Pathogenesis and Molecular Targets in Treatment of Diabetic Wounds

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Abstract

Wound healing in diabetes is remarkably delayed due to various underlying pathological processes. Diabetes alters all the stages of wound healing such as remodeling, proliferation, hemostasis, and inflammatory phases. In diabetic patients, minor skin lesions may lead to unhealed chronic ulcers, and ultimately result in infection, gangrene, even amputation. Physiological factors responsible for the delay of wound healing include impaired growth factor and cytokine production, angiogenic response, macrophage and neutrophil function, collagen accumulation, and variation in the ratio of collagen types leading to weakened healing response. Key molecular targets for the local/pharmacologic treatment of wound healing include growth factors and other molecules, absorbable biomaterials, and cell regeneration therapy.

Keywords

Pathogenesis · Diabetic wound · Molecular targets · Healing · Diabetic foot ulcer

Introduction

Diabetes is a major cause for slower healing in every population. Between 2019 and 2045, the global expenditures for diabetes treatment is expected to grow from 760 billion U.S. dollars to 845 billion U.S. dollars (Elflein 2019).

Diabetes is a chronic metabolic disease that affects more than 463 million persons, and it is estimated that 20% of them develop complicated diabetic wounds or foot ulcer (IDF Diabetes Atlas 9th edition 2019; Nunan et al. 2014; Patel et al. 2019). Most acute wounds heal without issue; however, as age increases, impaired blood circulation and other conditions like smoking, obesity, and chronic diseases, such as diabetes, lead to slower healing. Diabetic complications resulting from diabetes include neuropathy, arterial damage, and ischemia, which may complicate diabetic wounds (Nunan et al. 2014). Unhealed wounds are prone to infection and lower-limb amputation. Diabetes is one of the principal causes of nontraumatic lower-extremity amputation.

In diabetes, each stage of healing, i.e., hemostasis, inflammatory, proliferation, and remodeling phase, is altered. Diabetic wounds show signs of impaired healing due to an uncoordinated healing process. Elongated inflammatory phase with hindrance in the mature granulation tissue formation and reduced wound tensile strength is observed in diabetic wounds (Patel et al. 2019).

Normal Wound Healing

Multiple sequential cellular and biochemical phenomena are necessary to restore damaged tissue. Hemostasis and clot formation is conventionally the starting point, triggering the inflammatory phase, commanded by neutrophils and macrophages, during which debris and bacteria are eliminated and growth factors are secreted. True repair only begins with the proliferative phase, including revascularization (angiogenesis) and build-up of the cellular and noncellular matrix. The maturation phase is responsible for tissue strength, encompassing remodeling of the local architecture and vascular abundance (Fadini et al. 2014; Singh et al. 2011, 2013). Differently from acute injuries, chronic wounds are more indolent and don't follow the same phases. As a consequence, healing can be delayed for 12 weeks or more (Anisha et al. 2013; Mohandas et al. 2015).

Diabetic Wounds

In diabetic patients, a minor skin wound often leads to chronic, nonhealing ulcers and ultimately results in infection, gangrene, even amputation. Damage of numerous layers of dermal tissue involving epidermis, dermis, and sometimes the subcutaneous tissue is not uncommon. The prevalence of foot ulcers ranges from 4% to 10% with a lifetime incidence as high as 25% (Dadpay et al. 2012). It is the most common complication of

diabetes, greater than retinopathy, nephropathy, heart attack, and stroke combined.

In the Asian continent, the diabetic foot represents a significant health problem, provoked by the high frequency of infection and the ever-rising incidence of diabetes. Insufficiency of diabetic foot care centers, deprived foot care information, approach and practice among diabetic patients, deferred recommendation or reporting to the podiatry centers, and limited income and educational status of the patients contribute significantly to the increased frequency of diabetic foot complications (Viswanathan et al. 2005).

Etiology and Pathogenesis

There are scores of reported physiologic disorders reportedly responsible for wound healing deficiencies in diabetes. Some representative ones are listed below:

- Impaired growth factor production (Qi et al. 2018)
- Impaired cytokine production (Zubair and Ahmad 2019)
- Impaired angiogenic response (Galeano et al. 2011)
- Weakened immune response (Peleg et al. 2007)
- Decreased neuropeptide expression (Theocharidis and Veves 2020)
- Impaired macrophage and neutrophil function (Maruyama et al. 2007)
- Increased serum matrix metalloproteinase-9 (Li et al. 2013)
- Impaired collagen accumulation and variation in the ratio of collagen types (Stolarczyk et al. 2018)
- Dysregulation of procalcitonin, fibrinogen, and IL-6 (Korkmaz et al. 2018)
- Aberrant macrophage polarization and function in wound healing responses (Ganesh and Ramkumar 2020)
- Imbalance between extracellular matrix (ECM) components and remodeling by matrix metalloproteinases (Gooyit et al. 2014)
- Deficiency of thrombin-activable fibrinolysis inhibitor (Verkleij et al. 2010)
- Advanced glycation end products (AGEP) modification of platelet-derived growth factor (PDGF) (Nass et al. 2010)
- Decreased levels of chemokine receptor CXCR3 and its ligand 10, CXCL10 (Bodnar et al. 2009)

Diabetic Foot Ulcer

Peripheral vascular and neuropathy disorders are believed to be crucial for the development of diabetic foot ulcers, compromising the survival and well-being of diabetic patients (Jeffcoate 2011). Predisposing conditions include previous deformations of the foot, reduction in regional oxygenation and perfusion, poor eyesight, and obesity. Poorly compensated diabetes as well as bacterial colonization are aggravating circumstances, along with presence of resistant bacteria, which further worsen the prognosis and make the therapy expensive (Hariono et al. 2018).

Classification of Diabetic Foot Ulcer

- Wagner–Meggit
- Brodsky Depth—Ischemic
- University of Texas
- International Working Group (2019 Guidelines)
- SAD
- PEDIS
- Other classifications

Wagner–Meggit

This is one of the oldest classification systems, created for the dysvascular foot. It includes six grade systems (grade 0 to grade 5), which emphasize ulcer depth, concentration of tissue necrosis, and occurrences of gangrene (Mehraj 2018).

Depth Ischemic

This classification system is the modernized form of the Wagner–Meggit classification, aiming to show a clear difference between lesion and foot vascularity (Mehraj 2018). It consists of three grades which depend upon the presence or

absence of ischemia with total or partial gangrene.

University of Texas

The University of Texas San Antonio classification system (UTSA) measures diabetic foot wound based on the depth of wound, infection, and ischemia in the lower limb. The grading system depends upon the wound depth, while stages of the classification depend upon the ischemia occurrence, bioburden of lesions, or merger of both by eliminating neuropathy. Superior grades or stages of a wound are less prone to healing. As compared to the Wagner classification, this system looks more promising and accurate. Nevertheless, optimal use of this and other classifications is still debated (Bravo-Molina et al. 2018).

International Working Group (2019 Guidelines)

The International Working Group has updated its guidelines related to diabetic foot disease on prevention, offloading, peripheral artery disease, infection, wound healing interventions, and classification of diabetic foot ulcers. These six protocols are not included in Table 55.1; however, they can be searched in the literature.

The following variables are endorsed as relevant for classification: patient-related (end-stage renal failure), limb-related (peripheral artery disease and loss of protective sensation), and ulcer-related (area, depth, site, single, or multiple and infection). Thorough wound assessment, including severity of infection and arterial perfusion (need for revascularization), is underscored. Among existing classifications, SINBAD (Site, Ischemia, Neuropathy, Bacterial Infection, and Depth), WIfI (Wound, Ischemia, and Foot Infection), and the guidelines of the Infectious Diseases Society of America are recommended for certain purposes (Lipsky et al. 2012; IWGDF Guidelines Org 2019; Bus et al. 2020a, b; Monteiro-Soares et al. 2020).

SAD

This classification system (Size, Arteriopathy, Denervation) considers size, denervation, sepsis, and arteriopathy. It is designed for hectic clinical practice and doesn't require any specialist techniques. Even though quite old, it has been reasonably well-validated (Monteiro-Soares et al. 2014).

PEDIS

As the acronym indicates, it addresses Perfusion, Extent, Depth/Tissue Loss, Infection, and Sensation. The numerous classification grades make it complex for clinical practice use (Jain and Joshi 2013).

Other Classifications

There is no dearth of diabetic foot ulcer classifications. However, most methods were developed with relatively small series and were not widely adopted, consequently suffering from limited validation. Many are not particularly user-friendly (Monteiro-Soares et al. 2014). Current representative tools are depicted in Table 55.1.

Diagnosis and staging are fundamental; however, instrumental monitoring is not less of a priority. To this aim, neuropathy and autonomic dysfunction, peripheral vascular disease, and eventual osteomyelitis need to be investigated. 3D and hyperspectral wound imaging are equally emphasized as reliable tools for lesion measurement and ulcer (Fernández-Torres et al. 2020).

Major Factors and Key Molecular Pathways of Diabetic Wounds

Molecular factors/targets for the management of diabetic wounds include cytokines, growth factors, clotting factors, prostaglandins, free radicals, nitric oxide, insulin-like growth factor (IGF-1) signaling axis including gangliosides, neuropeptides, mi-RNAs, lactoferrin, stromal cell-derived factor (SDF-1α), Hypoxia inducible factors (HIFs), thymosin beta 4, substance P, endopeptidase cathepsin D, and RANKL (Martí-Carvajal et al. 2015; Dam and Paller 2018; Zubair and Ahmad 2019; Liu et al. 2020). These agents directly or indirectly modulate vascularization, innervation, matrix reconstruction, and

Table 55.1 Examples of classifications of diabetic foot wound

Wagner-Meggit classification system					
Grades	**Foot wound**				
0	No open wound or cellulitis				
1	Superficial ulcer				
2	Deep ulcer upto tendons and joint tissue				
3	Deep ulcer with abscess, osteomyelitis, and joints sepsis				
4	Local gangrene forefoot or heel				
5	Gangrene of entire foot				
Depth ischemic classification					
Depth grade	**Definition**		**Ischemia grade**	**Definition**	
0	At risk, foot with the previous ulcer that may cause a new ulcer		A	No ischemia	
1	Superficial noninfected ulcer		B		
2	Deep ulcer with tendon or joint exposed (±infection)		C	Partial forefoot gangrene	
3	Extensive ulcer with bone exposed or deep abscess		D	Total foot gangrene	
University of Texas Classification					
Stages	**Grades**				
	0	**1**	**2**	**3**	
A	Healed pre- or post-ulcerative lesion completely epithelialized	Superficial wound not involving bone, tendon, or capsule	Wound penetrating tendon or capsule	Wound penetrating to bone or joint	
B	With infection	With infection	With infection	With infection	
C	With ischemia	With ischemia	With ischemia	With ischemia	
D	With infection and with ischemia	With infection and with ischemia	With infection and with ischemia	With infection and with ischemia	
(AD) SAD system					
Grades	**Area**	**Deep**	**Sepsis**	**Arteriopathy**	**Denervation**
0	Skin intact	Intact skin		Pedal pulse	Intact
1	Lesion <1 cm^2	Superficial (skin and subcutaneous tissues)	No infected lesions	Pedal pulse reduce or miss	Reduced
2	The lesion from 1 to 3 cm^2	Lesion penetrating to tendon, periosteum, and joint capsule	Cellulitis-associated lesions	Absence of both pedal pulses	Absent
3	Lesion >3 cm^2	A lesion in bone or joint space	Osteomyelitis-associated lesions	Gangrene	Charcot joint
PEDIS classification					
Risk factor group	**Characteristics**				
0	No neuropathy, no PVD				
1	Neuropathy, no deformity PVD				
2	Neuropathy and deformity, and/or PVD				
3	History pathology				

reepithelialization, including keratinocyte migration and proliferation on an extracellular matrix. All of these can be defective in diabetic wounds (Martí-Carvajal et al. 2015; Dam and Paller 2018).

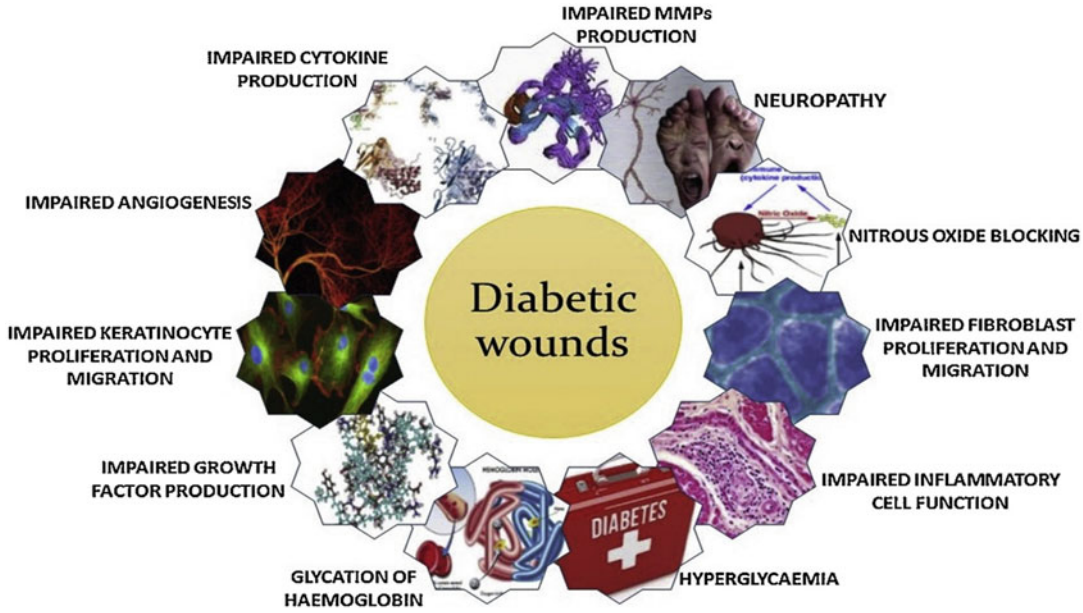

Fig. 55.1 Factors responsible for diabetic wounds [Adopted and reproduced from Patel et al. 2019]

It is important to emphasize that despite encouraging experimental results, few of these biomolecules have been investigated in large randomized trials. Indeed, a Cochrane Systematic Review a few years ago, addressing 11 growth factors for diabetic wounds, concluded that all increased the likelihood of healing of foot ulcers in diabetic patients. Such optimism notwithstanding, the disclaimer was that protocols suffered from high risk of bias, and side effects were possibly underreported (Martí-Carvajal et al. 2015). More clear-cut positive outcomes were detected for recombinant human epidermal growth factor (rhEGF), both intralesionally and topically applied, in a meta-analysis covering 6 trials and 530 patients (Bui et al. 2019). There are several factors that responsible for diabetic wounds are mentioned in Fig. 55.1.

Mitochondrial Overproduction of Reactive Oxygen Species

Advanced glycation end-products (AGEs) are a consequence of deranged glucose homeostasis. Transcription factors involved in inflammation and protein kinase C can be activated in such circumstances, and nerve protein glycation can occur, in conjunction with tissue oxidative stress and ischemia. Wound healing can be impaired because of these aberrations, including diminished local sensation which potentially results in additional injury (Patel et al. 2019; Shaikh-Kader et al. 2019).

Alteration of Growth Factors

Growth factors are pharmacologically active polypeptides. In all phases of wound healing, they promote relevant biological and molecular events. In the granulation phase of tissues, growth factors contribute to the early inflammation stage (Patel et al. 2019). Compromised wounds often demonstrate a defect in the kind and quantity of growth factor, due to alteration in the occurrence, enhancement in the degradation, reduction in the trapping, release, and production. Extracellular matrix (ECM) synthesis is categorized by a balance between matrix formation and matrix degradation, for optimal healing. Some of the factors influencing the formulation of ECM are VEGF,

IGF-I, IGF-II, TGF-β23, KGF24, PDGF25, EGF26, FGF27, TNF-α, and IL-6, which can be diminished in diabetic person, including suppression of receptors along with quick degradation of growth factors (Fui et al. 2019; Su et al. 2019; Patel et al. 2019; Zubair and Ahmad 2019).

Platelet-Derived Growth Factor

At the early stage of wound healing, platelets synthesize the platelet-derived growth factor (PDGF). PGDF is an essential mitogen that promotes the proliferation of fibroblast, matrix production, along with the maturation of connective tissue. In all stages of wound healing, PDGF continuously activates various cellular responses. PGDF binds with a receptor of tyrosine kinase and triggers various signaling pathways, leading to the enhancement of migration and proliferation of the cell. For the inflammatory cell and fibroblast, PDGF acts as a chemoattractant and encourages the production of collagen, glucosamine, and proteoglycan. In diabetic wound patients, there is a reduction in the expression of PDGF and PDGF receptors (Ishihara et al. 2019).

Vascular Endothelial Growth Factor

The wound healing process is affected by the concentration of VEGF as it supports the rate-determining steps in angiogenesis and vasculogenesis. With the help of the protease, it causes the degeneration of a three-dimensional network of an extracellular macromolecule of active vessels. In the case of diabetic wounds, it could enhance the density of capillary and develop the perfusion rate of blood along with the metabolism in wounded tissue. In a small series of diabetic foot ulcers, managed by hyperbaric oxygen therapy (HBOT), VEGF became elevated. The author defends that HBOT aids lesion epithelialization, both directly and indirectly, through VEGF upsurge and TNF-α downturn (Semadi 2019).

Transforming Growth Factor Beta

It has been reported that in diabetic patients there is a decrease in the amount of TGF β in wounded tissue that leads to retardation of the wound healing process. At the promoter site, MMP-encoded genes show the TGF-β1-dependent inhibitory element with a decrease in gene expression. The decrease in expression of TGF-β and upregulation of MMPs lead to destruction of growth factor transcription factors like Smad-2, Smad-3, and Smad-4, which also activate and repress TGF-β target genes. TGF-β1 activates Smad-2 and Smad-3 for the production of collagen (Hozzein et al. 2015). The decrease in the level of TGF-β1 causes increased recruitment of activated inflammatory cells, predisposing to a delayed inflammatory phase till the proliferation phase of the healing process in DWs (Heublein et al. 2015). Decreased levels and expression of those growth factors could contribute to poor and prolonged wound healing processes in diabetes (Patel et al. 2019).

Matrix Metalloproteinase 9

The central role of the extracellular matrix in the tissue remodeling processes involved in wound healing has already been alluded to. Endopeptidase enzymes degrade such matrices, and inappropriate conduction of this phenomenon can seriously affect the healing sequence. Matrix metalloproteinase 9 (MMP9) is actually a cluster of different crystal structures highly expressed in diabetic foot ulcer healing. Inhibitors have been identified and their binding mode was elucidated. In this sense, they could play a pharmacologic role in the handling of such complication (Hariono et al. 2018).

Defective Cytokine Production

Elevated interleukin-6 (IL-6) in diabetic foot ulcers has been demonstrated, and these levels decrease as the ulcers heal (Korkmaz et al. 2018). In experimental animals, similar lesions treated with IL-22 heal more rapidly, due to better vascularization, reepithelialization, granulation tissue formation, and VEGF release, with less keratinocyte differentiation. Pharmacologically, interleukin-22 seems to be superior to PDGF and VEGF, because of gene induction related

to reepithelialization, innate host defense mechanism, and tissue remodeling (Zubair and Ahmad 2019).

Abnormal Cellular Activity

At the start of the healing process, neutrophils appear, followed by monocytes which differentiate into macrophages. Endothelial cells, fibroblasts and keratinocytes, are analogously involved in the restoration of damaged tissue (Patel et al. 2019; Krzyszczyk et al. 2018). Macrophages and neutrophils are often increased in diabetic wounds. Macrophages in diabetic patients have reduced clearance activity; reduced capability to phagocyte the dead cells. Decreased T cells, increased B cells, dysregulation of the proliferation of macrophages, fibroblasts, endothelial cells, and keratinocytes are all reported. Infiltration of macrophages and neutrophils is prolonged in diabetes, and macrophages produce a reduced level of cytokines.

Mesenchimal stem cells are a promise to overcome such overlapping deficiencies, given their potential for multilineage differentiation. In-vivo and in-vitro protocols have availed themselves not only of direct cell therapy, but also of indirect intervention with the help of micro RNA (miRNA) and long noncoding RNA (lncRNA) (Li et al. 2020).

Neuropathy

Peripheral neuropathy mainly alters the sensory, motor, and autonomic function. An insensate foot can lead to injury, including skin irritation and pressure sores. Alteration of autonomic function predisposes to a delayed healing process due to arteriovenous shunting, impaired circulation, and edema (Theocharidis and Veves 2020). Due to the lack of protective sensation, many wounds remain unnoticed and progressively become worse.

The polyol pathway, along with many others, has been implied in the pathogenesis of diabetic neuropathy. In this sense, they could serve as potential targets for pharmacotherapy (Dewanjee et al. 2018).

Nitric Oxide Interventions

In diabetes, hyperglycemia can decrease the production of nitric oxide by inhibiting endothelial nitric oxide synthase (NOS) activation, which can favor the accumulation of reactive oxygen species, mainly superoxide. In the presence of metals ion like ferrous or cuprous ions, reactive oxygen species are converted to the highly reactive and damaging hydroxyl radical. In addition, they are also involved in the oxidization of sulfhydryl groups in proteins, lipid peroxidation, and the generation of reactive aldehydes and nitrogen oxide. These radicals disrupt the endothelium, which affects vascular function like vasoconstriction response, platelet aggregation, abnormal growth, and inflammation. Nitric oxide is a potent vasodilator, and local administration could positively influence indolent wound healing.

The hypothesis that a deficiency of NOS in diabetic patients leads to poor vascularization, peripheral neuropathy, and foot ulcers has been entertained (Walton et al. 2019). There is evidence that the genotype eNOS distribution is not different between diabetics with and without foot ulcers. Incidentally, in the same experience VEGF gene polymorphism wasn't different either (Erdogan et al. 2018). Nevertheless, transdermal nitric oxide (NO) treatment, in the form of NO donors, iNOS induction, and other pathways, has received attention in the recent literature (Erdogan et al. 2018; Walton et al. 2019).

Vascular Disease

The association of vascular disease and nonhealing of foot ulcer is well-established. Such circumstances notwithstanding, indications

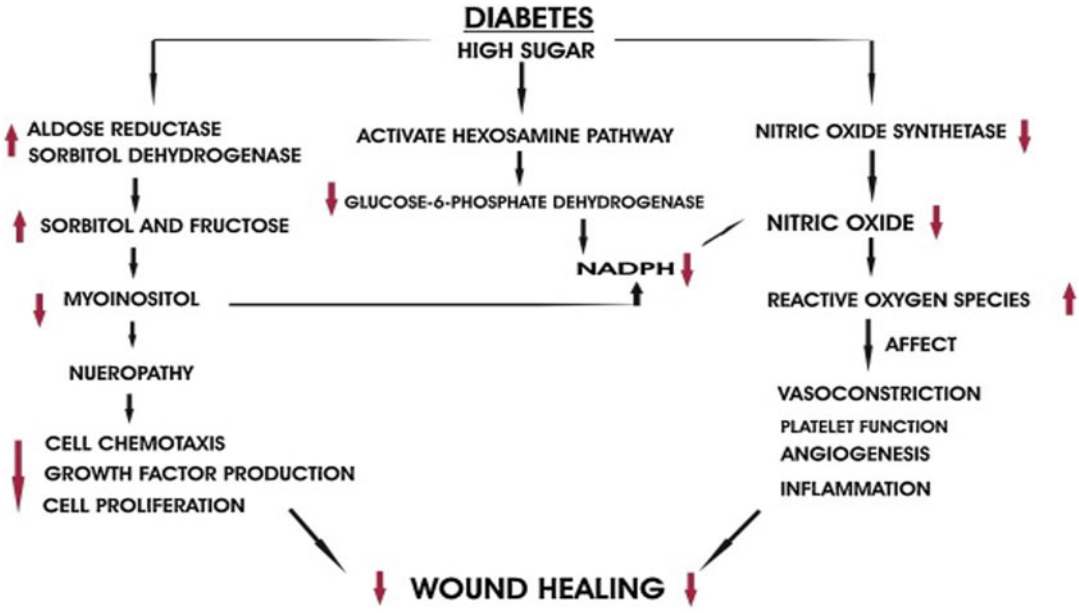

Fig. 55.2 Major pathways responsible for decreased wound healing in diabetes [Adopted and reproduced from Patel et al. 2019]

and outcomes of revascularization surgery are still controversial. One of the largest reviews, conducted by the International Working Group of the Diabetic Foot, covered over 13,000 patients. Results concerning part of this investigation point out towards fairly good response with both open and endovascular operations. Approximately 60% of all wounds were healed after 1 year, and amputation rate was about 10% after the same follow-up period. Revascularization interventions are, therefore, recommended when there is clear evidence of peripheral artery disease and ulcer; however, the best technique is still open to debate (Forsythe et al. 2020). The major mechanisms responsible for the decline in the wound healing process in case of diabetes are mentioned in Fig. 55.2.

Preventive Care

Traditional handling of diabetic foot ulcers is associated with inadequate efficacy, prolonged morbidity resulting in high direct and indirect cost, insufficiently documented side effects, and relapse rate as high as 50%. Hence, prevention of diabetic foot ulcers is the most important challenge.

According to the IWGDF guidelines (Bus et al. 2020b), screening of asymptomatic cases for peripheral neuropathy and arteriopathy is the first concern. If low risk is estimated, education and self-care by the patient should be highlighted, and pre-ulcerative signs should be treated. Additionally for those with moderate to high risk, footwear should be carefully selected, and monitoring of foot skin temperature is advised.

Indeed, footwear that relieves plantar pressure is useful for secondary prevention as well, reducing recurrence rates. Refractory cases should be surgically managed, and access to a multidisciplinary, integrated center should be a priority, whenever feasible (Bus et al. 2020b).

Therapeutic Approaches: Standard Care Versus Cells, Biomaterials, and Growth Factors

Many innovative local approaches are being assayed for patients with complex, recurrent, or refractory lesions, including stem cell therapy, photobiomodulation, and nanotherapy, in order to restore function and prevent amputation. Wound dressings, as well as scaffolds made of absorbable biomaterials, often impregnated with growth factors, nitric oxide and other pharmacologic agents, have been designed (Shu et al. 2018; Zarei et al. 2018; Erdogan et al. 2018; Zubair and Ahmad 2019;Walton et al. 2019).

Secondary infection is not uncommon, sometimes by resistant bacteria, and antibiotics are an integral part of the therapeutic arsenal in such context, within the recommendations of the Infectious Diseases Society of America (Lipsky et al. 2012). Revascularization surgery, as already mentioned, is indicated for those with demonstrated vascular impairment. Debridement and amputation, along with eventual flap rotation and skin grafting, once comparatively common among diabetics, should be reserved for carefully selected acute/chronic cases or for certain acute/urgent foot ulcers (Monteiro-Soares et al. 2014; Forsythe et al. 2020).

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