**). NO is a small gaseous mediator involved in many physiological processes and therefore, nanomaterial carriers for NO delivery need to have high loading capacity and extended release times[65]. After thorough investigation of NO carriers, the PLGA-PEI system was found to prolong NO release over the course of 6 days without any burst release. Although this approach has been proven in a non-diabetic model, its novel approach shows great potential in diabetic wound healing model. This PLGA-PEI system was also found to have antibacterial efficacy against methicillin-resistant *Staphylococus aureus* (MRSA) through the use of the polymeric vehicle, and wound healing activity through the decrease of reactive oxygen species (**Fig 4D**). Taken together, the therapeutic effects of the NO/PLGA-PEI nanoparticles was able to accelerate the wound healing process in comparison to untreated groups (**Fig. 4E**).
Chitosan is another widely used polymeric nanoparticle as a drug delivery vehicle. One approach involved use of drug-loaded chitosan nanoparticles to inhibit pro-inflammatory macrophages in order to accelerate wound healing[66]. Chitosan nanoparticles were used to deliver an otherwise unsustainable drug through the use of ionic crosslinking. The use of chitosan allowed for a simple preparation method of a stable and biocompatible drug vehicle that provides versatile routes of administration, such that this system can be used for a wide variety of wound healing applications.

Concluding remarks and future directions

The need for effective and safe strategies to treat wounds and ulcers in diabetic patients is pertinent. Non-healed wounds arise from a decreased angiogenic response, reduced growth factor recruitment, and collagen accumulation. Therefore, we must work towards an efficacious solution to manufacture pro-angiogenic and anti-inflammatory particles that will help accelerate wound healing into phases through increased growth factor recruitment and collagen accumulation. Among the various approaches that are being investigated, designed polymeric and non-polymeric nanoparticles have shown great promise and encouraging results have been obtained both *in vitro* and *in vivo* [11,67].
However, the nanoparticles outlined in this review have several challenges and limitations. These wound healing therapies have been produced in laboratory scale. The large-scale manufacturing of these nanoparticles may be problematic due to their often complex synthesis processes involving multiple steps, as well as decreased yield due to purification after each synthesis step[68]. Further, the large-scale syntheses of the outlined nanoparticles can become costly and laborious. Lastly, these nanoparticles have been tested *in vitro* and *in vivo* in various animal models and may not be entirely replicated in humans, in which the dosage and frequency of administration would have to be revaluated and optimised.
Taking into consideration the advantages of the materials into account, we discuss some of the potential applications and future perspectives of engineered nanoparticles for wound healing in diabetic patients. A summary of the advantages and limitations of the discussed materials has been presented in **Table 2**. Because bacterial infections pose a large problem in wounds and ulcers in diabetic patients, the risk of amputation is increased by the rate of bacterial infection occurring at the non-healed site. To address this problem in a clinical setting, AgNPs were used in numerous clinical trials in the therapy of various wounds, including burns and diabetic ulcers. Currently, there are some commercially available dressings containing AgNPs. One example of this is Acticoat©--wound dressings containing AgNPs[69]. This allowed for a flexible and absorbent coating that proved to be an efficient and effective barrier to bacterial penetration. Wound healing, infection reduction at the site of the wound, and pain reduction were all observed in most tested patients. Here, the use of the dressing allowed for sustained release of the AgNPs for at least 7 days, minimizing the need to change the dressing, and therefore increasing patient compliance[70].
Recent approaches to treating wounds in diabetic patients involve the use of microRNAs (miRNA). It has been shown that miRNA-146a and miRNA-200b play important roles in the regulation of growth factors and ECM protein production on a molecular level[71]. Antagonism or promotion of certain miRNAs can result in increased growth factors and fibronectin production, induce cell proliferation and migration, and inhibit apoptosis of endothelial cells[72,73]. The combination of miRNAs and nanoparticles can promote cellular targeting and uptake, increase circulation time, and decrease off-target effects[74]. The use of nanoparticles as nanocarriers for miRNAs has gained excitement in recent years and continues to be explored for the use of wound healing in diabetic patients.
Other studies have worked on developing nanocomposite scaffolds for sustained treatment of wounds in diabetic patients. For example, the advantageous properties of liposomes discussed earlier have been used in combination with a variety of hydrogels to produce topical liposome-hydrogel nanocomposites in the delivery of various cargo to ensure more sustained release[75,76]. One example of this is the use of electrospun PLGA-liposome fibers for the simultaneous delivery of microRNA and growth factors for the promotion of vascular smooth muscles *in vitro* and

in vivo[77]. In a recent study, a nanocomposite scaffold was synthesized of a main network of polyethylene glycol diacrylate (PEGDA) to form the scaffold, with a peripheral network formed between bioactive glass nanoparticles that contain copper and sodium alginate[78]. Bioactive glass has recently been shown to have a variety of biological properties including osteogenic ability, bone/soft tissue bonding activity, and promoting angiogenesis. Paired with copper, an element essential for the wound healing process, there is potential in observing increased angiogenesis, expressing and stabilization of skin proteins, and antibacterial activity. However, non-physiological concentrations of copper ions might increase the risk of ion poisoning, and therefore, the controlled release of Cu^{2+} is pertinent. Therefore, the use of a PEGDA scaffold would greatly enhance this system to create a self-healing antibacterial nanocomposite dressing in order to enhance the wound healing process in diabetic patients. As seen from the above examples, two or more of these approaches can be combined to exploit the advantages of various materials to develop superior delivery systems.

Other developing technologies in this area include conductive hydrogels[79]. Conductive hydrogels have recently become more widely used in healthcare recording electrodes, biomedical patches, implantable bio-devices, etc. These hydrogels are stimulated by external signals, which are converted to bioelectric stimulation after reaching the skin to achieve the purpose of the treatment. *Zhang et al.* developed a conductive hydrogel based on polyvinyl alcohol and chitosan to enable the hydrogels to perceive temperature and strain[80]. The activation of these hydrogels resulted in increased angiogenesis, collagen deposition, and inhibition of bacterial growth. Researchers are continuing to develop these sensory hydrogels to respond to wound environmental pH, ROS levels, and glucose concentrations. In the future, these hydrogels might also be studied as potential drug delivery systems that can encapsulate nanoparticles for the further promotion of wound healing in diabetic patients.

Lastly, a layer-by-layer (LBL) self-assembly technique is being studied for a wide range of biomedical technologies for delivery of a range of material surfaces[81]. These composite materials have good stability, mechanical properties, hydrophilicity, and most important, sustained drug release. This is a technique wherein charged polyelectrolytes are assembled from aqueous solutions to form nanostructured films to deliver a broad range of therapeutic molecules. This technique has been studied in relation to wound healing in diabetic patients to deliver materials that have the potential to induce varying therapeutic effects to accelerate wound healing in a diabetic model.

As mentioned previously, the risk of diabetes and wounds/ulcers in diabetic patients is increased every year. The strategies reviewed here can be used to produce safe commercial therapies either as free nanoparticles, nanoparticles as drug vehicles, or as nanocomposites in conjunction with dressings, hydrogels, or "smart" sensory-responsive systems. While some of the examples mentioned in this review have been used for non-diabetic wound healing models, they can also be applied to treat diabetic wounds. This is because the mechanism of uptake and design strategies of these nanoparticles allows them to have similar physiological effects in diabetic and non-diabetic wounds. We anticipate that these nanoparticles will exhibit superior therapeutic effects and accelerate the wound healing process in diabetic patients, lowering the risk of potential amputation.

Conflicts of interest

There are no conflicts to declare.

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References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nat Rev Endocrinol 2011 84*. 2011;8(4):228-236. doi:10.1038/nrendo.2011.183
2. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*. 2007;117(5):1219-1222. doi:10.1172/JCI32169

3. Guo S, DiPietro LA. Factors Affecting Wound Healing. *J Dent Res*. 2010;89(3):219. doi:10.1177/0022034509359125
4. A K, N S, S H, H Y, K T. Wound healing in mammals and amphibians: toward limb regeneration in mammals. *Curr Top Microbiol Immunol*. 2013;367:33-49. doi:10.1007/82_2012_305
5. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. *https://doi.org/101152/physrev000672017*. 2018;99(1):665-706. doi:10.1152/PHYSREV.00067.2017
6. None R, K S, MS G, RR I. Wound re-epithelialization: modulating keratinocyte migration in wound healing. *Front Biosci*. 2007;12(8):2849-2868. doi:10.2741/2277
7. Wallace HA, Basehore BM, Zito PM. Wound Healing Phases. *StatPearls*. Published online September 29, 2020. Accessed July 6, 2021. https://www.ncbi.nlm.nih.gov/books/NBK470443/
8. Kharroubi AT. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. Published online 2015. doi:10.4239/wjd.v6.i6.850
9. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet*. 2005;366(9498):1736-1743. doi:10.1016/S0140-6736(05)67700-8
10. Duque GA, Descoteaux A. Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. *Front Immunol*. 2014;5(OCT). doi:10.3389/FIMMU.2014.00491
11. Ezhilarasu H, Vishalli D, Dheen ST, Bay BH, Kumar Srinivasan D. Nanoparticle-based therapeutic approach for diabetic wound healing. *Nanomaterials*. 2020;10(6):1-29. doi:10.3390/nano10061234
12. McCarty SM, Percival SL. Proteases and Delayed Wound Healing. *Adv Wound Care*. 2013;2(8):438. doi:10.1089/WOUND.2012.0370
13. Diabetes. Accessed July 6, 2021. https://www.who.int/news-room/fact-sheets/detail/diabetes
14. Perez-Favila A, Martinez-Fierro ML, Rodriguez-Lazalde JG, et al. Current therapeutic strategies in diabetic foot ulcers. *Med*. Published online 2019. doi:10.3390/medicina55110714
15. Kruse I, Edelman S. Evaluation and Treatment of Diabetic Foot Ulcers. *Clin Diabetes*. 2006;24(2):91-93. doi:10.2337/DIACLIN.24.2.91
16. Giner-Casares JJ, Henriksen-Lacey M, Coronado-Puchau M, Liz-Marzán LM. Inorganic nanoparticles for biomedicine: where materials scientists meet medical research. *Mater Today*. 2016;19(1):19-28. doi:10.1016/J.MATTOD.2015.07.004
17. Lohcharoenkal W, Wang L, Chen YC, Rojanasakul Y. Protein nanoparticles as drug delivery carriers for cancer therapy. *Biomed Res Int*. 2014;2014. doi:10.1155/2014/180549
18. Van Norman GA. Drugs, Devices, and the FDA: Part 1: An Overview of Approval Processes for Drugs. *JACC Basic to Transl Sci*. 2016;1(3):170-179. doi:10.1016/j.jacbts.2016.03.002
19. Boniakowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-Mediated Inflammation in Normal and Diabetic Wound Healing. *J Immunol*. 2017;199(1):17-24. doi:10.4049/JIMMUNOL.1700223
20. denDekker AD, Davis FM, Joshi AD, et al. TNF-α regulates diabetic macrophage function through the histone acetyltransferase MOF. *JCI Insight*. 2020;5(5). doi:10.1172/JCI.INSIGHT.132306
21. Huang Y, Kyriakides TR. The role of extracellular matrix in the pathophysiology of diabetic wounds. *Matrix Biol Plus*. 2020;6-7:100037. doi:10.1016/J.MBPLUS.2020.100037
22. Maione AG, Smith A, Kashpur O, et al. Altered ECM deposition by diabetic foot ulcer-derived fibroblasts implicates fibronectin in chronic wound repair. *Wound Repair Regen*. 2016;24(4):630-643. doi:10.1111/WRR.12437
23. Dunnill C, Patton T, Brennan J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int Wound J*. 2017;14(1):89-96. doi:10.1111/IWJ.12557
24. MS B. Diabetes Impairs Angiogenesis and Induces Endothelial Cell Senescence by Up-Regulating

Thrombospondin-CD47-Dependent Signaling. *Int J Mol Sci*. 2019;20(3). doi:10.3390/IJMS20030673
25. Foroozandeh P, Aziz AA. Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles. *Nanoscale Res Lett 2018 131*. 2018;13(1):1-12. doi:10.1186/S11671-018-2728-6
26. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology 2018 161*. 2018;16(1):1-33. doi:10.1186/S12951-018-0392-8
27. Mudshinge SR, Deore AB, Patil S, Bhalgat CM. Nanoparticles: Emerging carriers for drug delivery. *Saudi Pharm J*. 2011;19(3):129-141. doi:10.1016/J.JSPS.2011.04.001
28. Yang J, Wang K, Yu DG, Yang Y, Bligh SWA, Williams GR. Electrospun Janus nanofibers loaded with a drug and inorganic nanoparticles as an effective antibacterial wound dressing. *Mater Sci Eng C*. 2020;111:110805. doi:10.1016/J.MSEC.2020.110805
29. Kang T, Kim YG, Kim D, Hyeon T. Inorganic nanoparticles with enzyme-mimetic activities for biomedical applications. *Coord Chem Rev*. 2020;403:213092. doi:10.1016/J.CCR.2019.213092
30. Jafari A, Hassanajili S, Karimi MB, Emami A, Ghaffari F, Azarpira N. Effect of organic/inorganic nanoparticles on performance of polyurethane nanocomposites for potential wound dressing applications. *J Mech Behav Biomed Mater*. 2018;88:395-405. doi:10.1016/J.JMBBM.2018.09.001
31. Wang X, Chang J, Wu C. Bioactive inorganic/organic nanocomposites for wound healing. *Appl Mater Today*. 2018;11:308-319. doi:10.1016/J.APMT.2018.03.001
32. Hussain A, Alajmi MF, Khan MA, et al. Biosynthesized Silver Nanoparticle (AgNP) From Pandanus odorifer Leaf Extract Exhibits Anti-metastasis and Anti-biofilm Potentials. *Front Microbiol*. 2019;10(FEB):8. doi:10.3389/FMICB.2019.00008
33. Dowd RDWDDRSE. Biofilms and chronic wound inflammation. *http://dx.doi.org/1012968/jowc200817830796*. 2013;17(8):333-341. doi:10.12968/JOWC.2008.17.8.30796
34. Leaper D, Assadian O, Edmiston CE. Approach to chronic wound infections. *Br J Dermatol*. 2015;173(2):351-358. doi:10.1111/BJD.13677
35. Sun Y, Dowd SE, Smith E, Rhoads DD, Wolcott RD. In vitro multispecies Lubbock chronic wound biofilm model. *Wound Repair Regen*. 2008;16(6):805-813. doi:10.1111/J.1524-475X.2008.00434.X
36. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. *Wound Repair Regen*. 2008;16(1):37-44. doi:10.1111/J.1524-475X.2007.00321.X
37. Velázquez-Velázquez JL, Santos-Flores A, Araujo-Meléndez J, et al. Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles. *Mater Sci Eng C*. 2015;49:604-611. doi:10.1016/J.MSEC.2014.12.084
38. SE J, HE J. Synthesis, Characterization, and Three-Dimensional Structure Generation of Zinc Oxide-Based Nanomedicine for Biomedical Applications. *Pharmaceutics*. 2019;11(11). doi:10.3390/PHARMACEUTICS11110575
39. Kaushik M, Niranjan R, Thangam R, et al. Investigations on the antimicrobial activity and wound healing potential of ZnO nanoparticles. *Appl Surf Sci*. 2019;479:1169-1177. doi:10.1016/J.APSUSC.2019.02.189
40. Chen S, Lu J, You T, Sun D. Metal-organic frameworks for improving wound healing. *Coord Chem Rev*. 2021;439(15):231929. doi:10.1016/j.ccr.2021.213929
41. Fu LQ, Chen XY, Cai MH, Tao XH, Fan Y Bin, Mou XZ. Surface Engineered Metal-Organic Frameworks (MOFs) Based Novel Hybrid Systems for Effective Wound Healing: A Review of Recent Developments. *Front Bioeng Biotechnol*. 2020;8:576348. doi:10.3389/fbioe.2020.576348
42. Xiao J, Zhu Y, Huddleston S, et al. Copper Metal-Organic Framework Nanoparticles Stabilized with Folic Acid Improve Wound Healing in Diabetes. *ACS Nano*. 2018;12(2):1023-1032. doi:10.1021/acsnano.7b01850

43. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and challenges of liposome assisted drug delivery. *Front Pharmacol*. 2015;6:286. doi:10.3389/fphar.2015.00286
44. Marasini N, Ghaffar KA, Skwarczynski M, Toth I. Liposomes as a Vaccine Delivery System. *Micro-Nanotechnol Vaccine Dev*. Published online January 1, 2017:221-239. doi:10.1016/B978-0-323-39981-4.00012-9
45. M S, D AS, T S, F S. Theranostic Liposome-Nanoparticle Hybrids for Drug Delivery and Bioimaging. *Int J Mol Sci*. 2017;18(7). doi:10.3390/IJMS18071415
46. Chhibber S, Kaur J, Kaur S. Liposome Entrapment of Bacteriophages Improves Wound Healing in a Diabetic Mouse MRSA Infection. *Front Microbiol*. 2018;9(MAR). doi:10.3389/FMICB.2018.00561
47. OE K, A A, JW G, et al. Topical α-gal nanoparticles accelerate diabetic wound healing. *Exp Dermatol*. 2020;29(4):404-413. doi:10.1111/EXD.14084
48. Tetteh-Quarshie S, Blough ER, Jones CB. Exploring Dendrimer Nanoparticles for Chronic Wound Healing. *Front Med Technol*. 2021;3:19. doi:10.3389/FMEDT.2021.661421
49. Deng X, Li X, Chen W, Zhao T, Huang W, Qian H. Design, synthesis and biological evaluation of peptide dendrimers with wound healing promoting activity. *Med Chem Res 2016 263*. 2017;26(3):580-586. doi:10.1007/S00044-016-1777-6
50. D Z, Q H. Encapsulation of Astragaloside with Matrix Metalloproteinase-2-Responsive Hyaluronic Acid End-Conjugated Polyamidoamine Dendrimers Improves Wound Healing in Diabetes. *J Biomed Nanotechnol*. 2020;16(8):1229-1240. doi:10.1166/JBN.2020.2971
51. Karolczak K, Rozalska S, Wieczorek M, Labieniec-Watala M, Watala C. Poly(amido)amine dendrimers generation 4.0 (PAMAM G4) reduce blood hyperglycaemia and restore impaired blood–brain barrier permeability in streptozotocin diabetes in rats. *Int J Pharm*. 2012;436(1-2):508-518. doi:10.1016/J.IJPHARM.2012.06.033
52. Hong S, Choi DW, Kim HN, Gwon Park C, Lee W, Park HH. Protein-Based Nanoparticles as Drug Delivery Systems. *Pharmaceutics*. 2020;12:604. doi:10.3390/pharmaceutics12070604
53. Jain A, Singh SK, Arya SK, Kundu SC, Kapoor S. Protein Nanoparticles: Promising Platforms for Drug Delivery Applications. *ACS Biomater Sci Eng*. 2018;4(12):3939-3961. doi:10.1021/acsbiomaterials.8b01098
54. Yu SM, Li Y, Kim D. Collagen mimetic peptides: Progress towards functional applications. *Soft Matter*. 2011;7(18):7927-7938. doi:10.1039/c1sm05329a
55. Luo T, David MA, Dunshee LC, et al. Thermoresponsive Elastin-b-Collagen-Like Peptide Bioconjugate Nanovesicles for Targeted Drug Delivery to Collagen-Containing Matrices. *Biomacromolecules*. 2017;18(8):2539-2551. doi:10.1021/acs.biomac.7b00686
56. Bruni R, Possenti P, Bordignon C, et al. Ultrasmall polymeric nanocarriers for drug delivery to podocytes in kidney glomerulus. *J Control Release*. 2017;255:94-107. doi:10.1016/j.jconrel.2017.04.005
57. Yeboah A, Cohen RI, Faulknor R, Schloss R, Yarmush ML, Berthiaume F. The development and characterization of SDF1α-elastin-like-peptide nanoparticles for wound healing. *J Control Release*. 2016;232:238-247. doi:10.1016/j.jconrel.2016.04.020
58. Gao F, Li W, Deng J, et al. Recombinant Human Hair Keratin Nanoparticles Accelerate Dermal Wound Healing. *ACS Appl Mater Interfaces*. 2019;11(20):18681-18690. doi:10.1021/ACSAMI.9B01725
59. Mahmoud Elsabahy, L. Wooley K. Design of polymeric nanoparticles for biomedical delivery applications. *Chem Soc Rev*. 2012;41(7):2545-2561. doi:10.1039/C2CS15327K
60. Li Y, Dong H, Wang K, Shi D, Zhang X, Zhuo R. Stimulus-responsive polymeric nanoparticles for biomedical applications. *Sci China Chem 2010 533*. 2010;53(3):447-457. doi:10.1007/S11426-010-0101-4
61. Saghazadeh S, Rinoldi C, Schot M, et al. Drug Delivery Systems and Materials for Wound Healing

Applications. *Adv Drug Deliv Rev*. 2018;127:138. doi:10.1016/J.ADDR.2018.04.008
62. FDA. FDA Executive Summary: Classification of Wound Dressings Combined with Drugs. In: *Meeting of the General and Plastic Surgery Devices Advisory Panel*. ; 2016.
63. Muyu Yu, Jinghuan Huang, Tonghe Zhu, et al. Liraglutide-loaded PLGA/gelatin electrospun nanofibrous mats promote angiogenesis to accelerate diabetic wound healing via the modulation of miR-29b-3p. *Biomater Sci*. 2020;8(15):4225-4238. doi:10.1039/D0BM00442A
64. Nurhasni H, Cao J, Choi M, et al. Nitric oxide-releasing poly(lactic-co-glycolic acid)-polyethylenimine nanoparticles for prolonged nitric oxide release, antibacterial efficacy, and in vivo wound healing activity. *Int J Nanomedicine*. 2015;10:3065. doi:10.2147/IJN.S82199
65. Choi HW, Kim J, Kim J, et al. Light-Induced Acid Generation on a Gatekeeper for Smart Nitric Oxide Delivery. *ACS Nano*. 2016;10(4):4199-4208. doi:10.1021/ACSNANO.5B07483
66. Sun M, Deng Z, Shi F, et al. Rebamipide-loaded chitosan nanoparticles accelerate prostatic wound healing by inhibiting M1 macrophage-mediated inflammation via the NF-κB signaling pathway. *Biomater Sci*. 2020;8(3):912-925. doi:10.1039/C9BM01512D
67. Kasiewicz LN, Whitehead KA. Recent advances in biomaterials for the treatment of diabetic foot ulcers. *Biomater Sci*. 2017;5(10):1962-1975. doi:10.1039/C7BM00264E
68. Vega-Vásquez P, Mosier NS, Irudayaraj J. Nanoscale Drug Delivery Systems: From Medicine to Agriculture. *Front Bioeng Biotechnol*. 2020;8:79. doi:10.3389/fbioe.2020.00079
69. Boroumand Z, Golmakani N, Boroumand S. Clinical Trials on Silver nanoparticles for wound healing (review). *Nanomed J*. 2018;5(4):186-191. doi:10.22038/nmj.2018.05.00001
70. K D, V E-J. The role of Acticoat with nanocrystalline silver in the management of burns. *Burns*. 2004;30 Suppl 1(SUPPL. 1). doi:10.1016/S0305-4179(04)90000-9
71. Pharmacology J of P and. miRNA-146a and miRNA-200b Antagomirs Accelerate Wound Healing through the Regulation of VEGF and Fibronectin. Accessed July 30, 2021. https://www.academia.edu/38832246/miRNA_146a_and_miRNA_200b_Antagomirs_Accelerate_Wound_Healing_through_the_Regulation_of_VEGF_and_Fibronectin
72. Feng B, Chen S, McArthur K, et al. miR-146a–Mediated Extracellular Matrix Protein Production in Chronic Diabetes Complications. *Diabetes*. 2011;60(11):2975-2984. doi:10.2337/DB11-0478
73. Waters R, Subham S, Pacelli S, Modaresi S, Chakravarti AR, Paul A. Development of MicroRNA-146a-Enriched Stem Cell Secretome for Wound-Healing Applications. *Mol Pharm*. 2019;16(10):4302. doi:10.1021/ACS.MOLPHARMACEUT.9B00639
74. Lee SWL, Paoletti C, Campisi M, et al. MicroRNA delivery through nanoparticles. *J Control Release*. 2019;313:80-95. doi:10.1016/J.JCONREL.2019.10.007
75. Levchenko TS, Rammohan R, Lukyanov AN, Whiteman KR, Torchilin VP. Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating. *Int J Pharm*. 2002;240(1-2):95-102. doi:10.1016/S0378-5173(02)00129-1
76. Wang H, Zhao P, Su W, et al. PLGA/polymeric liposome for targeted drug and gene co-delivery. *Biomaterials*. 2010;31(33):8741-8748. doi:10.1016/J.BIOMATERIALS.2010.07.082
77. Hu K, Xiang L, Chen J, Qu H, Wan Y, Xiang D. PLGA-liposome electrospun fiber delivery of miR-145 and PDGF-BB synergistically promoted wound healing. *Chem Eng J*. 2021;422:129951. doi:10.1016/J.CEJ.2021.129951
78. Li Y, Xu T, Tu Z, et al. Bioactive antibacterial silica-based nanocomposites hydrogel scaffolds with high angiogenesis for promoting diabetic wound healing and skin repair. *Theranostics*. 2020;10(11):4929. doi:10.7150/THNO.41839
79. Bai Q, Han K, Dong K, et al. Potential Applications of Nanomaterials and Technology for Diabetic Wound Healing . *Int J Nanomedicine*. 2020;15:9717-9743. doi:10.2147/IJN.S276001
80. Zhang J, Wu C, Xu Y, et al. Highly Stretchable and Conductive Self-Healing Hydrogels for Temperature and Strain Sensing and Chronic Wound Treatment. *ACS Appl Mater Interfaces*.

2020;12(37):40990-40999. doi:10.1021/ACSAMI.0C08291
81. Kurapati R, Groth TW, Raichur AM. Recent Developments in Layer-by-Layer Technique for Drug Delivery Applications. *ACS Appl Bio Mater*. 2019;2(12):5512-5527. doi:10.1021/ACSABM.9B00703

Table 1: *Examples of nanoparticles developed as therapeutic molecules or drug delivery vehicles for the application of wound healing in diabetic patients.*

Type of Nanoparticle	Material/ Nanoparticle	Modification to Nanoparticle	Incorporated Cargo	*In vitro Results*	*In vivo Results*	Ref.
Inorganic	AgNP	N/A	N/A	Increasing concentrations of AgNPs induced cell death Significant reduction in bio-film activity in a dose-dependent manner of AgNPs	N/A	[37]
	ZnO	N/A	N/A	ZnO NPs with larger particle sizes showed higher antimicrobial properties.	N/A	[39]
Liposome	α-gal NPs	N/A	N/A	N/A	Increased keratinocyte migration compared to saline treatment	[47]
	PLGA-Liposome nanofibers	N/A	miR-145 and PDGF-BB	Significantly increased tube formation of nanoparticles containing miR145+PDGF-BB compared to nanoparticles, which showed increased angiogenesis compared to untreated	Significantly decreased wound area with loaded cargo Increased therapeutic effect of loaded nanoparticles compared to free drug Decreased inducible nitric oxide synthase, indicating inhibition of inflammation in later stages of wound healing Increased angiogenesis	[77]
Dendrimer	Polyamidoamine (PAMAM) Nanoparticles	Connected to hyaluronic acid through substrate polypeptide of MMP-2 (Gly-PLGLAG-Cys)	Astragaloside (ASI)	Controlled release in the presence of MMP-2 (>70% release) compared to in the presence of PBS (~13% release) Significantly reduced levels of reactive oxygen species Increased cell proliferation and migration Increased expression of wound-repair-related genes	Nanoparticle was observed to be MMP-2 responsive at wound sites (overexpress MMP-2) Increased cell proliferation and migration Increased expression of wound-repair-related genes	[51]
Protein	SDF1α-elastin-like-peptide	Recombinant fusion of SDF1α and	N/A	Binding of SDF1-ELP to cell receptors was similar to that of SDF1, with longer retention	SDF1-ELP accelerated wound closure (21 days) compared to free SDF1 (42 days)	

		elastin-like-peptide		Increased intracellular calcium release observed with SDF1-ELP than with free SDF1, indicating decreased inflammation and increased hemostasis	SDF1-ELP resulted in thicker epidermal and dermal layer	[57]
	Recombinant keratin NPs	N/A	N/A	Cell proliferation Increased cell migration in scratch-wound-healing assays, observed by increased closure of scratch area	Accelerated wound closure compared to commercial dressing Decreased inflammatory cells (after day 7) Increased proliferation of fibroblasts observed (after day 7) Increased angiogenesis Higher collagen deposition	[58]
Polymeric	PLGA/Gelatin nanofibers	PLGA crosslinked with Gelatin	Liraglutide	Slow initial degradation, quicker degradation after 14-days compared to PLGA, allowing release of drug in later healing stages Continuous degradation over 30 days Increase in cell proliferation, migration, and growth factor secretion of endothelial cells Increased angiogenesis	Complete wound healing achieved in 14 days More new epithelium tissue, increased collagen deposition and collagen thickness, and increased blood vessel formation were observed in PLGA/Gelatin/Lira compared to PLGA/Gelatin and PLGA groups	[49]
	PLGA	N/A	Polyethylenimine-Nitric Oxide (PEI/NONOate)	Prolonged release of NO over 6 days without burst release using PLGA NP, compared to release over 12 hours with burst release with PEI/NONOate alone PEI/NONOate-PLGA NPs showed bacterial resistance in a dose-dependent manner against MRSA and *P. aeruginosa* Adhesion of PLGA to bacterial allowed for increased antimicrobial activity of cargo	PLGA-PEI/NONOate showed more than 60% reduced wound area on day 4 of diabetic and infected mice compared to untreated, which showed an increase in wound area Increased fibroblast-like cells and decreased pro-inflammatory macrophages in treated mice compared to untreated	[64]
	Chitosan	N/A	Rebamipide (RBM)	Prolonged drug release of encapsulated drug over 48 hours compared to free drug (released in 8 hours)	Wounds completely covered with epithelium after 3 weeks of chitosan-RBM treated wounds compared to untreated which	[66]

					showed tender wounds with some hemorrhage Decreased pro-inflammatory macrophages in treated wounds	

Table 2: *Advantages and disadvantages of the reviewed strategies for wound healing application in diabetic patients*

Type of Nanoparticle	Advantages	Limitations
Inorganic	Uniform size and shape Antimicrobial properties	Can cause an immunogenic response Low loading capacity
Liposome	Biocompatible High loading capacity Metabolised *in vivo*	Difficult to ensure bath-to-batch consistency in synthesis Costly Storage issues due to leakage of drugs
Dendritic	Controllable physical properties and size High loading capacity	Complex synthesis Tendency to aggregate Can be cytotoxic
Protein	Easy production of recombinant proteins in bacterial systems Biocompatible and Biodegradable Stable structure *in vivo*	Expensive Difficult to store—storage requires sub-zero temperatures Protein aggregation can cause storage issues
Polymeric	Sustained release of drugs Controllable mechanical properties based on crosslinking densities Can synthesize stimuli-responsive drug delivery vehicles	Synthesis and purification processes length and involve many steps Higher Cost Degradation time can be too slow

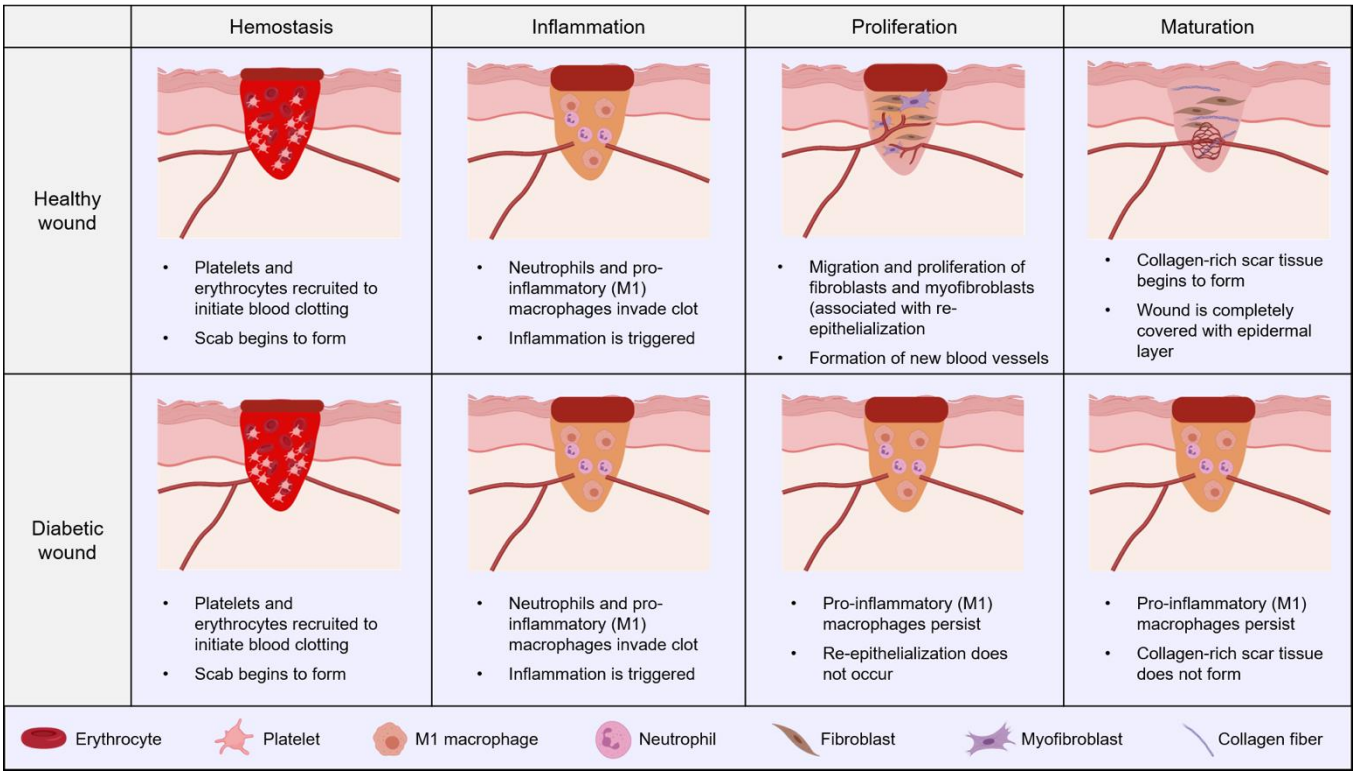


Figure 1. ***Key differences between the wound healing processes in non-diabetic and in diabetic individuals.*** Hemostasis in both situations initiates the healing process with the formation of blood clots, followed by the initiation of inflammatory phase. Pro-inflammatory cytokines are recruited to digest and engulf contaminants, causing inflammation in the area. Wound healing in non-diabetic patients proceeds into the proliferative phase where fibroblast migration and proliferation occur and new vascularization occurs, followed by the maturation phase when collagen-rich scar tissue closes the wound. In diabetic patients, the wound is chronically in the inflammation phase and does not proceed to the proliferative or maturation phases of the healing process.

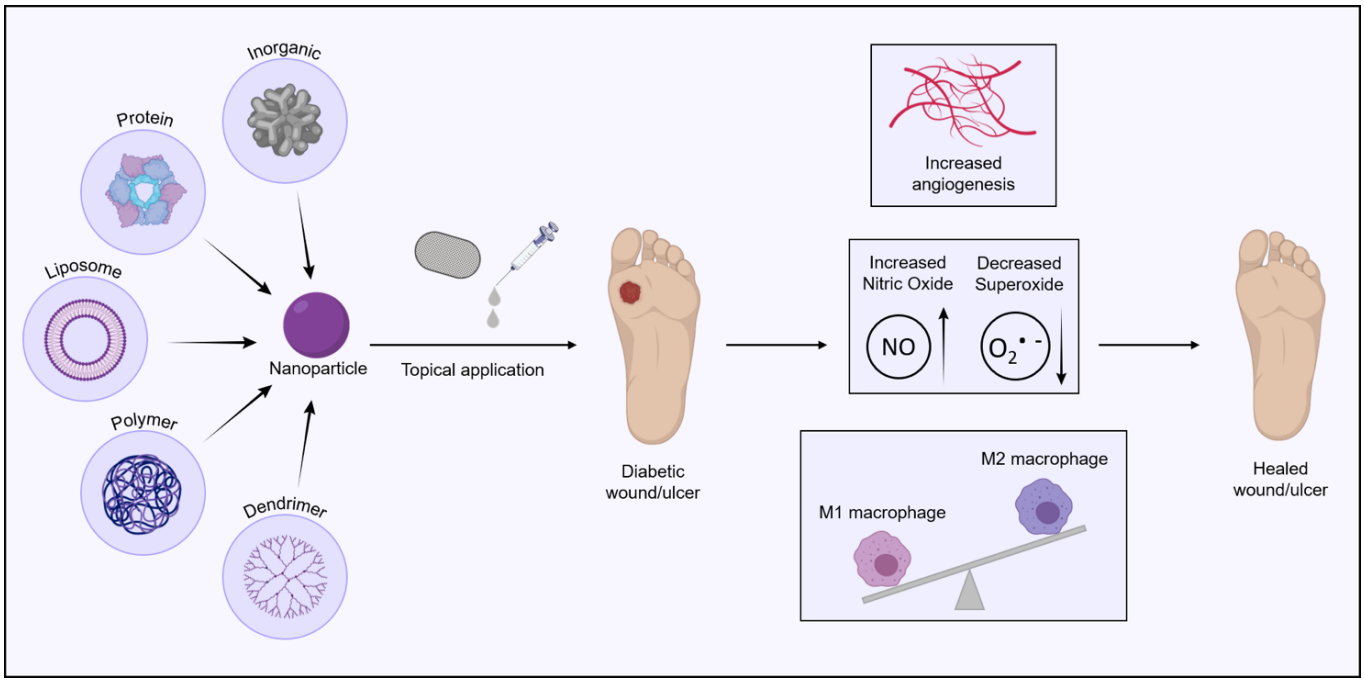


Figure 2. ***Overview of engineered nanotherapeutic strategies to accelerate wound healing in diabetes.*** The nanoparticles developed in recent years to treat wounds in diabetics are shown. Successful nanotherapeutics, when administered topically, will increase angiogenesis, decrease reactive oxygen species, and increase anti-inflammatory macrophages. Taken together, these therapeutic effects will improve and accelerate the wound healing process in diabetic patients.

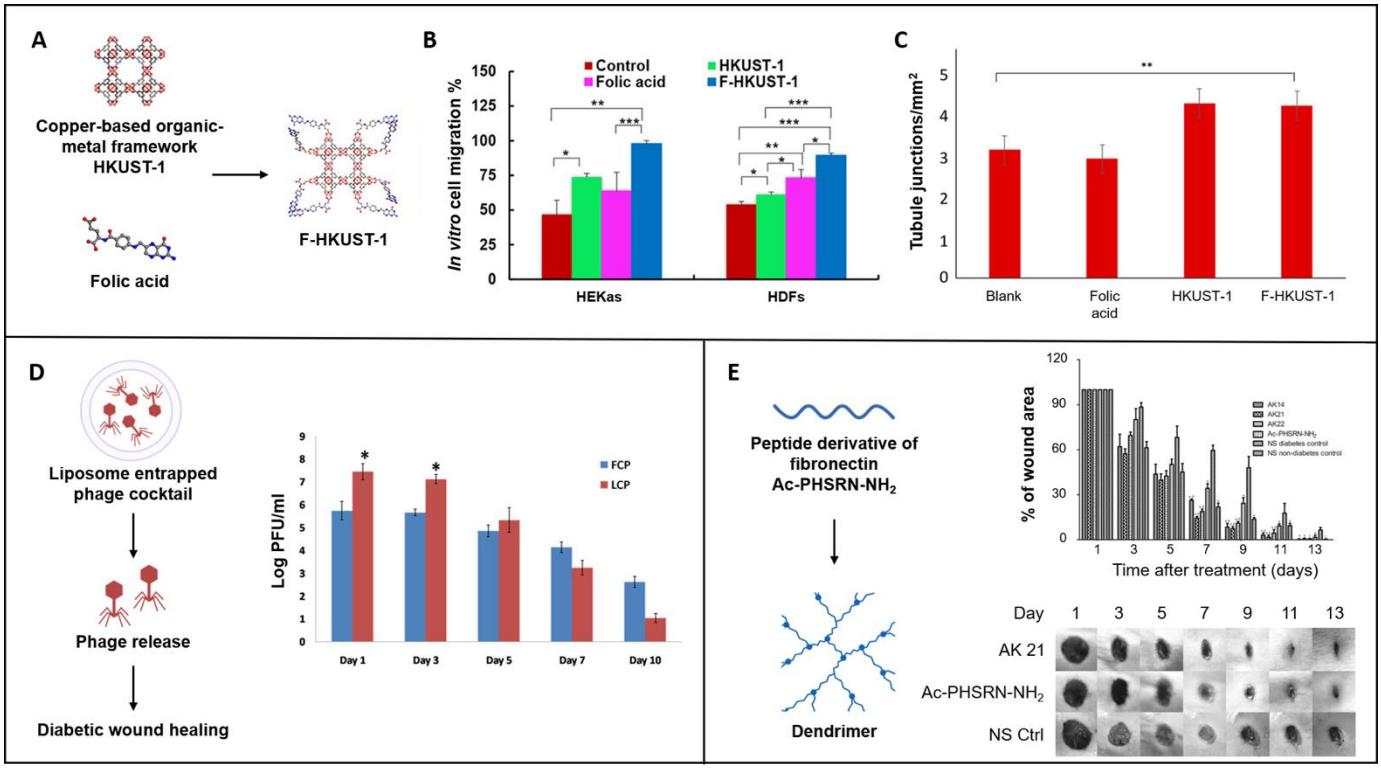


Figure 3. Design strategies and efficacy of nanoparticles on wound healing. (A) Schematic showing copper-based MOFs (HKUST-1) in combination with folic acid to synthesize folic acid-modified HKUST-1 (F-HKUST-1). (B) Percentage of cell migration in human epithelial keratinocytes (HEKs) and human dermal fibroblasts (HDFs) with after treated with PBS (control), HKUST-1, folic acid, and F-HKUST-1 for 24 hours. HKUST-1 showed a significant increase in cell migration compared to other experimental groups. (C) Quantification of tubule junctions in human umbilical vein endothelial cells (HUVECs) showed highest tubule junctions in HKUST-1 and F-HKUST-1, both expected to show contribution to angiogenesis and wound healing *in vivo.* Adapted from ref. 42 with permission from American Chemical Society, copyright 2018. (D) Schematic illustration of phage cocktail encapsulation and release in liposomes for diabetic wound healing. Liposomes entrapping the phage cocktail showed significant decrease in phage titer present in the wound of *S. aureus* with both the free cocktail of phages (LCP) and the liposome entrapped cocktail of phages (LCP). However, over the period of 10 days, FCPs showed lower phage titers than FCPs. Adapted from ref. 46 with permission from Frontiers Media, copyright 2018. (E) Schematic illustration of dendrimer formation using peptide derivatives of fibronectin. Treatment of these derivative dendrimers to diabetic wounds was evaluated by percentage of wound closure over 13 days and compared to treatment with normal saline (NS) and the peptide of fibronectin which served as negatice and positive controls, respectively. Treatment with dendrimers containing peptide derivatives of fibronectin showed significantly decreased the size of the wound compared to the saline control. Adapted from ref. 49 with permission from Springer Science+Business Media, copyright 2017.

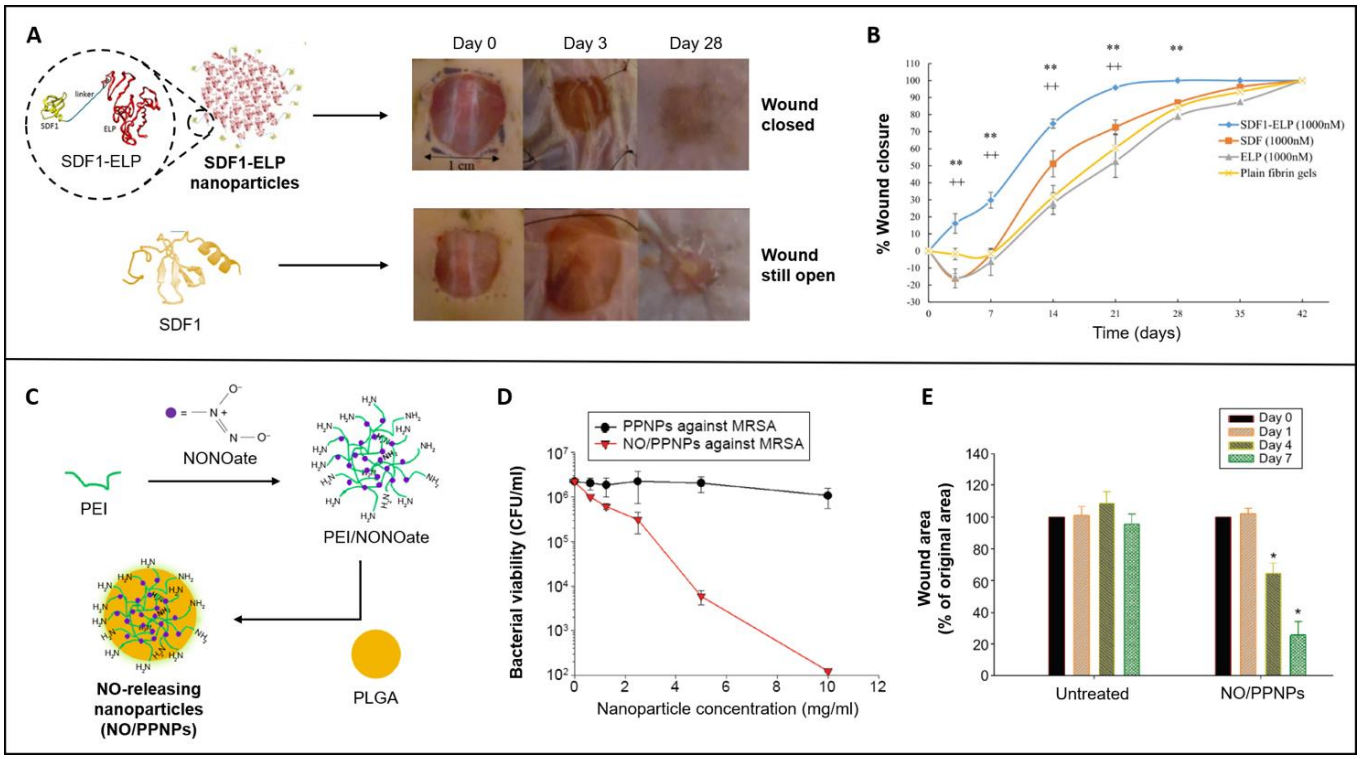
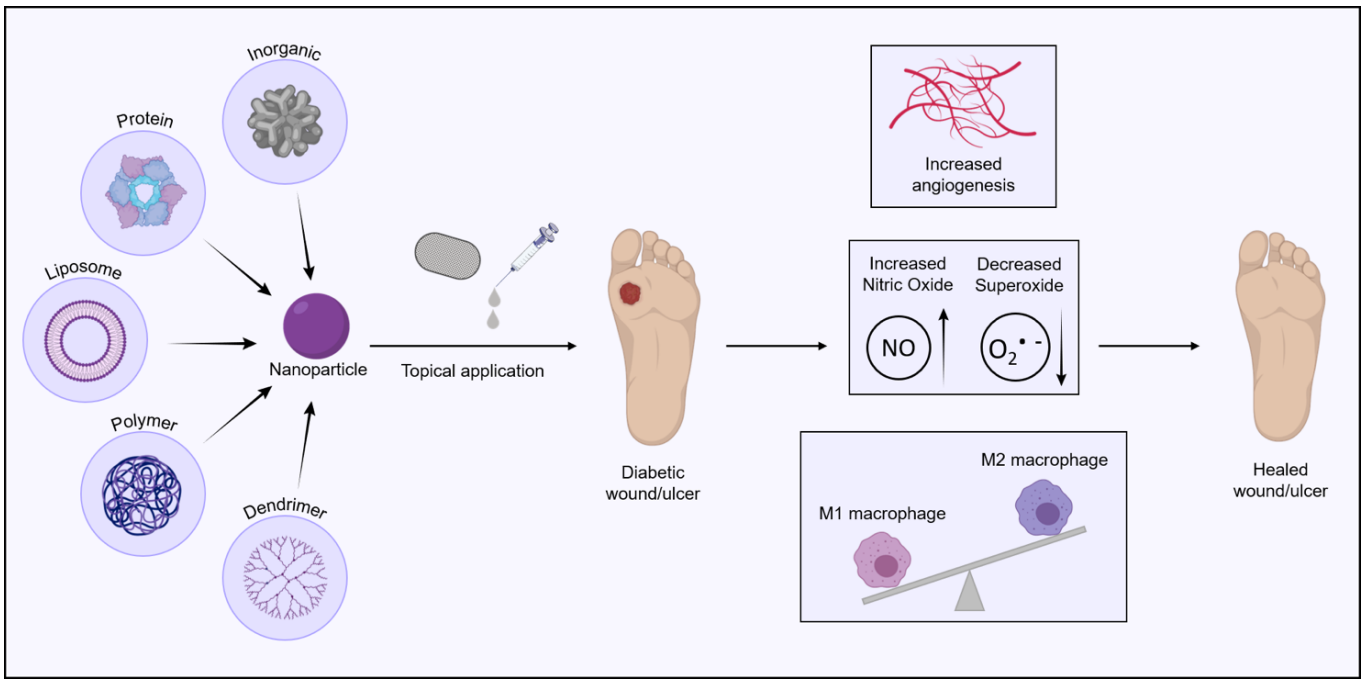


Figure 4. Efficacy of nanoparticles on wound healing. (A) Efficacy of recombinant SDF1-ELP nanoparticles compared to free SDF1 in wound-induced diabetic mice. SDF1-ELP nanoparticles accelerated wound healing in diabetic mice, allowing the wound to completely recover in 28 days. Free SDF1 improved wound healing slightly; however, the wound remained open by day 28. (B) Wound closure studies in diabetic mice showed complete recovery in all experimental groups.SDF1-ELP nanoparticles showed complete recovery by Day 28 compared to remaining groups showing complete recovery by Day 42. Adapted from ref. 57 with permission from Elsevier, copyright 2016. (C) Schematic illustration of PLGA nanoparticles loaded with crosslinked with Polyethylenimine-Nitric Oxide (No/PPNPs) to form NO releasing nanoparticles. (D) NO/PPNPs were tested for their antimicrobial resistance against MRSA compared to PLGA nanoparticles without the PEI-NO crosslinks. Bacterial viability decreased in a concentration-dependent manner compared to PLGA nanoparticles alone. (E) NO/PPNPs significantly accelerated wound healing in wound-induced mice over the course of 7 days compared to untreated mice. Adapted from ref. 64 with permission from the Dove Medical Press Limited, copyright 2015.



Graphical Abstract: *Engineering Nanoparticle Therapeutics for Impaired Wound Healing in Diabetes*