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REVIEW

Current and Emerging Therapies in the Management of Diabetic Foot Ulcers

Veera Venkata Satyanarayana Reddy Karri, Gowthamarajan Kuppusamy, Siddhartha Venkata Talluri, Karthik Yamjala, Sai Sandeep Mannemala, Rajkumar Malayandi

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Abstract

Background: Diabetic foot ulcer is one of the major causes of mortality in diabetic patients. Very few drugs and therapies have regulatory approval for this indication and several agents from diverse pharmacological classes are currently in various phases of clinical trials for the management of diabetic foot ulcers.

Scope: The purpose of this review is to provide concise information of the drugs and therapies which are approved and present in clinical trials.

Review Methods: This review was carried out by systematic searches of relevant guidelines, patents, published articles, reviews and abstracts in PubMed/Medline, Web of Science, clinicaltrials.gov, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and Google Scholar of all English language articles are considered as of 01 March 2015.

The following search terms were used: Diabetes, Diabetic foot, Diabetic foot ulcer, Diabetic wound, Diabetic foot infections, wound management, randomized controlled trials, Approved treatments, new treatments and Clinical trials.

Conclusions: The various drugs and therapies for the management of diabetic foot ulcers comprises of antibiotics, neuropathic drugs, wound dressings, skin substitutes, growth factors and inflammatory modulators. The majority of these therapies target the treatment of diabetic foot ulcer to address the altered biochemical composition of the diabetic wound. However, no single treatment can be definitively recommended for the treatment of diabetic foot ulcers.

REVIEW

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***Key words*:** diabetes; diabetic foot ulcer; diabetic foot infection; approved drugs; clinical trials; new treatments.

List of Abbreviations

ACT	:	Alpha Connexin carboxy Terminus
AhR	:	Aryl hydrocarbon receptor
BBSS	:	Bi-layered bioengineered skin substitute
BM-MNCs	:	Bone marrow derived mononuclear cells
BM- MSCs	:	Bone marrow derived mesenchymal stem cells
Cx	:	Connexin
CHD	:	Cultured human dermis
DF	:	Diabetic foot
DFIs	:	Diabetic foot infections
DFUs	:	Diabetic Foot Ulcers
DM	:	Diabetes Mellitus
DPN	:	Diabetic Peripheral Neuropathic
EGF	:	Epidermal growth factor
FGF	:	Fibroblast growth factor
GABA	:	Gamma amino butyric acid
GJs	:	Gap junctions
G-CSF	:	Granulocyte-colony stimulating factor
HBO_2	:	hyperbaric oxygen
IGF	:	Insulin-like growth factor
IL	:	Interleukin
LMWH	:	Low Molecular Weight Heparin
MMPs	:	Matrix metalloproteases
MOR	:	μ-Opioid receptor
MRSA	:	Methicillin-resistant *Staphylococcus aureus*

MSCs	:	Mesehchymal stem cells
NAC	:	N-Acetyl Cysteine
NIDDM	:	Non-insulin-dependent diabetes mellitus
NMDA	:	N-methyl-D-aspartate
ORP	:	Oxidative reductive potential
PKC	:	Protein kinase C
PSIS	:	Porcine small intestine submucosa
PRP	:	Platelet-rich plasma
P-T	:	Piperacillin-Tazobactam
rh-bFGF	:	Recombinant human basic fibroblast growth factor
rhEGF	:	Recombinant human epidermal growth factor
rhPDGF-BB	:	Recombinant platelet-derived growth factor-BB isomer
SBG	:	Soluble Beta-Glucan
SBP	:	Systolic blood pressure
TGF	:	Transforming growth factor
TJs	:	Tight junctions
TNF	:	Tumor necrosis factor
uPA	:	Urokinase-type plasminogen activator
uPAR	:	Urokinase plasminogen activator receptor
VEGF	:	Vascular endothelial growth factor
WBCT	:	White blood cell therapy

Abstract

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The various drugs and therapies for the management of diabetic foot ulcers comprises of antibiotics, neuropathic drugs, wound dressings, skin substitutes, growth factors and inflammatory modulators. The majority of these therapies target the treatment of diabetic foot ulcer to address the altered biochemical composition of the diabetic wound. However, no single treatment can be definitively recommended for the treatment of diabetic foot ulcers.

1. Introduction & Epidemiology

Diabetes Mellitus (DM) is a severe problem in both developed and developing countries with the reasons being globalization, urbanization, sedentary life style, socioeconomic and cultural changes [1, 2]. A large chunk of chronic diseases that occurred in 2010 were attributed to DM; in hindsight, it has been approximated that 382 million adults were diagnosed with diabetes and this statistic is estimated to rise to 592 million by 2035 [3, 4]. The cost of diabetes related healthcare expenditure was USD 548 billion in 2013, which accounts for 11% of the total adult healthcare cost, which is further projected to exceed USD 627 billion in 2035 [5]. Approximately 25% of DM patients are under risk of developing the diabetic foot ulcer (DFU) and have higher chances of amputation and mortality rate. Early recognition of the high-risk foot and timely treatment will save the lower limbs and improve patients' quality of life [6, 7]. The average cost of healing a single ulcer is USD 8,000 to that of an infected ulcer is USD 17,000 whereas major amputation is USD 45,000. More than 80,000 amputations are performed each year on diabetic patients in the United States [8]. Since, prevalence of diabetes is expected to grow to 592 million by 2035, the burden of diabetic wounds can be expected to increase accordingly [9, 10]. Current diabetic wound treatment hinges on patient education, prevention, and early diagnosis. However, once a wound has developed, invasive therapies are costly while noninvasive therapies are less effective [11]. The delayed DFU healing is due to collective complications such as peripheral arterial diseases, peripheral neuropathy, foot deformity and secondary bacterial infections [12]. Moreover, the abnormal wound microenvironment and pathogenic factors lead to delayed ulcer closure. Diagnosis and therapeutic intervention of DFU patients necessitates an integrated plan of treatment which includes effective local wound care and infection control, optimal diabetes control, restoring pulsatile blood flow and pressure assuaging strategies [4]. The present review discusses about the drugs and therapies approved for treating DFU along with those which are currently in various phases of clinical trials, including their mechanisms of action.

2. Methods

2.1 Search strategy

This review was conducted using a systematic search of relevant guidelines, patents, published articles, reviews and abstracts in PubMed/Medline, Web of Science, clinicaltrials.gov, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and Google Scholar of all English language articles and is considered as of 01 March 2015. Reference lists of original studies and narrative reviews were also searched manually.

2.2 Selection of Studies

We limited interventions to drugs and biologicals, excluding electric devices, procedures, preventative or educational interventions. Interventions were included if they provided or reported sufficient data. Only clinical trials that have been specifically studied in diabetic foot ulcers have been included.

3. Definition and Pathophysiology

Diabetic foot (DF), as defined by the World Health Organization (WHO) is "*The foot of a diabetic patient that has the potential risk of pathologic consequences includes 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*" [13]. DFU is a combination of various risk factors; among them, neuropathy plays a major role. The three major ethiopathologic conditions which are frequently associated DFU are ischemia, neuropathy and infection [14]. The pathogenesis is initiated by the combination of neuropathy and ischemia also called as neuro-ischemia, which is prone to infection. As a result of the absence of protective sensation in the foot, most of the minor injuries are caused due to built-up pressure, mechanical or thermal injury. The detailed mechanism of DFUs [15] is shown in Fig. 1.

3.1. Diabetic Neuropathy

Long-term preponderance of high blood glucose level causes nerve fiber damage, disrupted nerve signal transmission and diminished blood vessel wall strength, which carries oxygen and nutrients to the nerves. There are several pathways associated with diabetic neuropathy such as sorbitol, myoinositol and glutathione [16]. Ethiopathogenesis of Diabetic neuropathy involves many complex mechanisms (remains uncertain); among them nitric oxide blocking and Maillard reaction between sugars and amino acids are most common (Fig. 2). [17, 18]. The mechanisms by which nerve damage occurs includes the formation of advanced glycation end products, activation of protein kinase C, increased levels of reactive oxygen species and nitric oxide blocking [19]. These mechanisms directly or indirectly cause nerve damage (Diabetic neuropathy). Neuropathy may be sensory, motor or autonomic depending on the type of nerve that is damaged [20]. Sensory neuropathy leads to two types of conditions, *i.e.*, either a foot with severe pain or a foot without sensation [9]. The consequences of sensory neuropathy lead to distal symmetric sensory motor polyneuropathy (Loss of sensation), radiculopathy (leg pain) and entrapment syndrome (sensory impairment of plantar skin).

Muscle weakness, atrophy and paresis are associated with motor neuropathy. The imbalance between flexor muscles and extensors lead to claw and hammer toe deformities, which is termed as 'intrinsic minus foot'. Weakening of inter-osseous muscles of the foot causes the disability of intrinsic muscle to maintain the foot in its usual nature and contributes to foot deformity. If the foot undergoes deformation, then the pressure distribution over the foot is variable and abnormal pressure emerges at different points on the foot [21, 22]. It becomes dysfunctional to footwear usage and the abnormal point of contact becomes a pressure area. Due to recurring pressure, keratosis and callus formation occurs. Keratosis development takes place in the presence of sensory neuropathy. Callus formation is the sign of severity of the disease. Excessive pressure exerted on the callused areas damage the foot tissues and induces ulcer formation under the callus that further cause cracks on the foot. 20 times higher pressure is exerted on callus point than the surrounding skin [23-26]. Crowding of toes (crowded toes tend to overlie with each other on slanted direction which adjacent toes with other), Cock up toes (a toe could cock up and is not in the same level as the other toes of the foot; this makes them susceptible to trauma) and Clawing of toes (bending of toes) are the common deformities caused by motor neuropathy [27-31].

In autonomic neuropathy, reduction in the flair reaction to a noxious stimulus results in the restricted blood flow at the wound/infected sites [32]. Pseudo motor dysfunction and arterio-venous shunting in the foot are the complications that occur due to autonomic neuropathy. Malfunctioning of the sympathetic nerves supplying the sweat glands in the foot reduces the sweat and moisture in the feet. Therefore, the skin develops cracking due to the dryness and low moisture content in the foot [33]. This pathophysiological condition is called as pseudo motor dysfunction. The nerves in autonomic neuropathy produce an autosympathectomy like state that results in warm excessively dry feet susceptible to skin breakdown as well as functional alterations in microvascular blood flow [34]. Diabetic neuropathy leads to foot deformation or restricted joint mobility, which in turn develops the abnormal pressure in the foot followed by callus formation at the abnormal pressurized points. Local pressure in the foot is elevated due to the callus. Further, combining with unnoticed injury develops inflammation, necrosis and ulceration [35, 36].

3.2. Peripheral Vascular Diseases

Peripheral arterial disease is another prime reason for the formation of foot ulcers in almost 50% of diabetic patients [37]. . In the early stages of diabetes, microcirculatory deficiencies are developed, which may be reduce capillary size, thickening of basement membrane and arteriolar hyalinosis. Thickening of basement membrane disturbs the physiological exchanges, alters the leukocyte migration, lowers the maximal hyperemia and alters auto regulatory capacity [37-39]. Endothelial dysfunctioning reduces the nitric oxide synthetase but lumen size of micro vessels remains normal [40]. Although the lumen occlusion is absent in the blood vessels, the blood flow is altered, which leads to change in bone alignment and develops pressure in insensate foot. Accelerated atherosclerosis is most common in diabetic patients. The most affected sites are the tibial and peroneal arteries of the calf portion. Development of atherosclerotic plaque in these vessels decreases the blood flow followed by occlusion of larger vessels and further leads to stroke, myocardial infarction, ischemia and formation of non-healing DFU. Another form of ischemia is the decreased angiogenesis in the diabetic wounds. Persistent hyperglycemic state is developed due to endothelial dysfunction and smooth cell abnormalities and this is followed by vasoconstriction due to the reduction of vasodilators. Further, the elevated vasoconstrictor-thromboxane A2 and platelet aggregation agonist develops plasma hyper-coagulability [37]. Further, vascular extracellular matrix modulations will cause the development of stenosis in the arterial lumen. Vasculopathy and neuropathy are inter-linked in the development of DF. Altered blood flow arises due to the shunts in microcirculation, sympathetic nerve degeneration and autonomic neuropathy. Hypertension, cigarette smoking and hyperlipidemia are some of the contributing factors for the formation of the peripheral arterial disease. All the factors collectively contribute for the development of occlusive arterial disease that leads to ischemia and ulcer formation in diabetics [39-42]. Among the various classification systems that have been used for the DFUs, the Wagner classification system and Texas classification system are the most popular [43]. Also recently a risk stratification classification System: based on Wound, Ischemia, and foot Infection (WIfI) has been introduced for lower extremity threatened limb by the Society for Vascular Surgery (SVS) [44].

3.3. Diabetic foot infections (DFIs)

Foot infections are a common cause of death and disability in people with diabetes, accounting for at least half of all non-traumatic lower-limb amputations. Infections range in severity from simple cellulitis to deep soft-tissue infection, necrotizing fascitis, and chronic osteomyelitis. Among diabetic patients with deep soft-tissue infections, 70-83% of the infections are polymicrobial at the time of diagnosis [45, 46]. Therefore, empiric therapy should include broad-spectrum antibiotics. The most common pathogen in DFIs is *S. aureus* and groups A and B *streptococci*. Patients previously exposed to antibiotics are more likely to have methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* isolated from their wounds [17]. Armstrong et al. reported MRSA in up to 25% of all DFIs involving *S. aureus* [47]. Gram-negative, gram-positive aerobes and anaerobes are typically detected in a single tissue sample. In nearly all cases, anaerobes are found only in the presence of aerobes [45, 48]. As with milder infections, *staphylococci* are common pathogens in patients with severe foot infections. Also found in severe, mixed, soft-tissue infections are Proteus, Klebsiella, and *Escherichia coli (E.coli)*. Among the anaerobes, *Peptostreptococcus* and *Bacteroides* are the most frequent. *Bacteroidesfragilis* is common in chronic osteomyelitis [49, 50]. In treating diabetic deep soft-tissue foot infections, attention should be directed to adequate debridement of devitalized tissue and administration of antibiotics. For non-limb-threatening infections, debridement of necrotic soft tissue and any involved bone should be performed as soon as possible. Since it is sometimes difficult to determine the viability of local tissue, serial debridement maybe performed [45, 51, 52]. Depending on the location/type of infection, the treatment range from single-agent therapy with anti-staphylococcal and anti-streptococcal antibiotics for cellulitis, to treatment with broad-spectrum antibiotics those are effective against gram-positive, gram-negative, aerobic, and anaerobic pathogens for deep soft-tissue/chronic bone infections. The most commonly used broad-spectrum agents are carbapenems or β-Lactam, β-Lactamase inhibitor combinations such as piperacillin/tazobactam, ampicillin-sulbactam, and ticarcillin-clavulanic acid. Antimicrobial therapy along with surgical treatment or debridement is essential for treating any chronic deep infection in bone.

4. Current Standardized Treatment Approach to DFUs

At present, DFU care primarily comprises of diagnosis for vascular diseases, neuropathy and infections of the skin, soft tissue or bone [53]. While vascular disease and infections require vascular supply optimization and antibiotic treatment, neuropathic foot ulcers require pressure redistribution (off-loading) which is of critical importance. Pressure off-loading approaches are varied, that include wheelchairs, crutches, foot inserts and therapeutic shoes, bed rest, casts and surgical procedures [33]. Due to its association with high healing rates, the Total Contact Cast is deemed the 'gold standard' off-loading device world over; however, its use is restricted to staff well trained in application and removal, with accompanying limitations such as improper application leading to trauma, contralateral foot ulcer, significant arterial insufficiency, balance problems and contraindication in the event of infection [33]. Poor patient compliance, in most instances affects successful off-loading and as a consequence, these devices hinder with the performance of daily activities [54]. Debridement is an integral component of DFU standard care, permits the removal of necrotic tissue, reduction of bacterial biofilms and excess MMPs and callus and abnormal edge tissue [3]. The types of debridement include surgical, enzymatic, autolytic, mechanical and biologic of which surgical debridement is mostly preferred [12]. Tissue regeneration products (Apligraf™, Dermagraft™ and GraftJacket™) and growth factors are considered for superficial wounds. Hyperbaric oxygen and negative pressure wound therapy are used for deep seated and complicated wounds [55].

5. USFDA approved drugs and therapies for treating various complications associated with DFUs

5.1. Neuropathic and antibiotic Drugs (Table 1)

USFDA has approved three drugs for the treatment of diabetic peripheral neuropathy, *viz*., duloxetine, anticonvulsant pregabalin and opioid tapentadol [56]. Three drugs are approved for the treatment of DFIs in the complicated skin and skin structure infections (cSSSI) indication: piperacillin/tazobactam combination, trovafloxacin, and linezolid. Specifically for osteomyelitis associated with overlying DFIs, there are currently no drugs/agents that have been approved [57]. The detailed clinical trials published on various drugs and therapies specific to DFUs treatment are summarized in Table 2.

5.2. Bioengineered Skin Substitutes/ Soft Tissue Substitutes

5.2.1. Amniotic membrane

Natural human amniotic membrane has been used as a wound covering for over 100 years. It belongs to the innermost layer of placenta consists of epithelial layer, a basement membrane and an avascular stroma. They provide biologically active cells and important regenerative molecules along with structural support to ECM. Type IV, V and VII collagen acts as substrate which maintains structural integrity and also facilitates cellular infiltration and wound healing. Amniotic membrane contains essential growth factors and cytokines that may enhance the healing process. It also found to posses some antimicrobial activity and reduced inflammation at the site of application [58].

5.2.2. Autologous stem cell therapy

Stem cells are capable of self-renewal and multilineage differentiation has been studied in the damaged tissues of DFUs. Among the different types of stem cell therapies bone-marrow (BM)-derived mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) are most successful clinically. MSCs containing multipotent progenitors are capable of differentiating into cells of numerous tissue lineages. MSCs have previously proven clinically to repair or regenerate somatic tissues to treat severe graft versus host disease in allogeneic stem cell transplantation. MSCs fill the dermis of the skin and can alter the composition (dermal, vascular, and other components) of chronic wound which helps in optimal wound healing. BM-MNCs are a group of differentiated cells from many kinds of stem cells including hematopoietic stem cells, endothelial progenitor cells, mesenchymal stem cells, precursor cells, and their progeny. MNCs are abundant in bone marrow and peripheral blood. Therapeutic application of autologous stem cell based therapy has revolutionized the field of regenerative medicine [59, 60].

5.2.3. Bi-layered bioengineered skin substitute

It is a living, bi-layered skin substitute where the epidermal layer consists of well-differentiated stratum corneum developed by human keratinocytes. This skin substitute consists of human fibroblasts in a bovine Type I collagen lattice, matrix proteins and cytokines. It is devoid of macrophages, melanocytes, langerhans cells, lymphocytes, hair follicles and blood vessels. It is used for the treatment of DFU with a greater than 3 week's duration. It is recommended for the management of non-infected partial and full-thickness skin ulcers that extend through the dermis but without tendon, muscle, and capsule or bone exposure; those which haven't been effectively treated by conventional ulcer therapy and those with venous insufficiency of greater than 1 month duration [61].

5.2.4. Human fibroblast-derived dermis

It is a biological substitute synthesized from human fibroblast cells. The human fibroblast cells are produced from the newborn foreskin tissue, which consists of fibroblasts, extracellular matrix and it is available as a bio-absorbable polyglactin mesh scaffold. The fibroblast proliferate and generates the matrix proteins, human dermal collagen, cytokines and growth factors which fill into the interstices of this scaffold to construct a three-dimensional human dermal substitute consisting of metabolically active living cells. It is devoid of lymphocytes, macrophages, blood vessels or hair follicles. It is indicated in the treatment of long term and severe cases of DFU (Long term and severe cases where DFU is persistent for more than six weeks duration, and spreading through the dermis, but without tendon, muscle, joint capsule or bone exposure) [61] .

5.2.5. Porcine small intestine submucosa

Porcine small intestinal submucosa (PSIS) is an acellular, biological ECM consists of type I collagen, glycosaminoglycans and proteoglycans which simulate native ECM. The porous microstructure of PSIS enables the oxygen diffusion. It supports cellular infiltration, adherence, proliferation, and differentiation of numerous cell types. The bioactivity of SIS includes releasing growth factors (TGF-β1, VEGF and FBF-2), minimizing the destructive activity of MMPs, and inducing angiogenesis to support new blood vessel in growth. SIS is biodegradable and can be well incorporated into tissue [62].

5.3. Growth Factors

DFU is the major cause of morbidity in diabetic patients. Even though across-the spectrum conventional treatments are available, there remains a group of patients with non-responding wounds, usually resulting in amputation. These types of wounds may benefit from growth factors which cause molecular manipulation in the wound micro-environment. Local and systemic application of these growth factors appears to signal a significant role for their therapeutic use in the treatment of DFU.

5.3.1. Platelet Derived Growth Factor (PDGF)

It is synthesized using recombinant DNA technology by insertion of the gene for the B chain of platelet-derived growth factor (rhPDGF-BB) into the yeast, *Saccharomyces cerevisiae*. It is concerned with the chemotactic recruitment and segregates the cells which involve in wound repair and develop the granulation tissue. It forms granulation tissue in DFU, healing them by promoting the chemotactic recruitment, promotes angiogenesis and induces fibroblast proliferation and proliferation of cells involved in wound repair. It is indicated for treatment of infection which is into the deeper subcutaneous tissues or beyond the tissues having an abundant blood supply [63].

5.4. Debridement

DFUs are characterized with excessive necrotic tissue; as the wound is stagnated in the chronic inflammatory stage, this halts further wound healing and acts as barrier for application of topical therapeutics over the wound. Debridement is a crucial step in these types of chronic wounds. Debridement is a process of removing damaged and necrotic tissue for advancing the healing of the remaining healthy tissue. It may be by chemical, mechanical, autolytic and surgical.

5.4.1. Hydrogel

Hydrogels rehydrates necrotic tissue, liquefies hard eschar and loosen the slough thereby promotes debridement. They promote granulation, epithelialization, and autolytic debridement. Hydrogels also provides the optimum moist wound healing environment during the later stages of wound healing. Hydrogel as a method for debridement is more effective than gauze or standard care in healing diabetic foot ulcers [64].

5.4.2. Maggot Therapy

It is a stage I and II viable-larva of the green bottle fly *Lucilia sericata*, packed in a sterile bag. The larvae are developed from disinfected fly eggs. These open mesh polyester bags are available in different sizes based on the dosage (number) of larvae to be packed. The bag confines the larvae on the wound and avoids the displacement of larvae away from it. Sterile polyvinyl alcohol foam cubes present in the bio bags are the ‘spacers’ that provide free mobility for the larvae within. These small maggots consume necrotic tissue more effectively than standard surgical debridement within a day or two. Maggot therapy is indicated for cleansing the non-curative necrotic skin and soft tissue wounds, including pressure ulcerations, venous stasis ulcers, neuropathic foot ulcers, and non-healing traumatic or post-surgical wounds [65].

5.5. Products in Pipeline

5.5.1. Flowable Bovine Collagen

It is a well-refined fibrillar flowable bovine collagen. Unlike regular collagen in biological scaffolds (cross-linked collagen), it contains fibrillar collagen *i.e.*, non-cross linked collagen. Collagen flowable wound matrix is the most advanced wound care matrix and it is a flowable (liquid) version of collagen scaffold. The tunneled wounds are having uneven geometry which is difficult to treat effectively. The matrix is reconstituted with saline and administered through a syringe with a flexible injector on the tunneled/complicated wounds. This flowable wound matrix can fill into the wounds, deep crevices and provides maximum contact on the wound bed. The mechanism behind this collagen flowable matrix is same as that of conventional collagen scaffold, except its physical nature, which allows intimate contact between the grafting material and the wound bed (deep creviced wounds). This non cross linked collagen gel has the ability to have PDGF bind to it to help the migration of human dermal fibroblasts. It is triggered by a miniscule quantity of blood in the wound. An advantage in the lack of collagen crosslinking lies in the fact that it aids the regeneration of skin [66].

5.5.2. Recombinant human epidermal growth factor (rhEGF)

rhEGF is applied by intralesional injections directly in the wound site for peri- and intra-lesional infiltration. Injecting rhEGF deep into the wound base and walls would allow for greater pharmacodynamic response in terms of granulation tissue growth and wound closure. Systemic or local injections of rhEGF have produced clear-cut cytoprotective and proliferative responses, suggesting an intrinsic ability of rhEGF at supra-physiological concentrations to trigger biological events necessary for tissue repair. rhEGF can enhance healing of chronic wounds following repeated local infiltrations. rhEGF increased wound cell infiltration and accelerated healing in poor prognosis wounds towards a rapid and sustained response. Some of the other products cleared by FDA are Amnionic membrane (MiMedx), Porcine intestinal membranes (KCI), Bovine dermal membrane (Intergra Life Sciences), Autologous Stem Cell (Osiris Therapeutics Inc.).

6. Drugs and therapies in clinical trials

Various drugs and therapies are currently in clinical trials for the treatment of DFUs. These studies contain drugs, tissue biologicals, medical devices and procedures (Fig. 3). The majority of these studies were found to be of topical application and involved the use of novel therapies to address multiple and altered biochemical pathways of DFU. Most of them are in the initial stages of evaluation for their safety and efficacy. These studies are summarized in Table 2.

6.1. Neuropathic and antibiotic Drugs

6.1.1. Thioctic Acid (alpha-lipoic acid)

For diabetes and its related neuropathic symptoms including burning, pain, and numbness in the legs and arms, Thioctic acid is extensively used. Alpha-lipoic acid seems to delay or reverse peripheral diabetic neuropathy through its multiple antioxidant properties. Treatment with alpha-lipoic acid increases reduced glutathione, an important endogenous antioxidant. It aids in preventing certain forms of cellular damage in the body and also reestablishes vitamin E and vitamin C levels. Thioctic acid is recommended for diabetics and nerve-diseased diabetic patients. Alpha-lipoic acid, naturally occurring anti-oxidant agent involves in delay or reversal of peripheral diabetic neuropathy. It is a dithiol derivative involved in mitochondrial biological reactions by reducing the oxidation of stress in neurons and other tissues. It is strong reducing agent, which deactivates the reactive oxygen species and reduces other oxidized forms. It's well tolerated and well documented molecule for the diabetic neuropathy treatment, which is clinically effective at a dosage of 600 mg daily and also at 1800 mg daily for some patients [67-69]. Alpha-lipoic acid is available as over the counter drugs and also present in other neuropathy treatment products. It's evident that thioctic acid ameliorates the functioning and conduction of neurons [70, 71].

6.1.2. Daptomycin

Daptomycin, obtained by *Streptomyces roseosporus* fermentation, is an anti-bacterial agent comprising of a 13 member amino acid cyclic lipopeptide with a decanoyl side chain [72]. Although the specific mechanism responsible for its activity has not been elaborated upon, it is hypothecated to be active via the calcium-dependent insertion of its lipophilic tail into the bacterial cell membrane, effecting rapid depolarization and potassium ion expulsion [73]. It has also been demonstrated that daptomycin induces a calcium-dependent dissipation of the membrane potential (Δ psi) in *S. aureus* without actually impressing on the chemical gradient (Δ pH) across the membrane [74]. This unique hypothesis could be the reason for its bactericidal efficacy.

6.1.3. Gentamicin-Collagen Sponge (G-C Sponge)

G-C sponge is a hemostyptic, resorbable and biodegradable collagen sponge that contains the broad spectrum aminoglycoside antibiotic-gentamicin, for local protection from DFIs. This drug delivery system consists of gentamicin sulfate, a water soluble broad spectrum aminoglycoside antibiotic which is uniformly dispersed in a type I collagen matrix for the management of DFUs [75]. Gentamicin is active against most strains of aerobic gram-negative and gram-positive pathogens including MRSA [76]. Additionally, it is indicated for skin infections as creams and ointments, which contain 0.1% whereas gentamicin collagen sponge contains 27%. It has a concentration dependent mechanism of action which ensures a higher concentration of drug at the target wound tissue. High dose of gentamicin in this system is likely to reduce resistance and elevate the efficacy. Topical application of Gentamicin may develop resistance but a recent study proved that there was no resistance found when used as ocular antibiotics [77-79].

6.1.4. Moxifloxacin

Moxifloxacin belongs to the fourth-generation synthetic fluoroquinolone anti-bacterial agents. It is active against most aerobic and anaerobic gram-positive and gram-negative species. Moxifloxacin has significant antimicrobial action on aerobic (90.8%) and anaerobic (97.1%) microorganisms [80, 81].

6.1.5. Nemonoxacin

Current therapies for the treatment of drug resistant bacterial infection are becoming less effective. Nemonoxacin belongs to the class of non-fluorinated quinolone anti-bacterial drugs that acts by inhibiting bacterial DNA topoisomerase enzyme [82, 83]. It is available both in oral and intravenous formulations. Clinical trials reports revealed that nemonoxacin has significant anti-bacterial action against the virulent drug-resistant bacterial species such as MRSA, vancomycin-resistant enterococci and acinetobacter baumannii species. Community-acquired pneumonia and DFIs' treatment has been modulated to incorporate nemonoxacin for its specificity [84].

6.1.6. Pexiganan

Pexiganan, is an 22 amino acid peptide, naturally available from the skin of African Clawed Frog. It exhibits bactericidal activity by disruption of bacterial cell membrane. Further, pexiganan has distinct advantages over other anti-microbial agents by its broad spectrum (gram-positive, gram-negative, aerobic, and anaerobic bacteria) and fungistatic activity. Additionally, recent studies suggest that highly resistant bacteria such as vancomycin-resistant enterococcus and methicillin-resistant staphylococcus aureus are sensitive to pexiganan

6.2. Growth Factors

6.2.1. Granulocyte-colony stimulating factor (G-CSF)

Immunodeficiency condition by white blood cell dysfunction contributes to susceptible infections in patients with DFU. G-CSF is cytokine which can directly act on neutrophil restricted progenitor cells in their proliferation. It increases chemotaxis by enhanced binding of polymorphonuclear leukocytes to chemotactic peptides [85]. G-CSF specifically regulates proliferation and differentiation of neutrophilic granulocyte precursors and stimulates the function of mature neutrophils [86].

6.2.2. Platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) is a portion of blood plasma that is enriched with platelets. It is a fraction of autologous blood platelets, which contains various growth factors and cytokines that stimulate wound healing by attracting undifferentiated cells into the site of injury following triggering cell division [87]. PRP also has been referred as platelet-rich concentrate, platelet-enriched plasma, platelet releasate and autologous platelet gel [88]. It is long lasting and cost effective than the recombinant human growth factors and also being an autologous source, it is free from communicable pathogens. Signaling proteins of platelets in PRP attracts macrophages and plays a role in host defense mechanism at the wound site. PRP has also shown antimicrobial properties against *E. Coli*, MRSA, *Candida albicans* and *Cryptococcus neoforrmans* [87].

6.2.3. Recombinant human basic fibroblast growth factor (rh-bFGF)

rhbFGF is a recombinant human basic fibroblast growth factor (heparin-building single-chain peptide of 146 amino acids), which is being developed for the treatment of wound repairs. It is produced by recombinant DNA technology using *Escherichia coli* type B [89]. It has a ubiquitous distribution in mesoderm- and neuroectoderm derived tissues, this is a potent mitogen for all cell types involved in the healing process [90]. rhbFGF stimulates angiogenesis, cell proliferation, migration, differentiation, neo-vascularization, re-epthelialization and collagen disposition which contribute towards wound healing. rhbFGF is a potent mitogen for all cell types involved in the wound-healing process and is highly angiogenic and chemotactic for fibroblasts and endothelial cells [91]. It provides sufficient neuroprotection at the site of the wound. It stimulates chemotaxis of the mesodermal cells and growth for the extracellular matrix. It expedites both acute and chronic wound healing which in turn gives a scar-free cure [92].

6.3. Modulation of Inflammation

6.3.1. Alpha connexin carboxy terminus (ACT)-1

Connexins are the gap junction proteins, which are involved in organizing cell to cell communication via gap junctions (GJs). Due to the interactions between the gap junctional connexins with tight junctions (TJs), the resultant effect is cell-to-cell contact. GJs and TJs are concerned in controlling the regulation of cell proliferation, migration, differentiation, and tissue development [93, 94]. These proteins have a vital role in tissue refurbishment and inflammatory activity. The platform technology has been established and wide variety of proteins were developed and ultimately screened for their therapeutic values. ACT peptide, including the C-terminus sequences of connexins (Cx43, Cx37, Cx45, Cx40, and Cx26) has been synthesized by the technology platform. Wound healing is initiated by the intercellular communication via Cx43 GJs and research has been forwarded in developing the lead peptide ACT-1 [95, 96]. ACT-1 is a small peptide (25 amino Acids) that mimics the C-terminus of the trans membrane protein Cx43, which is prevalent among all connexins. ACT-1 has a unique feature that triggers the body's own healing response from inflammation followed by tissue regeneration. ACT-1 is multi-functional, where it stabilizes GJs (intercellular communication) as well as TJs (intercellular contacts) of endothelial cells during the wound healing, thereby, in the process promoting the cellular communication, dampened excessive inflammation response and normal tissue regeneration [97]. In a randomized clinical trial on 300 patients, application of ACT-1 (100μM) resulted in highly significant increases in mean percent wound closure at four and twelve weeks as well as incidence of 100% wound closure, along with significantly reduced time for complete wound closure. ACT-1 was also shown safe and well-tolerated with no drug-related systemic or local adverse events. However, this evidence has not been confirmed by publication of full trial results.

6.3.2. Antisense oligonucleotide

Antisense oligonucleotides are single strands of DNA or RNA. Antisense oligonucleotides are single strands of DNA or RNA. The gap junction protein Cx43 plays an important role in the wound healing process and hypothesized that its down-regulation would accelerate that process. This topically applied unmodified antisense oligonucleotide down-regulates the key gap junction protein Cx43 to dampen inflammatory responses and enhance healing [98, 99]. It was demonstrated on abnormal up-regulation of Cx43 at the edge of wounds in the streptozotocin-diabetic rat model. By reducing the Cx43 expression using Antisense oligonucleotide, it is possible to restore healing rates to normal or better [100]. Subsequent preclinical studies of biopsy samples from a wide variety of human chronic wound samples also showed a striking over-expression of Cx43 protein [101].

The other proposed mechanism was aryl hydrocarbon receptor (AhR) or dioxin receptor modulated cell migration and plasticity. Activation of this receptor results in severe skin lesions such as chloracne, contact hypersensitivity, and dermatitis. AhR absence will enhance keratinocyte migration, accelerating re-epithelialization of skin [102, 103]. Carvajal-Gonzalez JM et al. [104] have performed wound healing studies in a mice model using wild-type (Ahr+/+) and AhR-null (Ahr-/-). Results revealed that *in vivo* treatment with antisense oligonucleotides has down-regulated the AhR and thereby improved re-epithelialization. Hence, antisense oligonucleotides could be a potential new tool for the treatment of chronic wounds like DFU.

6.3.3. Chinese herbal medicine (*Radix rehmanniae* and *Radix astragali*)

Recently, traditional Chinese herbal medicine (CHM) is extensively applied is thought to be a substitute to mainstream medicine in different diseases. *Radix astragali* (*R.astragali*) and *Radix rehmanniae* (*R.rehmanniae*), the principal component herbs were efficient in increasing fibroblast proliferation, the major step in wound healing. *R.astragali* boosts the functioning of "Qi" which is concerned in wound healing and muscle re-formation, whereas *R.rehmanniae* involves in lowering the heat in blood, nourishing the "Yin" and elevating the body fluid production [105, 106]. According to the Chinese medicine theory, these two phytoconstituents possess high healing activity on ulcers and generate the anti-inflammatory effects [107]. Additionally, *R.astragali* shows improvement of insulin resistance and *R.rehmanniae* shows regressive effects in diabetic nephropathy [108]. In a study conducted by Chen M et al., [109] on clinical trials of CHM, it was concluded that CHM may be effective and safe as an adjunctive therapy for treating DFU. Nevertheless, a firm conclusion could not be reached because of the poor quality of the included trials.

6.3.4. Doxycycline Monohydrate

Doxycycline is a tetracycline antibiotic which has matrix metalloproteinase (MMP) inhibitory activity and tumor necrosis factor-α (TNF-α) converting enzyme activity [110, 111]. It is applied on the surface of the wound along with a secondary dressing or non-adhering dressing. The secondary dressing provides secure and moist environment to the wound. High levels of pro-inflammatory cytokines, TNF-α and Interleukin-1-beta (IL-1β), abnormal levels of proteinases (MMPs) and neutrophil elastase are found in the DFUs where these endogenous growth factors, enzymes and proteins prevent wound healing [112, 113]. Doxycycline is the molecule concerned with lowering the inflammation and anti-MMP activity, thereby leading to wound healing. In addition doxycycline is used as an antibiotic orally for the treatment of DFU [114].

6.3.5. HO-0303 (Protein Kinase C Inhibitor/Stimulant)

HO-0303, is a topical therapeutic agent for the management of chronic wound healing, including DFUs, venous leg ulcers, and pressure ulcers. It consists of protein kinase C (PKC) modulating agents and exhibits synergistic effects *i.e*., PKC_{α} (re-epithelialization) activation and PKC_{δ} (delayed re-endothelialization) inhibition to promote the wound healing [115]. It mediates by various several biochemical pathways which promote rapid and complete epidermal closure. These biochemical pathways include migration of the skin cells towards the wound site, accelerating the granulation tissue formation, proper matrix deposition for the dermal reconstruction and isolation of the pathogens, thereby diminishing the inflammatory response [116].

6.3.6. N-acetylcysteine (NAC) on diabetic foot oxygenation

Breathing of pure oxygen (100%) in a pressurized room is called hyperbaric oxygen (HBO_2) therapy [117, 118]. It will improve wound tissue hypoxia, reducing edema, enhances perfusion, promoting fibroblast proliferation, down regulating inflammatory cytokines, angiogenesis and collagen production. These advantages make use of hyperbaric oxygen therapy as an adjunct therapy for "problem wounds," such as DFUs. However, hyperbaric oxygen therapy in DFU patients, especially those with vascular diseases has variant tissue oxygen concentration level due to the vasoconstriction mechanism (decreased nitric oxide bioavailability) and exaggerated oxidative stress [119, 120]. NAC is a pre-cursor for the amino acid cysteine and an anti-oxidant. Hence, NAC administration may induce modulation of both parameters and there by improved ulcer oxygenation during hyperbaric oxygen therapy.

6.3.7. Soluble Beta-Glucan (SBG)

In DFU complications, the macrophages malfunctions and wound healing fails. Therefore, immunomodulatory beta-glucans will help in the treatment, as they are highly potent in resuming the normal actions of the malfunctioning macrophages. β-Glucans are the natural medicines which are known to sensitize the macrophages and helps in wound repair

[121, 122]. Beta-glucans specifically bind to the leucocytes, macrophages, dendritic cells, granulocytes and other cells present on the cell surfaces. SBG is of microbial origin, where it is synthesized by *Saccharomyces cerevisiae.* Beta–glucans have additional properties such as moisture maintenance and absorption of the exudates from the wound which promote wound healing. SBG shows slightly acidic (pH 6), which helps in wound healing and decreases the protease activity in the wound. SBG stimulates secretion of cytokines and modulates inflammation. As SBG stimulates macrophages in inflammatory stage but also coordinate in other stages in wound healing [123]. SBG is involved in modulation of immune response by acting on cellular receptors in cells of innate immune system [123]. In vivo studies proved that SBG is a potent enhancer of immune functions and this can be clinically translated for the development [124, 125].

6.3.8. WH-1

WH-1 constitutes extracts from two botanical raw materials, Plectranthus amboinicus Lour. (Lamiaceae) and Centella asiatica Linn. (Umbelliferae). *P. amboinicus* and *C. asiatica* are proven to possess anti-inflammatory and healing properties relevant to wound treatment [126]. *P. amboinicus* is one among approximately 300 botanical species in the Plectranthus genus of the Lamiaceae family, which is identified for its myriad applicability, in particular as medicines for skin, infective, digestive and respiratory disorders. *In vivo* evaluation (rat model) of anti-inflammatory and anti-tumor activities of an extract of *P. amboinicus* leaves demonstrated a substantial edema reduction and confirmed the anti-inflammatory properties at specific dose levels [127]. This anti-inflammatory effect was attributed to antioxidant enzyme activity modulation in the liver and TNF-α generation.

6.4. Vasodilators

6.4.1. Nitric Oxide Releasing Patch

In normal conditions, nitric oxide is synthesized and secreted in the human body and is involved in the wound healing process [128]. It has a key role in collagen synthesis, chemotactic cytokines release, blood vessel permeation, enhancement of angiogenic actions, epidermal growth factors release and bacterial mitochondrial respiratory chain disruption [129-131]. However, nitric oxide is unstable and needs to be applied frequently. Therefore, this limitation provided a formulation strategy to use nitric oxide releasing polymeric groups like S-Nitrosothiols that gives constant nitric oxide (NO) release [132, 133]. NO stability and its release pattern has led to the development of a new NO releasing patch and this device is produced by the electro-spinning technique [134]. Various studies, performed in murine models, have demonstrated the role of NO in the healing process. The levels of the final metabolic products from NO (nitrite and nitrate) rise during the first two days more than subsequently in the liquid recovered from the sponges previously placed in the subcutaneous tissue of healthy subjects' wounds, increase that is not observed in diabetic subjects, suggesting an impairment in the cutaneous production of NO in diabetic individuals. The topical use of NO accelerates the wound healing process of excision wounds [135-137].

6.5. Debridement

6.5.1. Pirfenidone

DFUs can lead to excessive scarring. In the chronic wound healing process of DFUs reducing scarring is required for fibroblast migration, proliferation and epithelialization. Leftover scar tissue may also get infected. Pirfenidone is a pyridone analogue belongs to the class of anti-fibrotic drugs that acts by reducing fibrosis. It has a good effect to inhibit skin scarring of wounds by inhibiting the production of tissue erosion and improves tissue granulation and epithelialization in the proliferative phase of wound healing. Pirfenidone also have significant anti-inflammatory and antioxidant [138].

6.6. Others wound healing promoters.

6.6.1. Angiotensin analogue

It is analogous to angiotensin, the naturally occurring peptide. It has been synthesized and developed to promote wound healing without interrupting the angiotensin's physiological functions. The salient mechanism of this drug involves mesenchymal stem cells (MSCs) up regulation in the wound [139]. MSCs are produced from the embryo and are without a specific cellular phenotype. The cells distinguish into a variety of cells within the body, namely adipose cells, fibroblasts, osteocytes, muscle cells, and keratinocytes. It elevates the keratinocyte proliferation, extracellular matrix production and vascularization. Moreover, some histological studies reported that angiotensin analogue increases collagen deposition by six-folds. Extensive pre-clinical studies have demonstrated the ability of angiotensin analogue to accelerate healing and reduce scar formation [139].

6.6.2. Low Molecular Weight Heparin (LMWH)

A sulfated glycosaminoglycan, Heparin is extensively utilized as an injectible anti-coagulant by showcasing its anti-fibrinolytic and anti-thrombic effects [140]. Heparin is widely touted to possess honorable virtues on local tissue microcirculation and oxygenation, by means of the inhibition of thrombin generation and amelioration of fibrin gel porosity. This vastly favors fibrinolysis, thereby portraying its applicability in DFUs. Aside from its significant anti-thrombotic benefits, heparin is also reported to be viable *in vitro*, encouraging heparin sulfate synthesis in endothelial cell cultures [141]. In addition to this, increment of fibroblasts sourced from DFUs, forbidding endothelial basement membrane damage and enhancing the capillary strength and count are some of its reported merits [142]. Heparin treatment for venous thromboembolism as a cautionary measure appeared to have demonstrated substantial gains in diabetic patients with long-term foot ulcers [143].

6.6.3. Iroxanadine

Iroxanadine is one of the hydroxylamine derivatives. In DFUs, cells like fibroblasts and endocytes are in high stress levels and lose their integrity. The stress proteins/heat-shock proteins (molecular chaperones) are essential for the cell integrity sustenance during regular growth, in addition to during pathophysiological conditions, and therefore can be conceived as "homeostatic proteins". Establishment of eukaryotic chaperone molecules is facilitated by Iroxanadine by hyperbolizing the heat shock protein expression, thereby regularizing normal cellular protein repair mechanisms by the initiation or suppression of molecular chaperones in DFUs . It also has the ability of refolding the incompatible proteins into appropriate shape and non-toxic form [144].

6.6.4. Moist Exposed Burn Ointment

It is a phyto-drug complex comprising of alkaloid-sterol-flavonoid *i.e.,* berberine-betasitosterol-baicalin. It exhibits synergistic effects due to the triple combo of phyto-drug molecules. Berberine possess anti-oxidant, anti-bacterial, anti-microbial and vasodilator properties. Beta-sitosterol has anti-inflammatory activity. Baicalin is having anti-thrombotic, anti-oxidant, anti-bacterial and anti-inflammatory properties. The safety and efficacy data of MEBO wound ointment is not yet available [145] .

6.6.5. Phenytoin

Phenytoin is used as an anticonvulsant medication for effective control of convulsive disorders with a common side effect being gingival hyperplasia [146]. This unwanted stimulatory side effect of phenytoin suggests its use in wound healing. In addition, it may have the potential to alter the dynamics of wound healing through a stimulatory effect, which can induce the growth of connective tissue, and may have the ability to promote wound healing. Phenytoin promotes wound healing by various mechanisms that includes stimulation of fibroblast proliferation, enhancing the formation of granulation tissue, decreasing collagenase activity, inhibition of glucocorticoid activity and direct or indirect antibacterial activity by affecting inflammatory cells and neovascularization [147].

6.6.6. Urokinase

Urokinase, also termed as urokinase-type plasminogen activator (uPA), is a serine protease synthesized and secreted by keratinocytes. It is majorly expressed at times of wound repair by migrating keratinocytes [148]. Neither uPA nor its corresponding receptors are expressed in normal, healthy epidermal conditions and are down-regulated upon skin integrity restoration. When receptor bound, uPA induces focal extracellular proteolysis at the wound periphery during re-epithelialization phase. uPA stultification is stipulated by fibroblasts, delayed cellular infiltration, granulated tissue and re-epithelialization [149]. Surface application of uPA on profound wounds was described to hasten the healing process in vitiated diabetic mice and their conventional littermates [150].

6.6.7. White Blood Cell therapy (WBCT)

It is an assortment of white blood cells constituting neutrophils, monocytes/macrophages and lymphocytes. It contains a different array of active cells which can treat dissimilar wound types with varying phases of inclemency. These cells are obtained from young (18-40 yrs old), healthy blood donors. WBCT incorporates the functionally-active immune cell in the inflammatory environment for wound healing. These functionally active immune cells re-establish the usual environment at the wound area, release the growth factors and stimulate phagocytosis of bacteria and dead cells for wound healing. Among the white blood cells, activated monocytes have vital role in wound healing [151]. All strata of cellular and molecular mechanisms pertaining to wound healing involve macrophages *viz*., angiogenesis, chemotaxis, inflammation, synthesis of collagen along with its deposition and re-epithelialization. It also manages the proliferation process by secreting growth factors such as PDGF, transforming growth factors (TGF-a, TGF-b1), vascular endothelial growth factor

(VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF) and Insulin-like growth factor (IGF-1) involved in the wound healing process [152-154]. Initially, the macrophages secrete IL-1, which segregates the inflammatory cells from the blood circulation and diverts them to the wound site, elevating the phagocytosis process that helps in engulfing the bacteria and debridement, by the secretion of IL-6 from macrophages leading to endothelial cell proliferation and initiation of angiogenesis [155].

7. Discussion

Patient education, blood sugar control, wound debridement, advanced dressing, offloading, surgery and advanced therapies are still the standard care of therapies for treating DFUs. These gold standard cares has been successful in the management of most of the DFUs. However, there is a subset of patients who have high rates of failure in healing their DFUs ending with amputation. Furthermore, scientific investigation of such patients has shown microvascular dysfunction, decreased growth factor activity, hypoxic tissue environment and other factors which contribute to impaired wound healing. Perseverant research has ushered in contemporary therapeutic wound healing strategies to deal with the multifactorial pathogenesis in the DFUs. These include antibiotics, neuropathic drugs, biologicals, growth factors, skin substitutes and inflammatory modulators.

Although the pathogenesis of diabetic wound healing is multifactorial decreased expression of growth factors and increased levels of inflammatory cytokines are major causes of impaired wound healing of DFU. This altered molecular environment of DFU develops a proinflammatory state that may cause ulcers which fail to heal. Local or systemic treatment with immunomodulators can restore cutaneous homeostasis and have better wound healing. Growth factors cause molecular manipulation in the wound micro-environment; topical application of these growth factors appears to signal a significant role for their therapeutic use in the treatment of DFU. Nevertheless, only a single medication growth factor supplementation (PDGF) was approved by the FDA for topical application that has modest success. DFUs which do not show signs of improvement in the first few months are rarely transitioned to a therapeutic end unless supplemental combative strategies are prescribed; bioengineered skin substitutes have a major role in this form of stalled wounds. The major drawback of skin substitutes are expensive and cause infections. Additionally, clinical trials with many skin substitutes have showed variable success rates. If a wound fails to heal with standard therapy, surgical debridement followed by treating the wound with a bioengineered skin substitute or adding growth factors should be considered. Surgical method is still a standard way of debridement compared to other ways (autolytic and chemical) for hard to heal wounds. Neuropathic drugs can give symptomatic relief of pain but not have a direct impact in treating DFUs. Although acute foot infections seem susceptible to systemic antibiotics, the same cannot be said for chronic foot infections. Therefore, antimicrobial therapy in combination with surgical debridement is essential for treating any chronic deep infections. Bone infections are particularly difficult to treat and often require surgery.

Despite that fact that many reports having been published and promising compounds are presently undergoing clinical evaluation for DFU treatment, a conclusive set of evidences has not been established as on date. The foremost priority in DFU research revolves around deciphering the rudimentary mechanisms behind wound formation and progression. These conflated efforts dedicated in unraveling DFU’s concepts are still falling short. Further, the type of technology, intervention, and therapies those are suitable to promote healing, and the type of therapeutic strategy (single, adjuvant and combination) necessary remains questionable.

8. Conclusion

In conclusion, despite the fact that some of the new treatments mentioned above are promising, a quality based evidence of efficacy is lacking. Most of the described strategies are studied on uncomplicated or low grade ulcers but not on hard to heal ulcers, which fail to respond to conventional therapies and also the safety and efficacy of these treatment modalities are not established in large number of populations. Future studies should also take into the consideration of parameters such as complete healing, proportion of ulcers, rate of reduction in wound size, ulcers recurring, adverse events and quality of life. As a number of biochemical shortcomings /variations eventually cause DFUs, a single treatment strategy holds no promise. Hence, treatment alternatives of the future can only succeed if research expertise across the spectrum contributes equally to devise a therapeutic strategy for the menace and considers the inherent pathological complexities to ensure authentic redressal of the inadequacies arising out of DFUs.

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Figure Legends:

Fig. 1. Pathophysiology of diabetic foot ulcer

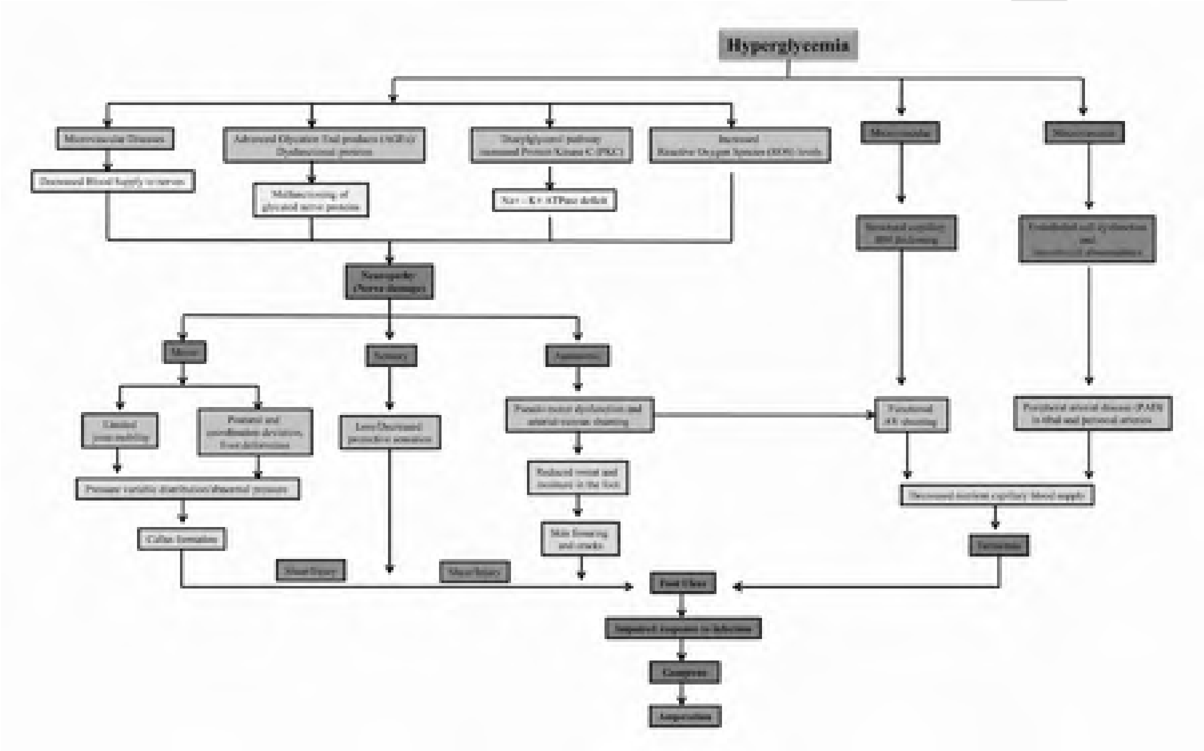

Fig. 2. Mechanism of nitric oxide blocking and maillard reaction in diabetic neuropathy

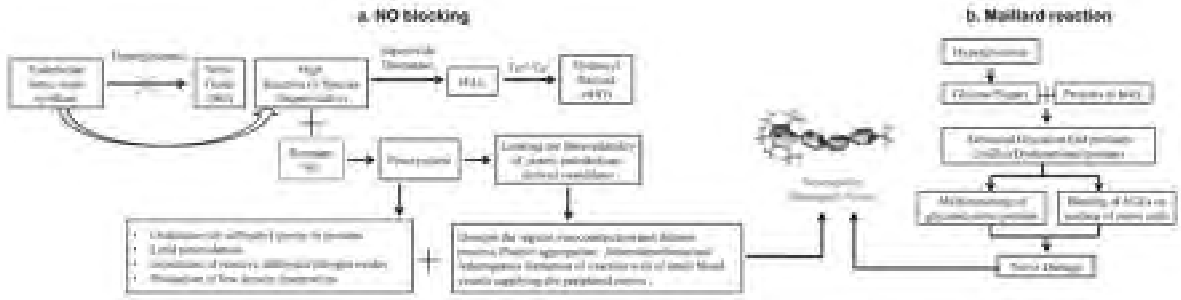

Fig. 3. Emerging drugs and therapies for diabetic foot ulcers

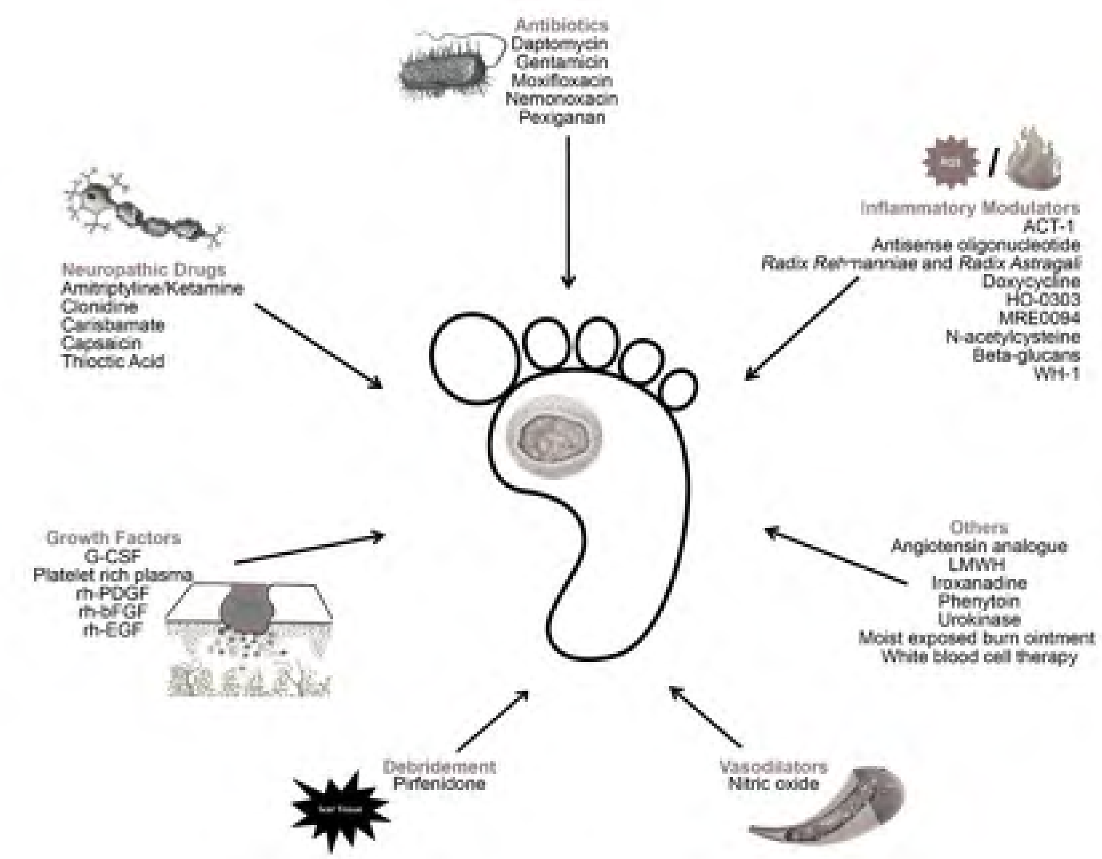

Table 1. USDFA approved Neuropathic and Antibiotic Drugs for treating various complications associated with DFUs

Drug	Class/ Category	Mechanism of action	Adverse effects	Indication
Duloxetine	Anti-anxiety and anti- depressant	Serotonin-norepinephrine reuptake inhibitor, elevation of serotonergic and noradrenergic activity in the central nervous system	Dry mouth, nausea, giddiness, hyperhidrosis, loss of appetite, constipation and Urinary retention.	DNP
Tapentadol	Analgesic	μ-Opioid receptor (MOR) agonist and as a norepinephrine reuptake inhibitor	Paresthesia, loss of equilibrium, memory loss, syncope, unconsciousness, dysarthria, presyncope and impaired gastric emptying	DNP
Pregabalin	Anticonvulsant	Regulation of alpha2-delta binding site (an auxiliary sub-unit of voltage-gated calcium channels) in central nervous system	Asthenia, accidental injury, peripheral edema, nerve damage, tremors, memory loss, anxiety, blurred vision, ataxia, confused state, abnormal gait, euphoria in coordination, over-thinking and dyspnea	DNP
Ertapenem	Carbapenem antibiotics	Binds to the penicillin binding proteins and inhibits the bacterial cell wall synthesis	Diarrhea, nausea, headache and infused vein complication	DFIs without osteomyelitis
Linezolid	Oxazolidinone derivatives	Inhibits the development of functional 70S initiation complex, which is involved in the bacterial translation process by binding to the site on the bacterial 23S ribosomal RNA of the 50S subunit	Lactic acidosis and loss of vision	DFIs without osteomyelitis
Piperacillin-Tazobactam	β-lactamase inhibitor	Retards septum development and cell wall formation in the bacteria	Diarrhea, constipation, nausea, headache and insomnia	ischemic DFIs
Trovafloxacin Mesylate	Fluoroquinolones	Inhibits the bacterial DNA gyrase and topoisomerase IV enzyme activity	Dizziness, nausea, headache and vomiting	DFIs

*DNP - Diabetic neuropathic pain, DFIs - Diabetic Foot Infections

Table 2.Summary of clinical trials published on various drugs and therapies specific to DFU treatment.

Drug/Therapy	Mechanism of Action	Grade/Type of wounds examined	No. of patients examined	Method of trial	Major Outcomes	Ref. No.
Bioengineered skin substitutes/Soft tissue substitutes						
Amniotic membrane	Provides structural collagen (extracellular matrix), biologically active cells and a large number of important regenerative molecules.	DFU size >1 and <25 cm^2 with no signs of infections	n=40	Randomized clinical trial	• At 12-week study period, 92.5% (37/40) ulcers completely healed. • Complete healing occurred at 50% vs. 90% by 4weeks in the biweekly and weekly groups (*p*=.014).	[58]
		DFU size >1 and <25 cm^2 with no signs of infections	Amniotic membrane=13, Control=12	RCT	• After 6 weeks of treatment the overall healing rate with amniotic membrane was 92% compared to 8% with standard wound care (*p*<.001). • Four patients in the standard wound care group and one patient in the Amniotic membrane group experienced adverse events (cellulitis, gastrointestinal bleed acute pyelonephritis and pneumonia).	[176]
BMCs	Promote cell proliferation, collagen synthesis, growth factor release, wound contraction, neovascularization, and cellular recruitment to wounds.	Less than Wagner grade 3	BMCs=12, Tissue repair cells=12.	RCT	• Improvements of microcirculation (transcutaneous oxygen pressure) and complete wound healing were observed in both the groups.	[162]

					• No adverse events regarding bone marrow aspirations and stem cells applications were observed.	
			BMCs=25, Control=23.	RCT	• After 12 weeks of study the average decrease in wound area was 36.4% (SD =0.48) in the BMCs group compared to 27.32% (SD=0.32) in the control group. • No adverse events were observed.	[160]
BSS	Provides structural and functional simulation as human skin	Low grade	BSS-112, Saline moistened gauze-96	RCT	• 56% of BSS-treated patients achieved complete wound healing compared with 38% in the control group (*p* = .0042). • Rate of adverse reactions (wound infection, cellulitis, osteomyelitis and amputations) was similar between the two groups with the exception of osteomyelitis and lower-limb amputations (less frequent in the BSS group).	[39]
		Difficult to heal ulcers	BSS-9, Control-8	RCT	• 56 % patients treated with BSS had complete healing compared to 37% control patients.	[169]

HFDD	Proliferate and generates the matrix proteins, human dermal collagen, cytokines and growth factors.	Low grade	HFDD -130, Control-115	RCT	• Complete wound closure by week 12, 30.0% (39 of 130) of patients in HFDD group were healed compared with 18.3% (21 of 115) incontrol group (*p*=.023). • Overall incidence of adverse events (local wound infection, osteomyelitis, and cellulitis) was significantly lower in HFDD group compared to control (*p*= .007).	[165]
		Low grade	HFDD -14, saline-moistened gauze -14	RCT	• Significant percentage of wound closure (*p*= .002) and number of ulcers (*p* = .003) in HFDD group than in the control group. • Lower rate of infection in the HFDD group. • Overall incidence of adverse events (local wound infection, osteomyelitis, and cellulitis) was less (*p*= .015) in HFDD group compare to control.	[159]
PSIS	Mimics the normal ECM And provides the support structure to wound.	Nonhealing ulcers for longer than 30 days	PSIS=37 PDGF gel=36	RCT	• At 12 weeks 49% of patients receiving PSIS were healed vs. 28% of patients receiving daily treatment of the PDGF gel (*p*=.055). • No significant difference was	[168]

					found in mean time to healing between treatment groups (*p*=.245).	
		Neuropathic foot ulcer	n=82	RCT	• At 12 weeks of treatment 54% of patients receiving PSIS were healed vs. 32% of patients in control group (*p*=.021). • Reported adverse events found to have no safety concerns.	[157]
Growth factors						
PDGF-BB	Synthesis of fibroblasts, Cell proliferation and angiogenesis	Low grade ulcers (not infected or ischaemic. No osteo-myelitis)	PDGF-BB-0.01%- 123, Control-127	RCT	• Complete wound closure was 43% in PDGF gel treated patients compared to 35% in control (*p* = .007). • The adverse events (cellulitis, osteomyelitis and infections) were similar in PDGFand control groups.	[175]
			n=922	Meta-analysis of 4 RCT's	• PDGF gel significantly increased (*p* = .007) the probability of complete healing compared with placebo gel. • Similar adverse events (infection, cellulitis, skin ulceration, and osteomyelitis) in all treated groups.	[172]
EGF	Stimulates cell proliferation and angiogenesis	Wagner grade 1 or 2	EGF-21, Placebo-19	RCT	• Significantly (*p*=.0003) faster wound closure and healing after	[173]

					12 weeks of therapy.	
PRP	Growth factor		n=325	Meta-analysis (9 RCTs and 2 DFU-specific RCTs)	• No statistically significant difference between the PRP and control in DFUs. • No significant difference of PRP vs. control by ulcer etiology or by the procedure used to obtain autologous PRP.	[166]
Debridement						
MDT	Consume necrotic tissue	Non-healing ulcers	MDT = 6, Conventional then MDT = 8, Conventional = 6	Retrospective controlled trial	• An association towards more rapid healingand granulation tissue formation.	[171]
		Non-healing ulcers	n=22	Non controlled trial	• Apparently more rapid healing as opposed to a historical control.	[65]
Hydrogel	Debride by providing a moist environment in wound, which promotes autolysis.	High grade ulcers	Hydrogel = 70, Control = 68	RCT	• Hydrogel increase the proportion of foot ulcers completely healed within 3–5 months with faster healing rate compared with standard care or gauze dressings. • Hydrogel appears to be more effective than standard wound care for wound healing and is associated with fewer complications in all the 3 studies.	[158]
		Wagner grade 2, no evidence of infection	Hydrogel = 14, Wet gauze=17	RCT		[161]
		All grades	Hydrogel = 15, Dry gauze = 14.	RCT		[174]
Inflammatory modulators						

TCM	Anti-inflammatory effects	Lower grade ulcers with mild DFU	n=16	RCT	• Daily rate of reduction in ulcer area was 3.55% in the TCM group and 1.52% in the placebo group (*p* = .062). • 6-month treatment with TCM was associated with improved wound healing. • TCM significantly decreased serum TNF-α levels (*p* = .034)	[163]
NAC	Improvement of HBO_2 effect on tissue oxygenation, by decreasing reactive oxygen species and there by increased NO availability	Non-healing ulcers (unresponsive to standard therapy after 8 weeks of treatment)	n=50	Randomized, cross-over trial	• Out of 50 subjects treated with HBO_2, 17 (34%) demonstrated insufficient increase in transcutaneous oxygen pressure. • NAC administration attenuated tissue oxygenation and improved HBO_2 outcome with a cure rate of 75%.	[22]
SBG	Inducers of immune function	Wagner grade 1 and 2	n=60	RCT	• The proportion of ulcers healed by week 12 for SBG was 59% compared to methyl cellulose (37%; Comparator product)- (*p* = .09). • Complete healing in the SBG group was observed at 36 days compared to 63 days with methyl cellulose (*p* = .130). • Four serious adverse events	[177]

					(three toe amputations/resections of metatarsal bones and one other surgical operation) in the control group, and none in the SBG group.	
WH-1	TNF-α generation	Wagner grade 3, post surgical debridement	WH-1=12, Hydrocolloid fiber dressings=12	RCT	• No statistically significant differences between two groups in wound size reduction. • Minimal adverse events with no serious adverse events observed.	[164]
Other wound healing promoters						
Angiotensin analogue	Up regulation of mescenchymal stem cells	Wagner Grade 1 or 2 (non-infected, neuropathic, or neuroischemic plantar ulcers)	DSC127 (0.03%) =26, DSC127 (0.01%) =27, Placebo=24.	RCT	• Percentage area of wound reduction at week 12 [40% in placebo; 67% inDSC127 (0.01%); 80% in DSC127 (0.03%)] and 24 week (23% in placebo; 53% in DSC127 (0.01%); 95% in DSC127 (0.03%)]. • Placebo-treated ulcers healed at 22 weeks compared with 8.5 weeks for 0.03% DSC127 (p = 0.04). • There were no significant effect ofDSC127 on safety and tolerance parameters, including BP changes.	[156]
Phenytoin	Induce the growth of	Low grade ulcers	Phenytoin=31	RCT	No statistically significant	[170]

	connective tissue		Control=34		differences in complete healing between the two groups (18 in the phenytoin group, 20 in the control group).	
		Low grade ulcers	Phenytoin-50 Dry gauze-50	Controlled clinical trial	• Control and phenytoin-treated groups showed wound healing with healthy granulation tissue appeared earlier in the phenytoin group ($P < 0.001$). • Overall percentage reduction of ulcer area was greater in the phenytoin group at 7th day ($P < 0.01$) and 14-35 days ($P < 0.005$).	[167]
LMWH	Inhibition of thrombin generation and improvement of fibrin gel porosity.	Low grade. Unresponsive to previous conventional therapy	n=10	RCT	• Ulcer improvement rates were 70.3% in the LMWH group and 45.5% in the placebo group. • Complete healing rates at 3 months were similar in both groups (35.1% vs. 33.3%) (p=.874).	[143]
Urokinase	Improves microcirculation in critical limb ischemia	DFU with critical limb ischemia	n=77	Non randomized, non controlled clinical trial	• Treatment for 21 days resulted in 33% of patients being alive, with no major amputation and completely healed ulcers after 12 months. • Total survival rate was 84.6%, amputation-free survival of 69.2% and rate	[150]

					of major amputation of 21.1%. • 11 patients experienced adverse events (cerebral bleeding, hypotension) • 7 patients experienced non-severe adverse events	

*BDM, bovine dermal membrane; bFGF, basic fibroblast growth factor;BMCs, Bone marrow derivedstem cells;BSS, bioengineered skin substitutes; TCM, Traditional Chinese medicine; DFU, diabetic foot ulcer;HFDD, Human fibroblast-derived dermis;LMWH, Low molecular weight heparin;MDT, Maggot debridement therapy; NAC, N-acetylcysteine; PDGF-BB, Platelet derived growth factor; PSIS, Porcine small intestine submucosa; PRP, platelet rich plasma; rhEGF, recombinant human epidermal growth factor; RCT, randomized controlled trial; SBG, Soluble Beta Glucan.