Diabetes Management

Nanotechnology as an innovative approach for accelerating wound healing in diabetes

Breanne Mordorski[1], Jaime Rosen[1,2] & Adam Friedman*[1,3]

Poor wound healing is a major source of morbidity and mortality for millions of diabetic patients. Diabetic wounds can lead to severe infections, prolonged hospitalizations, amputations and an overall markedly decreased quality of life. Diabetic wounds fail to heal secondary to several perturbations in the wound healing process. Here, we present a review of the direct barriers to diabetic wound healing as well as innovative therapeutic options to improve outcomes in diabetic wounds.

KEYWORDS
• diabetes • nanoparticles • nanoscaffold • nanotechnology wound dressings • wound healing

Wound healing & diabetes

The initial or inflammatory phase of wound healing is characterized by recruitment of neutrophils and macrophages, essential for clearing debris/bacteria via phagocytosis and release of reactive oxygen species (ROS) and matrix metalloproteinases (MMPs). However, the inflammatory phase must be sufficiently limited to prevent wound expansion [1]. In diabetic wounds, defects in phagocytosis and T-cell immunity cause inadequate bacterial clearance, leading to sustained elevation of proinflammatory cytokines and MMPs [2]. Additionally, hyperglycemia-induced ROS promote overexpression of macrophage inflammatory phenotypes, associated with sustained inflammasome activity [3]. Importantly, ROS decrease nitric oxide (NO) in the wound due to scavenging and decreased endothelial NO synthase activity. This is detrimental to healing as NO possesses broad spectrum antimicrobial properties and downregulates neutrophil adhesion. NO also stimulates fibroblasts and keratinocytes and promotes neovascularization via vasodilation, VEGF modulation and endothelial progenitor cell mobilization [4–6]. In the setting of diabetes-associated hypoperfusion, loss of NO-promoted angiogenesis further delays healing. The resulting hypoxia prolongs the inflammatory phase, increases susceptibility to infection and delays progression into the proliferative phase, as evidenced by decreased re-epithelialization, collagen and granulation tissue in diabetic wounds [7].

> "One of the most promising strategies for overcoming refractory diabetic ulcers is the use of nanotherapeutics – therapeutic agents engineered within the nanoscale (1–100 nm) – including nanoparticles and nanoscaffold wound dressings."

[1]Division of Dermatology, Department of Medicine, Montefiore-Albert Einstein College of Medicine, 1300 Morris Park Avenue, New York, NY 10461, USA
[2]Mt Sinai School of Medicine, 1428 Madison Avenue, New York, NY 10029, USA
[3]Department of Dermatology, George Washington School of Medicine & Health Sciences, 2nd Floor, South Pavilion, 2150 Pennsylvania Avenue Northwest, Washington DC 20037, USA
*Author for correspondence: Tel.: +1 202 741 2600; +1 240 483 0555; ajfriedman@mfa.gwu.edu

Future Medicine part of fsg

"...nanoparticles allow previously undeliverable drugs to be encapsulated and released in a sustained manner..."

A role for nanotechnology

Despite the billions of healthcare dollars allocated for diabetic wound care, treatment is an ongoing challenge. Our therapeutic armament consists of debridement, dressing changes, pressure off-loading devices and antibiotics, as well as advanced therapies such as bioengineered skin equivalents, growth factors, hyperbaric oxygen and negative pressure wound therapy [8]. Despite these interventions, diabetic patients continue to endure suboptimal outcomes. One of the most promising strategies for overcoming refractory diabetic ulcers is the use of nanotherapeutics – therapeutic agents engineered within the nanoscale (1–100 nm) – including nanoparticles and nanoscaffold wound dressings. Nanoparticles enable the targeted delivery of active drugs that may not otherwise be bioavailable *in vivo* due to poor solubility, short half-life, and/or leakage from the site of the wound. Nanoparticles have a high surface-to-volume ratio which increases the likelihood of interaction with their biological target, and can offer improved penetration into wounds, ideal for topical drug delivery. Furthermore, nanoparticles allow previously undeliverable drugs to be encapsulated and released in a sustained manner, and release rate can be conveniently controlled by altering nanoparticle composition. Sustained release allows continuous interaction of a drug with its intended target, without experiencing the rapid saturation and wash out of a bolus dose. This limits drug toxicity because the maximum amount of drug delivered is never in contact with the skin at one time [9,10].

In addition to nanoparticles, wound dressings with nanoscale fibers are being utilized for diabetic wounds. Such dressings have increased porosity and surface-to-volume ratio, and their structure simulates the topographic appearance of endogenous extracellular matrix (ECM), allowing attachment and spreading of both fibroblasts and keratinocytes, thereby facilitating collagen synthesis and re-epithelialization of wounds [11,12]. Here, we will review nanoparticles that deliver endogenous molecules such as NO and growth factors, natural products such as curcumin, and gene knockdown agents such as siRNA, as well as nanoscaffold wound dressings in the treatment of diabetic wounds.

Nanotechnology in diabetic wound healing

Nanoparticles allow the topical delivery of previously undeliverable substances, including those that are endogenously produced. One such substance is NO, a diatomic gaseous molecule with a half-life in seconds. Because the innate ability to generate NO is decreased in diabetic wounds, exogenous NO is an extremely promising therapeutic, though its utilization is limited due to the lack of effective delivery vehicles. One study found that topical NO-releasing nanoparticles (NO-np) accelerated wound closure in nonobese, diabetic, severe combined immunodeficiency mice, compared with both untreated controls and another NO-releasing agent, a diazeniumdiolate. NO-np treated wounds demonstrated fewer inflammatory cells, and more blood vessels and fibroblasts with increased fusiform morphology and organized collagen [5]. NO-np's wound healing effects were confirmed in another mouse study showing accelerated wound closure, similar histologic findings and increased prohealing TGF-β. *In vitro* human dermal fibroblasts also demonstrated accelerated migration, proliferation and collagen expression when treated with NO-np [13].

Growth factors are another group of endogenous molecules depleted in diabetic wounds, with challenging therapeutic delivery due to rapid degradation, a process accelerated in diabetic wounds due to elevated MMPs. In one study, recombinant human EGF (rhEGF) was incorporated into poly(lactic-*co*-glycolic acid) nanoparticles and applied to diabetic rat wounds, demonstrating accelerated healing with increased fibroblast proliferation, compared with both untreated controls and free rhEGF [14]. Another study incorporated rhEGF into nanostructured lipid carriers, offering occlusive properties to increase skin hydration, promote accumulation in the stratum corneum and facilitate penetration of the drug. In diabetic mice, rhEGF-loaded nanostructured lipid carriers accelerated wound closure with decreased neutrophil infiltration compared with untreated controls and free rhEGF. *In vitro* tests demonstrated increased fibroblasts and keratinocyte proliferation [15].

Nanotechnology also allows the encapsulation of plant-derived ingredients with wound healing and antimicrobial efficacy. Curcumin, the component of turmeric that provides its bright yellow-orange color, has known antimicrobial, anti-inflammatory and antioxidant properties. In diabetic rats, curcumin application led to earlier re-epithelialization of wounds, and increased fibroblasts, collagen and blood vessels associated with increased VEGF, TGF-β and endothelial

NO synthase [7]. However, its practical use has been limited due to poor aqueous solubility, short half-life and unaesthetic color. These shortcomings can be overcome with nanotechnology, as demonstrated by curcumin nanoparticles, which exerted potent *in vitro* antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *In vivo,* curcumin nanoparticles accelerated wound healing in methicillin-resistant *S. aureus*-infected mouse wounds, compared with untreated controls and solubilized curcumin [10].

Along with delivering gaseous molecules, growth factors and natural products, nanotechnology can be harnessed to augment gene expression. In both mouse and human skin, spherical nucleic acid–gold nanoparticle conjugates knocked down GM3 synthase via a siRNA pathway, without nuclease degradation or need for epidermal disruption, as typical with siRNA. GM3 is a sialylated glycosphingolipid related to insulin resistance and poor healing. The nanoparticle conjugates accelerated wound healing in diabetic mice, in addition to increasing keratinocyte migration and proliferation, neovascularization and IGF-1 and EGF receptor activation [16], thus presenting GM3 synthase as another future target in treating diabetic wounds.

While topical therapeutics demonstrate favorable outcomes in diabetic wound management, proper wound dressing is crucial for accelerating healing and lowering microbial burden. This can be accomplished with nanoscaffold dressings, a subject of recent study. Wu *et al.* investigated a bacterial cellulose nanofiber dressing impregnated with silver nanoparticles (Ag-np). Like NO, silver is a broad-spectrum antimicrobial that acts via multiple mechanisms, thereby decreasing the risk of microbial resistance. This dressing showed *in vitro* antimicrobial activity against *Escherichia coli, S. aureus* and *P. aeruginosa,* facilitated attachment and growth of keratinocytes, and created an ideal moist and absorbent barrier [12]. In addition, Shahverdi *et al.* investigated a poly(lactic-*co*-glycolic acid)/silk fibroin nanofiber dressing, which decreased wound size after 15 days compared with traditional dressings in diabetic rats. *In vitro* studies demonstrated fibroblast attachment and proliferation, which are observed when the nanoscaffold serves as a substitute for lost ECM, thus encouraging fibroblast homing and endogenous ECM formation, providing a stable foundation on which granulation tissue can form [11].

Safety

There is much debate about the potential toxicity of nanoparticles. To date, studies have found limited toxicity without evidence of systemic absorption. For example, Krausz *et al.* did not detect curcumin nanoparticle toxicity against human keratinocytes *in vitro,* or against embryonic zebrafish *in vivo* [10]. In contrast to previous Ag-np nanofiber dressings that found concentration-dependent Ag-np toxicity [17], Wu *et al.* found no toxicity against keratinocytes with Ag-np/bacterial cellulose nanofiber dressing because Ag-nps were synthesized directly onto nanofibers, allowing sustained release of silver ions while preventing cellular toxicity of free Ag-np [12]. Despite these advancements, each study utilizes different methods for assessing toxicity of nanotherapeutics. Moving forward, scientific researchers should seek standardized methodology for assessing toxicity, as well as the penetration of nanotherapeutics into the skin, which are essential to ensure safe wound treatments and alleviate fears of systemic absorption.

Conclusion

The above data represents a promising therapeutic strategy to accelerate diabetic wound healing. The use of nanotherapeutics has enabled the stabilization of previously unusable molecules, and has improved the efficacy of traditional dressings. Despite their promise, nanotherapeutics comprise a very young scientific field, and further studies are needed to elucidate their pharmacokinetics and potential for *in vivo* toxicity. Furthermore, there is a paucity of human clinical data, which is essential for determining clinical efficacy, as well as for translation of *in vitro* and *in vivo* studies for the benefit of diabetic patients. More work is certainly needed to capitalize on the extensive preclinical body of evidence, supporting the use of nanomaterials for this growing medical crisis.

"...the nanoscaffold serves as a substitute for lost extracellular matrix, thus encouraging fibroblast homing and endogenous extracellular matrix formation, providing a stable foundation on which granulation tissue can form."

Financial & competing interests disclosure

A Friedman is a co-inventor of NO-np, a technology that has been licensed to Nano Biomed, Inc. for commercialization. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Chiara G, Letizia F, Luca L *et al.* Nanoparticle therapies for wounds and ulcer healing. In: *Nanomedicine in Drug Delivery.* CRC Press, FL, USA (2013).

2 Guo S, Dipietro LA. Factors affecting wound healing. *J. Dent. Res.* 89(3), 219–229 (2010).

3 Mirza RE, Fang MM, Weinheimer-Haus EM, Ennis WJ, Koh TJ. Sustained inflammasome activity in macrophages impairs wound healing in Type 2 diabetic humans and mice. *Diabetes* 63(3), 1103–1114 (2014).

4 Schwentker A, Vodovotz Y, Weller R, Billiar TR. Nitric oxide and wound repair: role of cytokines? *Nitric Oxide* 7(1), 1–10 (2002).

5 Blecher K, Martinez LR, Tuckman-Vernon C *et al.* Nitric oxide-releasing nanoparticles accelerate wound healing in NOD-SCID mice. *Nanomedicine* 8(8), 1364–1371 (2012).

6 Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Int. J. Vasc. Med.* 2012, 918267 (2012).

7 Kant V, Gopal A, Kumar D *et al.* Curcumin-induced angiogenesis hastens wound healing in diabetic rats. *J. Surg. Res.* 193(2), 978–988 (2015).

8 Lipsky BA, Berendt AR, Cornia PB *et al.* 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis.* 54(12), e132–e173 (2012).

9 Kim BY, Rutka JT, Chan WC. Nanomedicine. *N. Engl. J. Med.* 363(25), 2434–2443 (2010).

10 Krausz AE, Adler BL, Cabral V *et al.* Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. *Nanomedicine* 11(1), 195–206 (2015).

11 Shahverdi S, Hajimiri M, Esfandiari MA *et al.* Fabrication and structure analysis of poly (lactide-*co*-glycolic acid)/silk fibroin hybrid scaffold for wound dressing applications. *Int. J. Pharm.* 473(1), 345–355 (2014).

12 Wu J, Zheng Y, Song W *et al. In situ* synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing. *Carbohydr. Polym.* 102, 762–771 (2014).

13 Han G, Nguyen LN, Macherla C *et al.* Nitric oxide–releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition. *Am. J. Pathol.* 180(4), 1465–1473 (2012).

14 Chu Y, Yu D, Wang P, Xu J, Li D, Ding M. Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats. *Wound Repair Regen.* 18(5), 499–505 (2010).

15 Gainza G, Pastor M, Aguirre JJ *et al.* A novel strategy for the treatment of chronic wounds based on the topical administration of RHEGF-loaded lipid nanoparticles: *invitro* bioactivity and *in vivo* effectiveness in healing-impaired db/db mice. *J. Control. Release* 185 51–61 (2014).

16 Randeria PS, Seeger MA, Wang XQ *et al.* Sirna-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown. *Proc. Natl Acad. Sci. USA* 112(18), 5573–5578 (2015).

17 Anisha B, Biswas R, Chennazhi K, Jayakumar R. Chitosan–hyaluronic acid/nano silver composite sponges for drug resistant bacteria infected diabetic wounds. *Int. J. Biol. Macromol.* 62, 310–320 (2013).