



## Understanding the implications of pharmaceutical excipients and additives in the treatment of diabetic foot ulcers.

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### ABSTRACT

A diabetic foot ulcer (DFU) is a consequence of Diabetes Mellitus (DM) and involves complex pathological processes. Among diabetic patients DFU is a major cause of deaths resulting from the amputation of the lower limbs. Various treatment strategies have been developed for the treatment of DFUs, but to this date unfortunately no single treatment fulfills the prerequisites necessary for treating this condition due to its complex, multifactorial pathophysiology. Additionally, costs associated with the treatment can be prohibitively high. Excipients are pharmaceutical agents which have diverse applications in the design of different dosage forms. Therefore, an ideal dosage form, with active excipients in combination or as adjuvants, which meet these requirements could be suited for treating DFUs. This review discusses the etiopathogenesis of DFUs and also the possible use of excipients and additives in various pathological cases of DFUs in designing medicinal products intended for the treatment of this condition.

**KEY WORDS:** Excipients, additives, diabetes, diabetic foot ulcer, diabetic wound, chronic wound

### INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder caused by hyperglycemia due to defects in insulin action or secretion. It is estimated that about 347 million people

worldwide suffer from diabetes and it is projected to be the seventh leading cause of death by the year 2030. Type 2 diabetes, at about 90% of all cases, is more common than type 1 (1). Approximately 25% of patients with DM may end up with DFUs during their lifetime and, if proper care is not taken, 50 % of these foot ulcers could lead to infections followed by amputation (2). In 2013 DM alone, was responsible for 5.1 million deaths, at a cost of USD 548 billion (3). The average cost for treating infected DFUs is USD 17,000 and for a

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major amputation it is USD 45,000. DFUs are the major cause of deaths in patients with DM (4). Current DFU treatment focuses on patient education, prevention and early diagnosis (5). Treatment strategies available for the management of DFUs include antibiotics (piperacillin, tazobactam, trofloxacin, linezolid), neuropathic drugs (duloxetine, pregabalin, tapentadol), dressings (collagen scaffolds), skin substitutes (Dermagraft<sup>®</sup>, Apligraf<sup>®</sup>), growth factors (rhFGF, rhEGF, PDGF), devices (negative pressure therapy, hyperbaric oxygen therapy) and surgery (reconstruction and amputation) (6, 7). However, once a DFU has formed, noninvasive therapies are less effective and, although invasive therapies are more effective, they are also costlier (5). A single treatment strategy (multi-mechanism based product) that addresses all the prerequisites to treat DFUs needs to be developed. The most important factor in minimizing the overall cost of therapy is the development of novel drug delivery systems which will not increase the net cost (despite their possibly higher unit cost), if their frequency of application is decreased (8).

Apart from active wound healing through therapeutic agents, slow healing wounds such as DFUs require more care including maintenance of a moist wound environment, removal of necrotic tissue, protection from microbes, maintenance of pH and temperature at the wound site, control of exudation, promotion of chemotaxis, adequate nutrient supply and substrate for controlled release of drugs as well as for infiltration of healing factors. Due to these distinct characteristics at different wound healing stages, a single therapeutic agent cannot fulfill all these requirements. However, it should be possible to develop and optimize a conceptual product using selective excipients to meet these requirements. Therefore, an ideal product, comprising active excipients in combination with active pharmaceutical ingredients (APIs) or as adjuvants could be developed into a suitable product for treating DFUs. The majority of pharmaceutical excipients are cost-effective, compatible, have

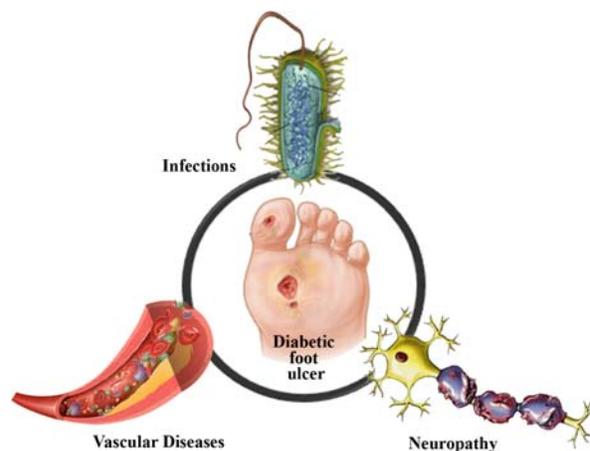
negligible toxicity profiles and possess either innate biological activity (active excipients) or may act to change the biological activity of the API (9). Developing them into a combination with therapeutic agents may increase the cost of a single unit but the overall cost of the therapy is likely to remain the same. This review focuses on the pathological factors to be considered when treating DFUs, as well as, on medicinal products that incorporate active excipients.

## PATHOPHYSIOLOGY

Diabetic foot, as defined by the World Health Organization, is “The foot of a diabetic patient that has the potential risk of pathologic consequences, including infection, ulceration, and/or destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral vascular disease, and/or metabolic complications of diabetes in the lower limb” (10). DFUs occur as a consequence of 2 major pathological factors, that is, neuropathy and peripheral vascular disease (PVD) which result in infections (also referred to as neuroischaemic foot) (Figure 1).

### Neuropathy

Chronic hyperglycemia causes damage to nerve fibers/neurons. The mechanisms by which nerve damage occurs includes the formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), increased



**Figure 1** Pathophysiology of DFUs

levels of reactive oxygen species (ROS) and nitric oxide blocking (11). These mechanisms directly, or indirectly, cause nerve damage (neuropathy). Neuropathy may be sensory, motor or autonomic depending on the type of nerve that is damaged. Sensory neuropathy leads to two types of conditions, i.e., either a foot with severe pain or a foot without sensation (12). In pain related neuropathy, drugs such as anticonvulsants, serotonin–norepinephrine reuptake inhibitors, tricyclic and tetracyclic anti-depressants can be used. In the latter case, the foot is highly vulnerable to injury because of the loss of sensation and is a far greater challenge for physicians to treat.

Motor neuropathy leads to muscle weakness and atrophy, which cause imbalances between the flexor and extensor muscles. This imbalance causes foot deformity and abnormal pressure distribution at different points of the foot (13). Due to the recurrence of pressure, callus formation is developed and any external force can cause the rupture of this callus, ultimately leading to DFU.

In autonomic neuropathy, restricted blood flow in the wound site occurs due to the reduction of 'flair reaction' as a result of noxious stimuli. Damage to sympathetic nerves, which supply blood to the feet, leads to the reduction in sweat and moisture, thereby leading to dryness of the foot. At this stage, the skin starts to develop fissures (14).

### Peripheral vascular disease

Peripheral vascular disease (PVD) is a condition of diabetes caused by atherosclerosis in the blood vessels of the lower extremities. PVD is one of the major risk factors contributing to DFUs. The tibial and peroneal arteries in the calf portion are most affected in PVD (15). Smoking, hyperlipidemia and hypertension are a few contributing factors for the formation of PVD. All the factors collectively contribute to the development of occlusive arterial disease that leads to ischemia and ulcer formation in diabetics (16). Symptoms associated with PVD

include intermittent pain, cramping, or aching in the calves, thighs or buttocks that are aggravated when walking or exercising and relieved by rest (17). There are several factors that must be considered when developing products for the treatment of DFUs as shown in Figure 2.

### PHYSICOCHEMICAL PROPERTIES OF EXCIPIENTS IN WOUND HEALING ACTIVITY OF DFUs

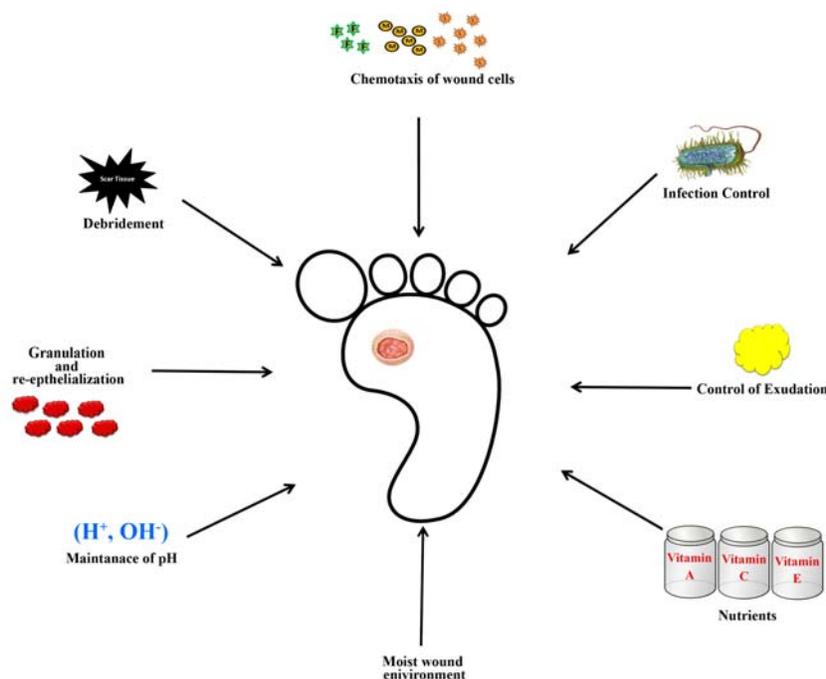
Excipients can play significant roles in the management of chronic DFUs and the most important ones, shown in Table 1, are related to specific treatment areas.

**Table 1** The role of excipients in the management of chronic DFUs

FACTORS IN THE MANAGEMENT OF CHRONIC DFUs	ROLE OF EXCIPIENT	NAME OF EXCIPIENTS
Debridement	Removes locally damaged or necrotic tissue and cleans the wound surface	Urea, H <sub>2</sub> O <sub>2</sub> , Glycolic acid and Sodium hypochlorite.
pH	Maintains optimum pH for wound regeneration and epithelialisation	Organic acids: citric acid, succinic acid, tartaric acid, malic acid, fumaric acid, adipic acid and maleic acid
Nutrient supply	Supports rapid tissue repair and regeneration	Vitamins: Vitamin A (from acetate and β-carotene), spirulina, lutein, zexanthine, cod liver oil, vitamin C/ascorbic acid (ascorbyltetraisopalmitate, ascorbylpalmitate), vitamin E (D-α-tocopheryl acetate) Proteins: Albumin, gelatin, thaumatin and zein.
Moist wound environment and Control of exudation	Absorb exudates from wound bed and provide moist environment	Foams, hydrogels, hydrocolloids, transparent films and alginate dressings.

### Removal of necrotic tissue (debridement)

Slow healing wounds, such as DFUs, are characterized by excessive necrotic or damaged tissue. Healing of DFUs should always begin with a debridement process, as leaving the necrotic tissue for a prolonged period of time on the wound site causes chronic inflammation, contributing to delayed re-epithelialization and wound healing (18).



**Figure 2** Factors that must be considered when developing pharmaceutical products for DFUs

Debridement is an essential process before the application of wound healing products since the efficacy of these products is highly dependent on the effective contact of the product with the actual wound bed. Efficient debridement should ensure the active removal of locally deceased bacteria and necrotic tissue (12). The debridement should be carried out at the early stages of necrotic tissue maturation, since the subsequently formed eschar (dry, black, thick necrotic tissue) is usually difficult to remove and may require surgical debridement. Debridement can be performed by surgical, autolytic, mechanical or chemical (enzymatic or non-enzymatic) methods (18).

Surgical debridement involves the surgical removal of necrotic tissue. The major downside of this process is considerable pain depending on the severity of the wound, hence it is carried out using local anesthesia.

Autolytic debridement is a natural process that occurs in the wound when using endogenous proteolytic enzymes (collagenase, elastase, acid

hydrolase, myeloperoxidase, and lysosomal enzymes) produced by neutrophils. This process is very slow and insufficient to remove the dead tissue in slow healing diabetic ulcers resulting in the accumulation of excessive quantities of exudates.

Mechanical debridement involves the non-specific removal of debris using mechanical force. This method is easy to perform but, due to its non-specificity, harm to adjacent healthy tissues can occur causing discomfort to the patient.

Chemical debridement is the most successful and highly selective method used in the treatment of DFUs. This kind of debridement can be enzymatic or non-enzymatic. Enzymatic debridement involves the use of pharmaceutical products containing proteolytic enzymes (papain, collagenase). Non-enzymatic debridement involves the use of chemicals such as urea, glycolic acid and sodium hypochlorite. In most of the chemical debridement products, urea is the most commonly used ingredient

associated with enzymes (19). This may be because it denatures the extracellular proteins which in turn increase their susceptibility to proteolysis by enzymes. Urea may also enhance the digestive potency (solvent action) of enzymes by exposure of enzyme activators (20). The combined use of urea with other enzymes has, in many instances, doubled the potency compare to using enzymes alone (18).

Pelle *et al.* used a 40% urea paste to demonstrate the debridement effects of urea in necrotic eschar. The study showed that small and large eschars were rapidly softened by the urea (19). This fast action resulted from its likely osmotic effect. Urea diffuses rapidly inside and surrounding areas of corneocytes and disrupts hydrogen bonding which leads to exposure of water binding sites. Urea also withdraws water from the tissues of the epidermal and dermal layers. This humectant character changes the hard damaged tissue into soft tissue (18). Pelle *et al.* also reported that following 8 to 16 weeks of urea application, none of the ulcers required the use of secondary debridement by surgical or chemical methods.

Chemical debridement using urea is inexpensive, painless and reduces wound traumatization (19). Hydrogen Peroxide ( $H_2O_2$ ) is another interesting debridement agent which has been used for wound irrigation. In a randomized controlled clinical trial carried out by Mohammadi *et al.* in patients with chronic-colonized burn wounds, the administration of 2%  $H_2O_2$  intraoperatively significantly increased the mean success rate of graft re-uptake (21).

## pH

pH is the most neglected parameter in the treatment of DFUs since the pH at the wound site plays a major role in healing (22). Factors including the activity of protease enzymes, angiogenesis and oxygen release at the wound site are highly dependent on wound pH. The pH of a chronic wound ranges from 7.15-8.9

(23). Wound healing is delayed by an elevated pH. Wounds at different stages have different pH. For example, wounds with epithelial tissue have  $pH < 6.0$ , compared with wounds without epithelial tissue, which have  $pH > 7.0$  (24). This could imply that the pH of a wound depends on the tissue type, but not on the stage/grade. Increased activity of Matrix Metalloproteinases (MMPs) is one of the prominent characteristics observed with chronic ulcers. These MMPs breakdown the components of the extracellular matrix. This activity is highly dependent on pH, with a higher pH favoring increased MMPs activity.

Oxygen distribution to the surrounding wound tissue also depends on pH. More oxygen release occurs at a lower pH. In addition to these factors, bacterial growth is generally favored in a basic environment. These observations suggest that the pH at the wound site should be considered when developing pharmaceutical products for the treatment of DFUs. The pH of the wound bed can be effectively altered by the application of topical preparations containing pH modifiers (25). A wide range of approved acidic pH modifiers such as citric acid, succinic acid, tartaric acid, malic acid, fumaric acid, adipic acid, maleic acid etc., are available. The incorporation of these pH modifiers into topical dosage forms intended for use in DFUs could modulate the pH of chronic wounds, thereby increasing the healing activity.

## Adequate nutrient supply

Another missing link in the conventional treatment of DFUs seems to be nutritional therapy. One of the major causes of DFUs is PVD. Atherosclerotic disease conditions of both microvascular and macrovascular blood vessels leads to an insufficient supply of blood to the wound site which creates an environment deficient in nutrients (vitamins, proteins and amino acids). Infected DFUs require a higher nutrient supply than acute wounds due to significantly greater tissue damage. Tissue repair and regeneration occurs faster in an ideal

physiological environment, i.e., an environment rich in nutrients and growth factors (26). Hence, topical application of nutrients may increase the rate of wound healing.

### **Vitamins**

Vitamins are essential for wound healing in various stages. Adequate supply of these nutrients could ensure an efficient wound healing process. It has been shown that vitamins A, C and E play vital roles in wound healing (26).

Vitamin A plays an essential part in immune system function, cellular differentiation and collagen activity. Vitamin C, or ascorbic acid, is the co-factor in the synthesis of collagen and other components of the intra- and extracellular matrix of the wound bed. It is involved in the hydroxylation of lysine and proline residues in procollagen synthesis. Deficiency of vitamin C leads to the abnormal formation of collagen fibrils and altered cellular matrix (27).

Vitamin E is the most popular agent used in skin care cosmetics. It functions as a major lipophilic antioxidant. However, mixed outcomes have been reported for the use of vitamin E in wound healing because of its altering effects in the presence of other nutrients (28).

### **Proteins**

Proteins are the precursors of collagen synthesis, a major component of extracellular matrix (ECM) that supports the construction of the wound bed. Protein loss through wound exudates should always be monitored. Excess wound exudates imply an excess loss of proteins. Some of the approved protein excipients used in topical formulations for wound healing include albumin, gelatin, thaumatin, collagen and zein (25).

### **Amino acids**

Research indicates that, compared to other amino acids, arginine and glutamine play vital roles in wound healing (26). These amino acids

stimulate the nitric oxide pathway, lymphocytic response, increases collagen synthesis and depositions as energy sources for inflammatory cells playing important roles in wound healing (29).

### **Dry foot**

Vascular dysfunction in autonomic neuropathy causes decreased sweating on the foot and leads to dryness and fissuring. In addition, deposition of AGEs coupled with increased pressure on the plantar surfaces of diabetic patients makes the foot vulnerable to trauma and skin damage. This condition ultimately increases the chance of bacterial infection, which is a major risk factor resulting in lower-limb amputation (30).

Dry skin can be moisturized using emollients or moisturizers. Emollients such as ointments, creams, gels and lotions contain varying degrees of lipids and water. Use of emollients in daily foot care improves skin integrity, resistance to injury from external agents and assists in the identification of areas susceptible to ulceration. Urea is a naturally occurring substance in the skin. The water holding property of urea makes the epidermis thinner without reducing the water content (31). Urea has also been reported to possess antimicrobial properties (32). Loden *et al.* showed that a formulation containing 15% urea enhanced dryness in the feet of persons with diabetes (33). Lactic acid is also an emollient which occurs naturally in the skin. It acts by promoting ceramide synthesis in the skin, improving its barrier function from damage (34).

An emollient diabetic foot spray (Medical Device Directive) (Aurena Laboratories, Sweden) has been approved by the European Medicines Agency. It contains petrolatum, liquid paraffin and hexamethyldisiloxane. It protects the damaged or irritated skin by creating a protective barrier, which reduces friction and soothes sensitive skin. It prevents further destruction of the skin and starts the process of healing. Since the product is a spray, the need to rub the petrolatum onto the skin is not necessary and therefore, damaged skin can be treated without touching the skin surface,

potentially reducing pain and risk of bacterial transmittance, ultimately increasing patient comfort.

### **Moist wound environment and control of exudation**

In autonomic neuropathy, the restricted blood flow of the DFUs occurs due to the reduction of the 'flair reaction', a consequence of exposure to noxious stimuli. Damage to sympathetic nerves, which supply blood to the feet, causes a reduction in sweat and moisture, eventually resulting in the dryness of the foot. At this juncture, the skin begins to develop fissures finally resulting in ulcer formation. Cells from a wound require moisture to migrate from the wound edges, therefore dryness of the wound restricts their movement.

A moist wound facilitates faster migration of epidermal cells in the wound bed which, in turn, supports angiogenesis and connective tissue synthesis. A moist wound environment also reduces nerve pain (neuropathy) due to the bathing of the exposed nerve ending in fluid, thus preventing dehydration of the nerve receptors (35). The major factor to be considered for moist wound therapy itself is appropriate 'moisture balance'. Because excessive moisture leads to a macerated callus at the edges of wounds favoring bacterial proliferation and deficient moisture, causing the wound to dry, thereby inhibiting cellular activities and formation of eschar.

The two therapies available to maintain a moist wound are negative pressure therapy and the use of moisture retention dressings. Negative pressure therapy involves suction of the blood from the surrounding tissues of a wound by applying a negative pressure to the wound area. This method is expensive and requires hospitalization. Hence, using moist wound dressings are gaining traction. These include foams, alginates, hydrogels, hydrocolloids, transparent films, and some topical treatments.

Exudation is a process of the migration of fluid, cells and cellular debris from blood vessels and their deposition in, or on, tissues usually resulting from an inflammation. Exudation and moisture are interrelated in wound healing. Infected wounds such as DFUs, are characterized by significant wound exudation that needs to be controlled to prevent the maceration of surrounding tissue. The moisture/exudates produced in the early stages of wound healing facilitate autolytic debridement and chemotaxis of inflammatory cells. However, in slow healing wounds, exudation is detrimental because the exudate of a chronic wound becomes copious over time and contains a mixture of elements (enzymes and cellular debris) which further prevent the wound from healing and hinder the application of topical treatments (36).

Sustained contact of the skin to elevated levels of moisture can cause hyper hydration of the outer layer epidermal cells, swelling and weakening the links between the layers of tissue. Controlling the level of exudates is one of the key components in the prevention of maceration and wound breakdown. Absorbent dressings are used to manage excess drainage. The ideal properties of a wound dressing are (i) maintaining high humidity, (ii) providing thermal insulation, (iii) removing wound exudates efficiently, (iv) permitting gaseous exchange, (v) adherence to the wound bed, (vi) impermeability to extraneous bacteria and (vii) non-toxic in nature (37). Types of wound dressings include semi-occlusive, moisture-retentive, and non-adherent, depending on the material used. Care must be taken in the selection of proper dressing for the absorption of exudates and at the same time, providing optimal moisture in the intact wound skin.

### **Hydrogel dressings**

Hydrogel dressings are water absorbent dressings manufactured using mixed hydrated polymers. They are clear, transparent, flexible, non-antigenic, permeable to water, and maintain a high moisture environment.

Hydrogels are designed to aid autolysis of necrotic tissue. The major advantage of hydrogel dressings is that they can be easily applied and removed because of their aqueous nature (38). These dressings are suitable for mild to moderate exudate wounds. Their application to DFUs is usually as an adjunct to sharp debridement of necrotic eschar (39). Amorphous gel, sheet hydrogel and impregnated gauze are the three types of hydrogels available (37).

### **Hydrocolloids dressings**

Hydrocolloids dressings are made of absorptive materials such as pectin and gelatin. These are occlusive dressings which do not allow water, oxygen, or bacteria into the wound and aid in facilitating angiogenesis and granulation. However, because of this occlusive (non-permeable) nature of these hydrocolloids, they cannot be used in wounds with infections and hence their use for treating DFUs is controversial (37).

### **Foam dressings**

Foam dressings are cushioning, highly absorbent, and conform with body surfaces. The extent of absorptive capacity depends on the polymer used and foam thickness. Due to their greater absorptive and protective properties, foam dressings can be usually left on the wound for up to seven days, making them more desirable candidates for DFUs treatment (38).

### **Alginate dressings**

Alginates (calcium or sodium) form gels when they come in contact with the wound fluid and they are also highly absorbent compared with other dressings. They have the capability of absorbing wound exudates up to about 20 times their own weight. As alginates are highly absorbent, they are not recommended for dry wounds. It has been shown that calcium alginate dressings are able to inhibit the growth of *Staphylococcus aureus* (*S. aureus*) *in vitro*, with no

increase in growth of *Pseudomonas* and *Streptococcus pyrogenes* (39).

## **THERAPEUTIC PROPERTIES OF EXCIPIENTS IN THE WOUND HEALING ACTIVITY OF DFUs**

### **Infection control**

PVD reduces blood flow resulting in more difficult treatment of infections. 50% of DFUs become infected (diabetic foot infections) and if the infections are left untreated, they extend to the deeper soft tissues and eventually the limb might have to be amputated. These infections are one of the most common complications of DFUs.

The most common pathogen in Diabetic Foot Infections (DFIs) is *S. aureus* and groups *A* and *B streptococci*. Patients who had previously received antibiotics were more likely to have methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* isolated from their wounds (40). Armstrong *et al.* reported MRSA in up to 25% of all diabetic foot infections involving *S. aureus* (41). Current antibiotic therapies available for treating DFIs include oral administration of flucloxacillin, doxycycline, clindamycin for *S. aureus* infections, vancomycin, teicoplanin, rifampicin, co-trimoxazole and linezolid for MRSA (42). There are several drawbacks with the systemic administration of antibiotics for the treatment of DFUs, including relatively low drug levels at the wound site, a potential risk of adverse reactions, the development of antibiotic resistance and the rapid decline of plasma antibiotic concentration to sub-therapeutic levels necessitating repeated dosing for a prolonged time. Therefore, localized delivery of antibiotics through the topical route is preferable. However, a topical application of antibiotics seems to be of limited use because of their direct toxicity to healing tissues despite their *in vitro* efficacy.

There are currently available a broad range of anti-microbials (acetic acid, benzalkonium chloride, cetrimide, chlorhexylenol, chlorhexidine gluconate, hydrogen peroxide,

hexachlorophene, phenyl mercuric salts (acetate, borate, nitrate), polyhexanide, povidone-iodine, sorbic acid and thimerosal) in various forms of semisolid dosage forms such as hydrogels, creams, sponges and dressings that are reported to be effective in treating infections at wound sites (shown in **Table 2**) (43). The localized application of these anti-microbials through the topical route is more promising, since it provides the desired concentration of anti-microbials at the wound site which makes the therapy more effective, with minimal or no side effects.

**Table 2** Topical antimicrobial agents available for treating DFUs

ANTIMICROBIAL AGENT	BACTERIAL SPECTRUM
Acetic acid (44)	Bactericidal against most gram-positive and gram-negative organisms, including <i>P. aeruginosa</i>
Benzalkonium chloride (45, 46)	Bactericidal against gram-positive ( <i>S. aureus</i> ) and less active against gram-negative organisms including <i>P. aeruginosa</i> , fungicidal.
Cetrimide (47)	Active against <i>S. aureus</i>
Chloroxylenol (48)	Active against gram-positive bacteria <i>S. aureus</i>
Chlorhexidine gluconate (47)	Active against gram-positive bacteria (eg, <i>S. aureus</i> ) and gram-negative bacteria, including <i>P. aeruginosa</i>
Hydrogen Peroxide (47)	Broad spectrum bactericidal (gram-positive and gram-negative), virucidal, and sporicidal.
Hexachlorophene (47)	Bacteriostatic against <i>Staphylococcus</i> species and other gram-positive bacteria
Phenyl mercuric salts (acetate, borate, nitrate) (49)	Bactericidal against <i>S. aureus</i> , less active against <i>Pseudomonas</i> species, especially <i>P. aeruginosa</i> , fungicidal.
Polyhexamide (50)	Active against <i>S. aureus</i> as well as MRSA
Povidone Iodine (42)	Broad spectrum includes <i>S. aureus</i> and MRSA.
Sorbic acid (51)	Anti-microbial, anti-fungal.
Thimerosal (49)	Broad spectrum active against both gram positive and negative bacteria.

### Granulation and re-epithelization

Any form of wound treatment strategy should culminate in the wound transitioning from the chronic inflammatory phase to the healing stage. The indication of this transition is the beginning re-epithelialization or granulation.

Wound healing, being an elaborate process of concurrent stimulation of soluble mediators, ECM, blood cells and parenchymal cells, can be divided into 5 major phases: homeostasis,

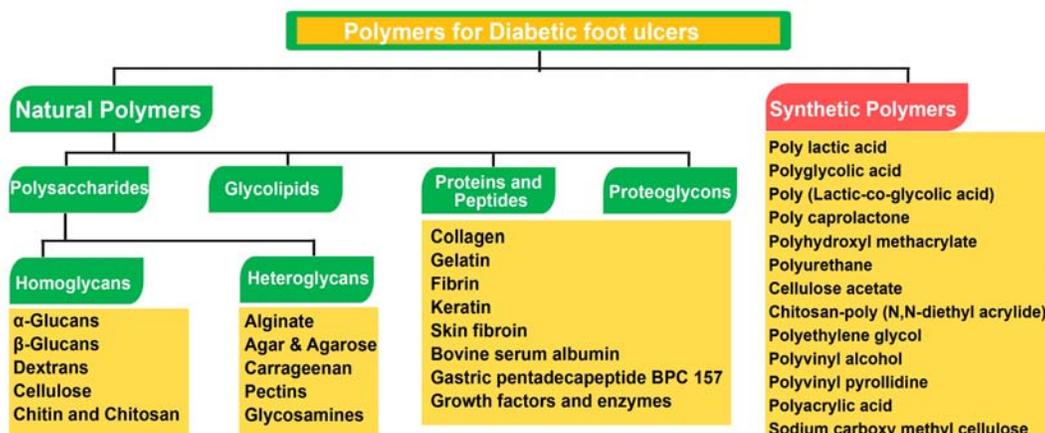
inflammation, proliferation, re-epithelialization and remodeling (52, 53). Of these, the regenerative phase is of the utmost importance, occurring as a result of platelet derived growth factors (PDGF's) influence in the inflammatory and proliferative phases (54). As a result, migration and proliferation of keratinocytes and fibroblasts occurs at the wound site for the development of the ECM that results in wound occlusion. With time, a collagen matrix supersedes the initial ECM by a process of new blood vessel growth called angiogenesis (attributed to angiogenic factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and PDGF). Complete closure of the wound area results in the termination of angiogenesis, accompanied with dermis regeneration (55).

These phases of wound healing need not occur in the same order as they would in acute wounds. Interchange generally occurs in chronic wounds as in DFUs, where the healing process is arrested in one or more phases (13).

Growth factors are usually used for skin regeneration in chronic wounds. However they are expensive and are often associated with antigenicity and stability problems. The widespread use of polymeric materials lies in their advantage of promoting chemotaxis. Usually these polymers are employed in the form of dressings for active tissue regeneration with pharmacological agents (active dressings) or in their absence (passive dressings). A wide variety of polymeric materials with potential DFUs applicability are available for commercial use, some of which have been approved for their enhanced healing properties (see Figure 3).

### Natural polymers

The field of regenerative medicine regularly incorporates natural polymers (wound and burn dressings) taking advantage of their biodegradability, biocompatibility and ECM mimicking properties. Natural polymers such as polysaccharides (alginates, chitin, chitosan, heparin, chondroitin), proteoglycans and



**Figure 3** Polymers for designing products to treat DFUs

proteins (collagen, gelatin, fibrin, keratin, silk fibroid, eggshell membrane) are extensively used in managing wounds and burns. Additionally, these polymers have been known to participate in many innate functions within the body, e.g., in addition to storage polysaccharides facilitate intracellular communication in cell membranes and proteins can operate as catalysts and structural scaffolds (56). Through stimulation and the induction of the wound healing process, natural polymers participate in damaged tissue repair and skin regeneration (57).

### **Polysaccharides**

Polysaccharides and protein based polymers possess the ability to influence migration, growth and cellular constitution during tissue regeneration and wound repair in order to ensure stability of encapsulated and transplanted cells (58).

#### **Chitin and chitosan**

Chitin is a linear polysaccharide (N-acetyl-D-glucosamine (2-acetyl-amino-2-deoxy-D-glucose) units linked by  $\beta$ -(1-4) glycosidic bonds insoluble in water and therefore can be converted to chitosan for application in medicinal products (38). Cross-linking chitosan with other synthetic polymers enhances its chemical and mechanical

properties. Chitosan is structurally similar to glycosaminoglycans found in extracellular matrices. It is involved in various steps of wound healing such as regulating the secretion of inflammatory mediators, including Prostaglandin E, interleukin 8, interleukin 1  $\beta$  and others (59). Chitosan enhances the infiltration of polymorphonuclear leukocytes (PMN) and fibroblasts at the wound bed, a vital step in wound healing (60). These features enhance granulation and re-epithelization of the wound. Additionally chitosan exhibits mild gelation and film forming properties which make it suitable for prolonged application in chronic wounds. Many studies that incorporate chitosan for DFU treatment have been reported. For example, Wang *et al.* studied the effect of chitosan cross linked with collagen on streptozotocin (STZ)-induced diabetic rats and found that faster wound healing occurs with collagen deposition and dermal cell proliferation (61). Lee *et al.* showed that chitosan increased hydroxyproline levels and collagen deposition, together with some antibacterial properties in STZ induced diabetic rats (62).

#### **Cellulosic derivatives**

Cellulose is a linear molecule ( $\beta$ -1, 4 linked D-glucose units) obtained from plant cell walls (63). Cellulose is not reabsorbed by body tissues due to the absence of the enzyme

cellulase. Microbial cellulose derived from bacteria possesses antibacterial activity together with wound healing properties (38). Studies have shown that cellulose enhances wound healing through stimulating a number of growth factors such as the epidermal growth factors (EGF), FGF and PDGF (64). Choi *et al.* used carboxymethyl cellulose hydrogel in db/db mice and found increased tissue regeneration and reduced wound area (65). Ulrich *et al.* studied the effect of oxidized regenerated cellulose on human subjects with non healing DFUs and found a decreased expression of plasmin, elastase and gelatin proteases (66).

### **Hyaluronic Acid**

Hyaluronic acid is a naturally obtained polysaccharide and is the major component of ECM. The application of hyaluronic acid hydrogel scaffolds has been shown to result in rapid tissue regeneration (67). The degradation products of hyaluronic acid promote endothelial cell migration and proliferation, regulate angiogenesis and inflammatory actions (68, 69).

Matsumoto and Kuroyanagi, demonstrated that the application of hyaluronic acid foam enhanced the epithelialisation and reduced the wound size in STZ-induced diabetic rats (70). Bayaty *et al.* showed that high molecular weight hyaluronic acid gel promoted the collagen regeneration and wound re-epithelization by increasing fibroblast and macrophages migration (71).

### **Proteins**

#### **Collagen**

Collagen is the most abundant protein present in humans and it is the major component of ECM of connective tissues (72). During a normal wound healing process, collagen is secreted by fibroblasts which involves several molecular and mechanical cascades of wound healing. Collagen interacts with cells in

connective tissues and is involved in essential transducer signals that regulate cell anchorage, migration, proliferation, growth and survival (73). Due to its unique chemotactic role, collagen is a vital component in each phase of wound healing. It attracts cells, such as keratinocytes and fibroblasts to the wound site which promote angiogenesis, debridement and re-epithelialisation (74).

Chikazu *et al.* demonstrated such wound healing effects of collagen gel in a db/db mice model (75). Arul *et al.* studied the effect of collagen matrix in STZ diabetic rats and showed improved cellular proliferation and rapid wound contraction (76). Hou *et al.* studied the effect of bone marrow mesenchymal stem cells conjugated with collagen, which provided significant progress in angiogenesis and wound healing (77). It was previously believed that collagen can be used for structural support. Previous research has shown that collagen and derived fragments influence many molecular and cellular functions (78). Commercial collagen based products used for treating DFUs include Unite<sup>®</sup>, BCG Matrix<sup>®</sup>, Promogran Prisma<sup>®</sup>, Dermalcol/Ag<sup>™</sup> and Fibracol<sup>®</sup>. Some of the products approved by USFDA for treating diabetic foot ulcers are Apligraf<sup>®</sup>, Excullagen<sup>®</sup> and INTEGRA<sup>®</sup>.

#### **Gelatin**

Gelatin is a natural protein polymer obtained from collagen. As a result it possesses low antigenicity and similar properties to collagen (79). In most cases, gelatin is used in the form of microspheres or as cross-linked products with other polymers such as collagen, alginate, hyaluronate etc., Kawai *et al.* demonstrated that basic Fibroblast Growth Factor (bFGF) loaded gelatin microspheres enhanced fibroblast proliferation and capillary formation (80).

#### **Fibrin**

Fibrin is a non-globular fibrous protein obtained from fibrinogen. As an important element in blood clots, fibrin is known to play a

vital function in later responses of wound healing (81). Fibrin contains particular sites for cell binding and hence could be a good substrate for cell migration, adhesion and growth (58). Fibrin hydrogels and fibrin glue are the two most widely used scaffold forms of fibrin. Pedroso *et al.* demonstrated the wound healing effects of fibrin gel on CD34+ cells in STZ-induced diabetic mice (82). Breen *et al.* observed an increased inflammatory response, combined with endothelial nitric oxide synthase expression, in fibrin scaffold treated alloxan diabetic rabbits (83).

Other natural polymers such as dextran, alginate (calcium, sodium), agar, carrageenan and pectins have been less studied for their tissue regeneration, rather the focus has been on their use in engineering tissue scaffolds because of their mechanical strength, bio-compatibility and bio-degradation.

### Synthetic polymers

Synthetic polymers have been used in designing scaffolds for tissue engineering due to their unique mechanical strength and long shelf life compared to natural polymers. The major disadvantage of these materials is their lack of cell-recognition signals. Hence, their application is limited to tissue engineering rather than active regeneration. However, manufacturing processes are now being developed that incorporate cell-adhesion peptides and biomaterials which are known to be involved in cellular interactions (84). Synthetic polymers, such as biomimetic extracellular matrix micro/nanoscale fibers (examples include polyglycolic acid, polylactic acid, poly-acrylic acid, poly- $\epsilon$ -caprolactone, polyvinyl pyrrolidone, polyvinyl alcohol and polyethylene glycol) manufactured by electro spinning, affect re-epithelialization, thereby demonstrating *in vitro* and *in vivo* wound healing efficacies. Through features such as tunable structure and good mechanical properties, they provide an

optimal microenvironment for cellular differentiation, proliferation and migration (81).

### CONCLUSION

The therapeutic management of DFUs remains confusing, partly due to the large number of contributory and promulgatory factors, as well as, the paucity of products that combine treatment options for all these factors. Local treatment of DFU becomes onerous and clinically unpredictable if different products that address single different factors are employed. In depth discernment of the underlying mechanisms and factors involved in DFUs may help to reveal therapeutically valuable and commercially viable solutions for its treatment. Excipients are a group of relatively inexpensive dosage form enhancers that may possess diverse applicability in DFU dosage form design by virtue of their diverse physiological properties that can be used to treat many of the above factors. Incorporating multiple excipients in a single local dosage form would address the multitude of beneficial properties required of a DFU product and could aid in ameliorating inconsistency in treatment. Despite excipients having been portrayed as commoditized 'inactive excipients', such examples of 'active excipients' may dispel the misguided myth of excipients not possessing any physiological activity or efficacy and drive regulation toward productive and progressive (rather than striving for compliance) ends. The design of a dosage form for DFUs requires the amalgamation of several factors for a beneficial, clinically predictable final result. A combination of these factors, with the specific impetus in the incorporation of active excipients can yield an economical, commercially and clinically viable DFU treating dosage form.

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### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest involved in this study. The authors alone are responsible for the content and writing of the paper.

### AUTHOR CONTRIBUTIONS

V V S Narayana Reddy Karri was the lead author and synthesized the literature. V V S Narayana Reddy Karri, Shashank Mulukutla and Sumeet Sood were involved in drafting the paper. Gowthamarajan Kuppusamy and Rajkumar Malayandi provided conceptual input, design and critical revision of manuscript. All the authors read and approved the final paper.

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