International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 29 No. 1 October 2021, pp.109-137

Updates of Diabetic Foot Ulcer (DFU) Management Critical Review

Maged Naser[1], Mohamed M. Nasr[2], and Lamia H. Shehata[3]

[1] Mazahmiya Hospital, Ministry of Health, Kingdom of Saudi Arabia, Department of ob/gyn.
[2] King Fahd Hospital, Ministry of Health, Kingdom of Saudi Arabia, Department of Surgery.
[3] Care National hospital, Department of Radiology.

Abstract – Diabetic foot issues are the critical reason involving non-traumatic lower limb amputation internationally. Most amputations in diabetes are preceded by foot ulceration. Therefore, an uncompromising understanding of the causes, evaluation and management of ulceration is essential. This review provides a concise description concerning the key factors contributing to the pathophysiology of the diabetic foot. The review moreover outlines an evidence-based strategy regarding the clinical evaluation and management primarily based on recently published guidelines. The importance of evaluating the presence and severity regarding wound(s), ischemia and foot infection is emphasized also, the evaluation will direct medication strategies such as, offloading, treatment of peripheral artery disorder and treatment of infection. The review additionally gives the diagnosis of osteomyelitis of the diabetic foot and discusses briefly Charcot neuroarthropathy. Finally, highlights updates amongst diabetic foot treatment.

Keywords – Osteomyelitis, Chronic limb-threatening ischemia, WIfI Foot ulceration, Offloading, Charcot neuroarthropathy, In Situ 3D Bioprinting, Biocompatible ECM-Based Hydrogels, and Bioactives of acellular3D-PrintedWoundDressings.

Key Points

- Diabetic foot complications are the frequent purpose of "non-traumatic" lower limb amputation. 85% concerning it, amputations are preceded by foot ulceration.
- Diabetic neuropathy is the just primary persistent complication concerning diabetes, affecting at least 50% regarding all diabetic patients all through their lifetime. The pathogenesis in regard to diabetic neuropathy is complex, multifactorial and not fully understood. Metabolic abnormalities so a whole are implicated of the pathogenesis in regard to diabetic neuropathy collectively together with non-enzymatic glycosylation regarding neural structures, malfunction regarding polyol metabolism, activation of the hexosamine pathway and protein kinase C (PKC) isoforms.
- Approximately 50% of patients complaining of a diabetic foot ulcer, have coexisting PAD (Peripheral arterial disease). PAD in diabetes tends to occur more distally than smoking-related PAD and is mainly common below the knee. These atherosclerotic lesions have an aptitude within consequence remain multilevel together with an excessive incidence in regard to long occlusions.
- Conventional methods assessing tissue perfusion of the peripheral circulation are often unreliable in patients with diabetes, and as a result it is difficult to determine the perfusion shortage in patients with diabetic foot ulceration.
- WIfI classification remain used for limb staging in the diabetic foot and is based on grading Wound, Ischemia and foot Infection regarding scale strip over 0 to 3. WIfI execute stand chronic in imitation of decide the risk as regard to major limb amputation at 1year and moreover the necessity of revascularization.

Corresponding Author: Maged Naser

- The standards of management of diabetic patients with foot ulcers encompass offloading, wound management, management of infection, assessment concerning perfusion and revascularization if required.
- Negative pressure wound remedy continue to be regarded in patients with diabetes and a post-operative (surgical) wound on the foot. Infection severity guides the preference of the empiric antibiotic regimen and its routes of administration. The presence of osteomyelitis has essential diagnostic, therapeutic and prognostic implications. The current IWGDF pointers recommend cure related to diabetic foot osteomyelitis together with antibiotic remedy of no longer than 6 weeks.
- Assessment concerning perfusion in the diabetic foot stay challenging. The level of perfusion required to heal a foot ulcer relies upon multiple factors, as ulcer size and location, presence/extent regarding gangrene and infection. The Global Vascular Guidelines regarding the regime as regards chronic limb-threatening ischemia recommends an evidence-based method according to diabetic patients with foot ulceration.
- Charcot neuroarthropathy is a serious but often overlooked scenario among people with diabetic neuropathy. The hallmark involving that surroundings is a warm, swollen and erythematous foot misinterpreted as acute infection, gout or osteomyelitis. Early cure regarding Charcot requires immobilization and non-weight-bearing in a cast until the acute inflammatory process subsides.

I. Introduction

Diabetes is a most important public health undertaking worldwide, which is associated with a range regarding problems consisting of cardiovascular, kidney, eye and foot diseases. It is an important cause regarding mortality, morbidity, value (to health systems and the patient) then incapacity worldwide. The wide variety over adults residing including diabetes global has quadrupled over the last 35 years and will continue to rise [1]. In 2013, approximately 382 million people had diabetes yet this quantity is expected in accordance with upward rise to 592 million by 2035 [2].

Diabetic foot might also moreover keep described as infection, ulceration or destruction of tissues over the foot related alongside neuropathy and/or peripheral artery disease in the lower extremity of people with diabetes [3]. It is estimated that patients with diabetes have a 34% lifetime risk related to developing foot ulcer with extra than 50% of these ulcers turn concerning infected and many of them requiring hospitalisation [4, 5]. The costs upon care of diabetic patients with a lower extremity ulcer is an essential economic burden for society and individual patients [6, 7, 8, 9, 10]. These costs are extraordinarily prolonged at present ulcers became infected or when patients needed extended inpatient therapy and amputation [11].

Diabetic foot complications are the most common cause of non-traumatic lower limb amputation internationally [5, 12]. A history concerning foot ulcer is considerably associated with negative outcomes. The risk of death at 5 years due to the fact of a patient inclusive of a diabetic foot ulcer is 2.5 times as high the as diabetic patient with who does not have a foot ulcer [13]. Of all amputations of diabetic patients, 85% are preceded by means of foot ulceration any therefore a cease end result deteriorates to gangrene or infection [14, 15]. Mortality on the grounds that diabetes-related amputation is notoriously high; 70% at 5 years for all patients with diabetes and 74% at 2 years for those undergoing dialysis [5, 16].

More than three quarters of patients with diabetic foot ulcers can achieve primary recovery within 1 yr. [5, 17, 18]. Unfortunately, patients with a DFU history have a high risk concerning re-ulceration [17]. Approximately 40% of patients have a recurrence within 1year of the wound healing, almost 60% within 3 years, and 65% within 5 years [5]. Thus, it is more useful to expect the patients who have achieved wound closure as like being in remission rather than being healed [5].

Foot ulcers with DM hold a significant effect on health-related quality of life, especially along observance among conformity on physical functioning and role limitations due to physical and emotional issues [19, 20]. They moreover signify an essential use related to health resources, incurring fees not only for dressings, however also staff costs, tests and investigations, antibiotics and specialist footwear.

1. Pathophysiology

The pathogenesis of foot ulceration is complex and requires interest on the role of several contributory factors, inclusive on peripheral neuropathy, peripheral arterial disorder (PAD), biomechanical problems along limited joint mobility and susceptibility in accordance to infection.

1.1 Neuropathy and Biomechanical Abnormalities

Diabetic neuropathy is some about the well-known chronic complications of diabetes, affecting at least half concerning all diabetic patients for the duration of their life-time [21]. It creates an enormous liability concerning each the affected patients and the healthcare system [22, 23].

The pathogenesis of diabetic neuropathy is complex, multifactorial and at current now not absolutely understood. There are a range of metabolic abnormalities so much are implicated in the pathogenesis concerning diabetic neuropathy including non-enzymatic glycosylation of neural structures, malfunction related to polyol metabolism and activation regarding the hexosamine pathway and protein kinase C (PKC) isoforms. Collectively such metabolic abnormalities cause an imbalance amongst the mitochondrial redox state lead to extra building regarding mitochondrial and cytosolic reactive oxygen species(ROS) promoting neuronal damage [21, 24, 25, 26]. The polyol pathway is possibly the most well-studied metabolic abnormalities. Excess glucose is transformed into sorbitol by using ability regarding capability related to aldose reductase and results in osmotic imbalance in the cell. This prompts a compensatory efflux in regard to myoinositol and taurine. Myoinositol is an essential component of sodium/potassium (Na/K) ATPase and its loss impairs normal nerve physiology. The expand within aldose reductase activity, who additionally depletes cellular stores of NADPH, is needed for nitric oxide generation and regeneration concerning the essential antioxidant glutathione. This effects over the generation of cytoplasmic ROS and consequent cellular dysfunction [21].

Another factor contributes to the pathogenesis regarding diabetic neuropathy is associated with impaired insulin signalling. Although insulin is not involved among glucose uptake into neurons, up to expectation has been proven consequently so has integral neurotrophic consequences promotion neuronal growth and survival. Reduction concerning such neurotrophic signalling due to insulin deficiency in Type 1 diabetes promotes cellular injury.

The state concerning blood relation insulin deficiency (due to peripheral insulin resistance), may additionally need according to among quantity additionally maintain contributing in accordance with the aetiology of DN (diabetic neuropathy) among Type 2 diabetes [22, 27]. Recently within so much area has been interest in appreciation the bioenergetics profile of the peripheral nervous system, among interplay between axons and Schwann cells (SC) and their association along neuropathy.

There is increasing evidence that, the SCs (Schwann cells) are fundamental sensors regarding axon activity and provide energy for axon activity [28, 29]. It is speculated hence within diabetes SCs at last now not only default their performance to provide energy between consequence together with myelinated and unmyelinated axons then again moreover transfer toxic lipid species to the axons they contact [22].

The supporter respect as regards the more than assured pathways implicated among the pathogenesis in regard to diabetic neuropathy varies together with cell type, disease profile and time. As a likely end result related to variations amongst the underlying mechanisms, tight glucose control can minimize neuropathy of type 1 diabetic patients but appears currently at present no longer efficacious in type2 patients [30, 31].

Diabetic neuropathy influences the proximal and distal, somatic and autonomic nerves. Most relevant to the pathophysiology regarding diabetic foot ulcers is sensory neuropathy with loss of protective sensation (LOPS), motor neuropathy resulting among foot deformity and autonomic neuropathy related along with pseudo motor dysfunction

Contributing to dry skin which is larger bend according to cracking and wound development.

Peripheral neuropathy should be severe before leading to LOPS and when present, increases vulnerability to physical and thermal trauma [4]. With an inability to distribute forces that are applied to planter surface, with the insensate foot is exposed to extended pressures therefore encourage tissue damage leading to ulceration [32].

Motor neuropathy is believed in consequence which leads to weakness preferentially affecting the intrinsic muscle of the foot, inflicting imbalance between flexors and extensors of the toes. Atrophy of the small muscles responsible for metatarsophalangeal plantar flexion is thought to lead to hammer toes, claw toes, prominent metatarsal heads, and pes cavus, and these structural deformities and restriction joint mobility are associated with accelerated peak plantar pressures [32, 33, 34].

Assessment involving gait and dynamic plantar pressures are valuable to understand the biomechanical abnormalities that contribute to formation and persistence of diabetic foot ulceration. Recent studies have shown that diabetic patients with foot ulcers have distinguishing gait parameters including decreased range of movements of joints; larger vertical and horizontal ground reaction

forces and smaller walking speeds with smaller step lengths [35] and greater plantar pressures than diabetic controls with no ulceration [36]. This gives supportive proof regarding the pressure-offloading in the management of diabetic foot ulcers.

1.2 Peripheral Artery Disease (PAD)

PAD is frequent in patients with diabetes [37, 38, 39], and approximately 50% regarding patients together with a diabetic foot ulcer have coexisting PAD [18, 40, 41]. PAD amongst diabetes occurs predominantly in the infra-inguinal vasculature and is dissimilar to PAD in patients without diabetes in its characteristics, remedy then outcomes. The atherosclerotic lesions tend to be multilevel and especially severe below knee vessels (popliteal and tibial arteries), with long prevalence of long occlusions [38, 42]. The predilection for multiple crural vessels involvement mixed with extensive calf arterial calcification will increase the technical challenges associated with revascularisation using either open bypass or endovascular approach [41]

Furthermore, within patients with diabetes, a similar anatomical arterial disease can result in an extra severe perfusion deficit due to conformity with the reality regarding poverty regarding collateral vessels so as well as an impact of physiological factors related to diabetes, such as arteriolar shunting [43]. The presence of PAD amongst patients with foot ulceration is related with unfavourable outcomes certain as like poor wound healing or greater rates of lower extremity amputation [17].

Assessing foot perfusion is usually hard among patients with diabetes. This population generally lacks typical symptoms related to vascular insufficiency such consequently claudication and rest pain [44]. However, distinction of perfusion is a necessary step in the management of patients with diabetic foot ulceration, in order to estimate the risk of amputation, likelihood of wound healing without vascular intervention, but possibly benefit of revascularization.

Foot perfusion needs to be measured and then assessed in phrases as regard to worldwide or provincial perfusion deficits rather than an absolute measurement. Quantification of blood flow required to heal a foot lesion depends on several factors including the presence of infection, extent of tissue loss, abnormal mechanical loading of the foot during walking, and co-morbidities such as renal failure [45, 46, 47]. Conventional techniques on assessing tissue perfusion within the peripheral circulation are frequently unreliable in patients with diabetes and it may be difficult to determine the perfusion deficit in patients with foot ulceration.

In summary, the mixture regarding foot deformity, loss of protective sensation, dry skin, insufficient off-loading, and repetitive minor trauma can lead to tissue damage and ulceration. Once an ulcer has formed, healing may be delayed or not occur, usually if significant ischemia is present.

2. Clinical Assessment regarding the Diabetic Foot

2.1 History and Physical Examination

An uncompromising data of physical examination involving each patient imparting along diabetic foot pathology need to include history related to duration of diabetes then adequacy of diabetic control, medical co-morbidities and a records concerning pedal wounds, prior amputations, and lower extremity vascular interventions [48]. A history of foot tingling, burning and/or numbness, may be useful resource according to identify these patients with neuropathy. A history concerning claudication or other walking impairment, ischemic rest pain, or non-healing wounds are noticeably suggestive peripheral artery disease.

Physical examination keeps in conformity with a systematic method and the patient should be examined including both feet. Foot deformities (e.g., claw toes, hammer toes), bony prominences and limited joint mobility as a contributions of accordance with a high risk of ulceration. It is indispensable since hold a look at inside entire regarding the toes. Footwear must be inspected due to the fact appropriateness. Any wounds assignment in conformity with state for being carefully assessed, and correct documentation performed related since wound location, size, depth, characteristic of the wound base and margins, exudate (amount and type) and the presence and severity of infection.

The possibility of peripheral neuropathy after vascular insufficiency must be assessed. Sensory examination concerning the 10 g (5.07 Semmes-Weinstein) monofilament and/or tuning fork (128 Hz) is imperative for assessment of pressure perception, vibration perception then tactile sensation. Lower extremity vascular examination encompasses palpation regarding lower extremity pulses (i.e., femoral, popliteal, dorsalis pedis, and posterior tibial), auscultation for femoral bruits, and inspection concerning the legs and feet. Pulse palpation is fundamental but not sufficient to assess perfusion.

2.2 Assessment of Foot Perfusion

2.2.1 Ankle-Brachial Index (ABI)

ABI is the ratio concerning systolic pressure at the ankle to accordance that of the arm; if the hand pressures are disparate, the greater concerning the two, should be the denominator. It is a quick, simple and non-invasive test used to document PAD [49]. In addition to the presence of PAD, the ABI in addition is an indicator of generalized atherosclerosis [50, 51]. Patients with ABI ≤0.90 are identified with PAD. Diabetic patients with an ABI above 0.9 may possibly endure PAD and should undergo further assessment if clinical suspicion is present. Values > 1.40 are abnormal and indicate so the arteries are calcified and not be able to be compressed, whosoever is greater frequent amongst patients with diabetes mellitus and/or advanced chronic kidney disease [52, 53]. Specific and suspected incompressible ABI values, extra checking out need after stand undertaken. Individuals with diabetes repeatedly hold calcium deposition among the arterial media; a situation recognized as much medial arterial calcification (MAC), whoever almost within much instances hold an effect on the calf arteries [54].

This state causes arterial wall stiffness, namely outcomes among vessels consequently are greater strong to occlude the calf and ankle. The end result is an artefactually immoderate ankle pressure and ABI [55, 56]. This assignment in addition state suspected additionally with near normal pressures if the Doppler arterial waveforms are blunted.

2.2.2 Toe Pressure and Toe: Brachial Index

An alternative in conformity with ABI, is in imitation of measure toe pressures (TPs) or the toe: brachial pressure index (TBI). These may additionally keep extra beneficial measures concerning perfusion of the diabetic person because MAC normally spares the pedal arteries [57, 58]. Toe pressures are obtained by means of putting a cuff around the base on the toe, ideally the hallux, together with a digital flow sensor beyond the cuff. Toe pressures may be measured by photo plethysmography (detecting pulsatile flow or producing a pulse wave curve) or laser Doppler (detecting changes within wavelength when the laser encounters red blood cells) [59]. TBI is the ratio in toe pressure and the very best over the two brachial pressures. A TBI ≥0.75 is generally considered within the ordinary range, while a TBI < 0.25 is consistent along severe PAD [60, 61]. An absolute systolic toe pressure of 30 mm Hg or greater has been correlated with a significantly greater probability concerning foot ulcer healing in diabetic patients [45].

2.2.3 Doppler Waveform Assessment

Audio and visible analyses regarding Doppler waveforms are excellent tools because assessment regarding the presence of PAD. A normal Doppler waveform in the lower extremities has a virtue triphasic pattern, drawn up specifically on a systolic forward-flow phase, a late-systolic reverse flow phase, and a smaller, diastolic forward-flow phase [21]. Detection regarding a triphasic pedal Doppler arterial waveform together with a handheld Doppler provides strong proof for the absence of PAD [62]. The availability concerning monophasic flow with an isolated forward systolic waveform together with diminished amplitude is related with significant PAD.

2.2.4 Transcutaneous Oxygen Pressure and Skin Perfusion Pressure

Transcutaneous oxygen tension (TcPO2) measures the transfer regarding oxygen molecules to skin surface, allowing objective quantification of the degree of limb perfusion [63]. TcPO2 maps the actual oxygen supply available for the skin tissue and moreover responds after microcirculatory events. The measured PO2 within the derm is displayed into millimetres concerning mercury, along an everyday healthful value into the foot because of an individual breathing normobaric air being >50 mmHg [63, 64]. Skin perfusion pressure (SPP) is the blood pressure so is required to restore flow to capillaries following controlled occlusion then subsequent flow return.

TcPO2 and SPP values be able to predict vascular disease then the probable success of healing an ulcer and major/minor amputations with or without revascularization. TcPO2 measurements with an oxygen challenge are additionally utilized so an indicator over whether or not hyperbaric therapy intention keep in every probability to keep encouraged in wound healing [60]. TcPO2 degrees of less than 25 mmHg are indicative regarding severely decreased blood flow to the area concerning assessment and high recommend so revascularization will stay required in imitation of gain healing. Patients with a TcPO2 ≥ 25 mmHg then SPP ≥ 40mmHg hold a higher probability about wound healing in contrast according to wounds including invulnerable concerning a more severe perfusion deficit [45, 65, 66].

3. Diagnosis of Osteomyelitis

The correct prognosis of foot sepsis and in particular osteomyelitis (OM) within the diabetic foot is crucial for planning adequate treatment and influences prognosis. The occurrence regarding bone involvement is variable, relying about the context. It is found that 60% of patients hospitalized because of diabetic foot infection (DFI) and 10–20% about curiously much less severe infections imparting within an outpatient hospital [67, 68]. The differentiation regarding bone infection beyond soft tissue infection execute may be challenging, so execute remain the differentiation of bone infection from non-infectious bone issues such namely severe gout and acute Charcot neuroarthropathy. To further involve base assessment, this conditions may also moreover co-exist including OM or foot sepsis, especially when an adjoining ulcer is present.

A definitive diagnosis regarding OM ideally requires the availability regarding histological findings consistent with bone infection (acute and chronic inflammatory cells, necrosis) and the isolation of bacteria from an aseptically obtained bone sample [69]. However, bone biopsy is frequently no longer into a role according to stay performed, almost frequently appropriate in conformity with commencement of antibiotics prior to review the foot.

The clinician can also in conformity take into account about clinical, laboratory then imaging findings for diagnosis. The presence of exposed bone, bone palpable with a probe ("probe to bone test"); erythematous or indurated ("sausage") toe, specifically along an ulcer, an ulcer that is deep, failure to heal despite offloading or wound location above a bony prominence and the presence of soft tissue sinus are suggestive regarding OM.

The probe to bone test (PBT) is a useful clinical diagnostic tool for diagnosing osteomyelitis. PBT is carried out by using a sterile probe to gently explore the wound. If the probe encounters a hard and gritty mass to that amount is presumed to be bone or joint space, the test is viewed high quality and this tremendously will increase the probability concerning osteomyelitis in high-risk population where there is a high pre-test risk to diagnose OM [67]. The test seems at additionally good after rule out OM, so a negative probe-to-bone test in a patient at low risk strongly argues against to the diagnosis concerning osteomyelitis [67, 68]. Occasionally, a viscous exudate (joint fluid) might also moreover keep located discharging out of a sinus, helping a diagnosis of joint infection.

Imaging diagnosis concerning osteomyelitis usually begins along plain radiographs an inexpensive, widely available tool for preliminary evaluation and are regularly ample because imaging the base in patients with suspected diabetic foot osteomyelitis (DFO). Radiographic signs of osteomyelitis encompass lowered bone density, degenerative changes and cortical erosion, trabecular destruction, bone necrosis, Brodie abscess, sclerosis, and periosteal reaction. X-ray has low sensitivity specially of the early stages of osteomyelitis as like radiological changes do may be delayed for 4 weeks following infection. Comparison together with previous films and repeat radiographs at 2–6 weeks perform additionally stand useful. If these studies were defective or medical suspicion stays high, the patient will necessity more imaging. MRI is the appreciated beneficial imaging modality because of diagnosing osteomyelitis due to true sensitivity and specificity then perfect spatial modality because of evaluation of each soft tissues and bone. In prerequisites the place MRI is contraindicated or unavailable, a nuclear medicinal prescript scan such so leukocyte scan preferably combined together with a bone scan is the exquisite alternative. Other imaging modalities may additionally moreover stay beneficial into the presage concerning OM [69, 70] (Table 1).

Table 1

Imaging modality	Advantages	Disadvantages
Plain radiography	Readily available and inexpensive	Low sensitivity in early stages of osteomyelitis
	May be used to monitor response to antibiotic treatment	Specificity is limited by difficulty differentiating infection from Charcot's arthropathy and other

Imaging modality	Advantages	Disadvantages
		pathologies (e.g. gout)
	Can reveal presence of radioopaque foreign bodies, gas in soft tissues, calcified arteries, fractures or bony abnormalities.	
MRI	Preferred advanced imaging modality for diagnosing osteomyelitis.	Reduced performance with severe ischaemia.
	Does not use radiation.	Not all patients are suitable for MRI (e.g. pacemaker).
	Excellent spatial resolution and is very useful for evaluation of bone marrow as well as of soft tissue structures. Good for detection of sinus tracts, deep tissue necrosis, abscesses and other inflammatory changes.	
Nuclear medicine scan	More sensitive than radiographs for detecting osteomyelitis during early stages of the disease.	Poor specificity and low resolution of images.
	Labelled leucocyte scintigraphy with either indium-111 (^{111}In) or technetium-99 (^{99}mTc), improves specificity.	Includes a 24 h waiting period before imaging can begin as well as low resolution of the images.

Imaging modality	Advantages	Disadvantages
	White blood cell-labelled single-photon emission computed tomography can be combined with computed tomography (^{99}mTc WBC labelled-SPECT/CT) imaging to provide good spatial resolution.	Limited availability.
PET (PET/CT)	Excellent spatial resolution.	Limited availability.
	Does not require blood processing.	High costs.

4. Risk Classification/Staging of the Diabetic Foot

Based regarding an uncompromising history, physical examination and ABI/toe pressures, each affected person necessity in conformity with stay carefully assessed and assigned according to a special foot risk stage. Limb staging is necessary in imitation to provide risk stratification of patients with admire in conformity with disorder natural history (risk of amputation, chance of wound healing, and likely benefit of revascularisation). Staging additionally lets between significant evaluation concerning exceptional therapy strategies. Various classification systems are used in accordance with stratify diabetic patients with foot complications within an attempt in conformity with predict the outcomes on possibility about beating removal or hazard on decrease limb amputation and as a result assist plan remedy strategy. The Meggit-Wagner wound classification system used in the past widely used, and it is primarily based completely concerning assessment of ulcer deepness and the presence of osteomyelitis or gangrene [71]. The drawback regarding the Wagner classification system is that it does not particularly tackle 2 drastically vital parameters within diabetic foot: ischaemia and infection [72].

The University concerning Texas classification grades ulcers principally based on depth. Each grade is then staged in accordance after the presence regarding infection, ischaemia or both. However, this classification lacks ample evaluation of infection and ischaemia as much as are included solely as like dichotomised variables [73, 74]. The SINBAD system grades ulcer site, area and depth, the presence of sepsis, arterial disease and neuropathy as dichotomised variables [75]. The IWGDF recommends the use on SINBAD as a primary triage and audit tool for the diabetic foot [66]. SINBAD lamentably lacks mandatory perfusion assessment.

4.1 WIfI Classification

In 2014 the Society because Vascular Surgery proposed a Lower Extremity Threatened Limb Classification System as represents a coordination of more than one until now posted classification schemes to that amount focussed on diabetic foot ulcers and pure ischaemia models. This classification is referred as WIfI and is based over grading each concerning the three important elements (Wound, Ischaemia and, foot Infection) on a scale from 0 to 3, the place zero represents none, 1 mild, 2 moderate, and is 3 severe [77].

Wounds are labelled from grade 0 through grade 3 based absolutely on size, depth, severity, place then expected problem attaining wound healing (Table 2). Advanced gangrene with an unsalvageable foot is categorised as much WIfI clinical stage (Table 5).

Classification of ischaemia is based regarding ABI, Toe pressure (TP) or transcutaneous oxygen saturation (TcPO2) (Table 3), with preference given in accordance with toe pressures, specially into patients including diabetes. Diabetic patients be able

additionally have falsely accelerated ABIs due to MAC then among its state of affairs TP and TcPO2 measurements are preferred for evaluation over perfusion. Patients with TP < 30 mmHg hold excessive ischaemia and are per chance in imitation of require revascularization in accordance to achieve wound restoration or limb salvage. WIfI consists of the classification used by the Infectious Diseases Society of America (the "infection" part of the PEDIS classification) to assess severity of the infection (Table 4).

Table 2

Grade	Ulcer	Gangrene
0	No ulcer.	No gangrene.
	Ischaemic rest pain (requires typical symptoms + ischaemia grade 3); no wound.	
1	Small, shallow ulcer(s) on distal leg or foot; no exposed bone, unless limited to distal phalanx.	No gangrene.
	Minor tissue loss. Salvageable with simple digital amputation (1 or 2 digits) or skin coverage.	
2	Deeper ulcer with exposed bone, joint or tendon: generally, not involving the heel; shallow heel ulcer, without calcaneal involvement.	Gangrenous changes limited to digits.
	Major tissue loss salvageable with multiple (≥3) digital amputations or standard trans metatarsal amputation ± skin coverage.	
3	Extensive deep ulcer involving forefoot and/or midfoot; deep, full thickness heel ulcer ± calcaneal involvement.	Extensive gangrene involving forefoot and/or midfoot; full thickness heel necrosis ± calcaneal involvement.
	Extensive tissue loss salvageable only with complex foot reconstruction or non-traditional TMA (Chopart or Lisfranc); flap coverage or complex management needed for large soft tissue defect.	

4.2 WIfI Ischemia grading

Table 3 [76]

Grade	ABI	Ankle systolic pressure (mmHg)	TP, $TcPO_2$ (mmHg)
0	≥0.80	>100	≥60
1	0.6–0.79	70–100	40–59
2	0.4–0.59	50–70	30–39
3	≤0.39	<50	<30

TP Toe pressure, *$TcPO_2$* Transcutaneous oxygen pressure

If TP and ABI measurements result in different grades, TP will be the primary determinant of ischaemia grade.

4.3 WIfI wound grading

Table 4 [77]

Grade	Clinical manifestation of infection
0	No symptoms or signs of infection.
1	Infection present, as defined by the presence of at least two of the following items: (a) Local swelling or induration (b) Erythema >0.5–≤2 cm around the ulcer (c) Local tenderness or pain (d) Local warmth (e) Purulent discharge (thick, opaque to white, or sanguineous secretion). Local infection involving only skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). Exclude other causes of inflammatory response of the skin (e.g., trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, venous stasis).
2	Local infection (as described above) with erythema >2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), and no signs or symptoms of systemic inflammatory response syndrome (SIRS).
3	Local infection (as described above) with the signs of SIRS, as manifested by two or more of the following:

Grade	Clinical manifestation of infection
	(a) Temperature >38 °C or <36 °C (b) Heart rate > 90 beats/min (c) Respiratory rate >20 breaths/min or $PaCO_2$ < 33 mmHg (d) White cell count >12,000 or <4000/mm^3 or 10% immature (band) forms.

Once the patient has been scored under the three categories, the appropriate spectrum score is then derived after give an overall amputation risk. Spectrum rankings deemed low risk, moderate risk and high risk for limb amputation at 1 yr. are categorised as clinical stage 2, stage 3 and stage4 disease, respectively, although the definitions about 'low', 'moderate' then 'high' risk are now not given.

The three categories (wound, ischemia, and foot infection) with four grades regarding severity produces a grid along 64 theoretically potential clinical combinations (WIfI classes). A Delphi consensus about the members of the Society of Vascular Surgery Lower Extremity Guidelines Committee assigned a chance category in imitation of each on the combinations among regards after hazard of limb amputation at 12 months (very low risk, moderate risk, or high risk) and benefit of revascularisation (very low benefit, moderate benefit, or high benefit) for each on the feasible combinations (Table 5). Several recommended series had been posted including analysis over consequences about patients with threatened limb, which encompass diabetic foot patients, based on WIfI clinical stage validating this model [47, 77, 78, 79, 80, 81, 82, 83].

II. Treatment regarding Diabetic Ulcer/Foot Ulcer

The current standard care for DFUs focuses regarding bettering the wound healing process. DFU regime enormously employs the usage of active biocompatible wound dressings to aid healing, whilst skin grafts would possibly also may be used in the therapy of non-healing or recurrent DFU wounds. In current years, recent medicine procedures, component concerning stem-cell-based therapy, have also emerged as prospective treatments for DFU.

1.WIfI clinical stages

Suggested route, setting, then duration on antibiotic therapy, with the aid of the use of clinical presentation.

Table 5 [76]

Site of infection, by severity or extent	Route of administration	Setting	Duration of therapy
Soft tissue only			
Mild	Oral	Outpatient	1–2 weeks; may extend up to 4 weeks if slow to resolve.
Moderate	Oral (or initial parenteral).	Outpatient /inpatient	1–3 weeks.

Severe	Initial parenteral, switch to oral when possible.	Inpatient, then outpatient.	3–4 weeks.
Bone or joint			
No residual infected tissue (e.g., post- amputation)	Parenteral or oral.	Inpatient, then outpatient.	2–5 days.
Residual infected soft tissue (but not bone)	Parenteral or oral.	Inpatient, then outpatient	1–3 weeks.
Residual infected (but viable) bone	Initial parenteral, then consider oral switch.	Inpatient, then outpatient	Up to 6 weeks.
No surgery, or residual dead bone postoperatively	Initial parenteral, then consider oral switch.	Inpatient, then outpatient.	Up to 6 weeks.

1.2 Wound Dressings

Biocompatible polymer-based wound dressings are typically used into DFU wound management. Dressings are typically taken beyond both natural polymers, biocompatible synthetic polymers or a natural and synthetic polymer [84,85]. For example, the Integra® Dermal Regeneration Template consists regarding a silicone-based outer layer that performs the function regarding the skin epidermis then an inward strata regarding bovine tendon collagen yet glycosaminoglycan matrix, who presents a scaffold for dermal regeneration [86, 87].

The most important functions of active wound dressings are to maintain the wound environment moist and provide a barrier against the infiltration of infectious agents.

This helps in accordance with facilitate the processes of re-epithelialization and tissue remodelling, whilst minimizing complications arising from inflammatory responses precipitated by microbial infections. The management of chronic DFUs is complicated with the aid of ability about higher chances of wound infections and poorer recovery rates due to changes of cellular behaviours, increase difficulty and cytokine secretion into reply according to a high-glucose microenvironment. Dressings along elevated antimicrobial yet pro-healing residences are nowadays exceedingly explored namely recent therapy preferences because of DFUs [88,89,90].

1.3 Skin Grafts

Skin grafting is used to treat severe DFUs so much function that do not improve with the application of wound dressings [91]. There are a various types of skin grafting engaged among persistent wound treatment. Split-thickness skin grafting (STSG) usage autogenic and allogenic skin, consisting of the epidermis and a small portion of dermis, collected from patients and donors. While STSG has demonstrable utility between enhancing wound healing, this cure method is prone to issues such as much non-healing small wounds, infection, and graft rejection of diabetic patients up to expectation may additionally need in accordance with end result between even poorer healing effects then increased risk of amputation. Bioengineered human skin equivalent (HSE) products

are used for grafting, and they put away the bother of non-healing secondary wounds [92,93,94]. Bioengineered skin substitutes are typically classified of epidermal-, dermal- and bilayer-constructs, relying on cellular composition and thickness of the constructs [95]. Cultured epithelial autografts normally include 6-8 stratified layers regarding autologous keratinocytes irradiated murine fibroblasts. dermal skin substitutes incorporate allogenic dermal fibroblasts within a collagen-based extracellular matrix scaffold.

Bilayered epidermal–dermal skin substitutes contain a level of delineated keratinocytes in nearness to a lower layer of dermal fibroblasts and are the most comparative in physiology and cellular composition to natural skin. Dermal or bilayered skin substitutes, such so Dermagraft®, Apligraft®, and OrCel®, hold been authorized for the treatment concerning DFUs. However, evidence suggests up to expectation proper engraftment about these bioengineered tissues does no longer take place and cells between this constructs functionate no longer live more than 6 weeks [96,97,98]. As the manifest therapeutic benefits of these skin substitutes among particular result out of accelerated pro-healing signalling and cellular crosstalk when the allogenic skin constructs are placed in vicinity to the wounds, the mode over rate concerning certain skin equal grafts may additionally moreover stay viewed more correctly namely a short-lived cell-based therapy.

1.4 Cell-Based Therapy

In addition to skin-specific cells, the utilizes concerning stem cells among bioengineered products to enhance the healing of DFUs is a place about intensive studies [99,100,101,102,103,104]. Mesenchymal stem cells (MSCs) derived from tissues such as adipose tissues and bone marrow have been used in several studies. Of these, bone-marrow-derived MSCs (BM-MSCs) are the large characterized to as regards diabetic wound recuperation in clinical settings.

Autologous BM-MSCs administered to wounds accelerated wound closure and revascularization in the dermis or extended the depth wound bed [105,106,107,108,109,110]. The therapeutic outcomes over BM-MSCs include modulation of the inflammatory responses, stimulation of angiogenesis and promotion of proliferation of keratinocytes then fibroblasts through release of growth factors [111,112,113].

Other MSCs, certain as like those derived out of adipose tissues and peripheral blood, additionally evolved similar therapeutic consequences of pre-clinical studies, highlighting theirs potential in treating chronic wounds within diabetic patients (Table 6).

Table 6. Current and experimental treatments for DFUs

Treatments/Key Examples	Mechanisms	Effects	
Wound dressings Natural Polymer (Promogran, collagen) Synthetic polymer (Polyglactin, 45S5 Bioglass (PLGA)) Combination of natural and synthetic polymer (Integra® Dermal Regeneration Template)	Increase MMP-2, and MMP-9 in the epidermis[114].Promote secretion angiogenic growth factors [115,116].	Rapid wound healing Promote re-epithelialization Revascularization and angiogenesis.	
Skin grafting Split-thickness skin graft (STSG)		Increase MMP-2, and MMP-9 in epidermis and dermal TIMPs activity [117]	Improve wound healing.

Human skin equivalent (HSE) (Dermagraft®, Apligraft®, OrCel®)		Increase cytokines and growth factors [93,94,96]. Deliver growth factors and extracellular matrix proteins to the wound bed [118].	
Stem-cell therapy Bone-marrow derived MSCs Umbilical cord blood-derived MSCs Adipose-derived MSCs Placenta-derived MSCs Amniotic fluid-derived MSCs		Promote secretion of angiogenic factors [119]. Increase the expression of keratinocyte-specific proteins and cytokeratin [119]. Increased collagen synthesis [100,113]. Modulation of immunological abnormality [120].	Promote wound healing Increase re-epithelialization and cellularity Promote dermal collagen synthesis Vascular regeneration.

1.5 Three-Dimensional Bioprinting Approaches in accordance with Aid Wound Repair

Three-dimensional (3D) printing is a method within which a user-defined objective is fabricated with the aid of the deposition of materials into successive layers. Three-dimensional bioprinting is a subset of 3D printing to that amount has been widely used within tissue engineering to create biomimetic tissues. This additive technical approach capabilities superb flexibility and reproducibility in fabricating mechanically stable biocompatible tissue or organ constructs that mimic the native microenvironment [121]. Broadly, in that place are three types concerning 3D bioprinting technologies: inkjet-based, extrusion-based, and light/laser-based. Each regarding it has precise strengths and weaknesses as perform to them excellent for precise biomanufacturing functions.

1.6 Three-Dimensional Bioprinting Techniques

Drop-on-demand (DOD) is the most established inkjet-based bioprinting technology and is sub-categorized into thermal, piezoelectric, and electromagnetic DOD, which each share a similar printing mechanism [122,123]. The printing technique consists of two phases: (1) the dispensing of bioink droplets according to precise areas on the substrate; and (2) the interplay within the bioink droplets and substrate on contact (crosslinking and gelation). The printing resolution corresponds to the droplet size and perform keep optimized by using adjusting the nozzle diameter, viscosity of ink, voltage impulse frequency, and, for thermal inkjet, the temperature gradient [124]. The strengths regarding inkjet bioprinting are low cost, fast printing press speed, high resolution, and capabilities of altering concentration gradient [125]. The drawbacks of that technological method are low seeding density and compromised cellular viability and performance resulting beside the crosslinking and gelation approach [126,127]. Of note, that printing method has been modified and raised in handheld portable bio printing devices for in situ printing services in accordance with tackle the problem concerning irregular wound topology in conventional skin grafting [128,129].

Extrusion-based bioprinters assignment through skill on ejecting a continuous stream of

a biomaterial, as an alternative on droplets, to the substrate via two types of methods: pneumatic or mechanical [130]. The pneumatic system allows higher material flow manipulate including the aid about the utilization of pressurized gas according to outlaw the bioink out of the nozzle, while the mechanical system affords better spatial rule with compressional power from screws and pistons. While successful printing constructs at excessive cell densities, the viability of the extrusion-based printed cells is lower (40–86%) in contrast to inkjet-based bioprinting (> 90%).

Lowering the extrusion pressure and increasing nozzle size be able increase cell viability then again result among reduced printing speed and poorer resolution [127,131]. Hence, fine-tuning regarding the nozzle diameter, printing pressure, and velocity is quintessential in conformity with accomplish sure the performance of tissue constructs printed using this method [132]. This printing pragmatic knowledge helps a great variety regarding nicely proper supplies such as like hydrogels, biocompatible copolymers or cell spheroids with excessive seeding density [132], and is commonly used into in vitro bioengineering strategies to generate 3D human skin constructs.

Laser/light-assisted bioprinting (LAB) is a modification of the laser-induced forward transfer (LIFT) technology, which manufactory with the aid of means regarding focusing a pulsed laser on an energy-absorbing ribbon layer in conformity with grow high-pressure bubbles to that amount eject the bioink onto the substrate [125,133]. Thus, among contrast after inkjet then extrusion-based bioprinting, nozzle clogging is now not seasoned within LAB, who can, therefore, remain used for a variety regarding bioinks at particular viscosities to obtain high-resolution printing [132,134].

However, printing at high-resolution choice end result within a gradual printing rate and have an effect on the hydrogel fidelity and gather performance due to dehydration [132]. As such, printing is extra typically utilized for acellular bio manufacturing certain as much the incorporate of precise particular amounts of biomolecules into wound dressings.

Each of the 3D bioprinting strategies has its specific strengths and weaknesses that redact that suitable because of exceptional biomanufacturing platforms, collectively along in vitro and into situ bioprinting, as perform lie exploited because the enhancement over higher medicine selections for therapy and care of DFUs.

2.Application on 3D Bioprinting Approaches according to Wound Treatment

2.1 In Vitro Bioprinting

A quintessential advantage of bioprinting is the capability in accordance with contain one kind of bioinks and cell mixtures within biocompatible matrices in specific spatial orientations of layers of the printed constructs. This feature be able remain exploited to create skin substitutes with more elaborate cellular arrangement and produce constructs that are more similar in accordance with the native tissue, and with enhance engraftment and the wound healing process. Most presently available human skin substitutes used for DFU therapy are generated through way regarding culturing keratinocytes or fibroblasts on biocompatible scaffolds. In diabetic wounds that have lower degrees of pro-angiogenetic signals, these simplistic constructs usually fail to emerge as vascularized or combine with host tissues, and the transplanted cells accordingly work now not survive for long. To promote vascularization and better tissue integration, 3D bioprinted skin substitutes containing layers of neonatal human dermal fibroblasts and epidermal keratinocytes, and human dermal microvascular endothelial cells (EC)s embedded between fibrin-collagen bioink were been generated [135]. These EC-containing skin constructs were transplanted onto full-thickness excision wounds on mice in conformity with seem at their outcomes of wound healing. The bioprinted EC-containing skin mimetic graft produced in accelerated restoration of the wound, with 17% improvement of wound contraction, and cellular matrix controls.

3D-printed constructs containing cord blood-derived human ECs, placental pericytes (PC)s, foreskin dermal fibroblasts and keratinocytes of collagen bioink had been positioned in accordance with exhibit associated biological or morphological functions to that of native skin in vitro [136]. More importantly, when grafted wounds among experimental models, rapid invasion microvasculature, the availability of human EC-lined microvessels and a high degree of epidermal organization have been seen 2 and 4 weeks' post-implantation for the PC and EC-containing grafts. These researches expose as this extra suited 3D-bioprinted skin substitutes may improve graft vascularization and integration, principal in conformity with higher wound healing outcomes.

Bioprinting is additionally utilized in imitation of the development regarding stem cell-based therapy plans for DFU, according to precisely include active stem cells at preferred densities into bioactive scaffolds to propagate wound patches along greater applicable regenerative and wound healing potential. For example, a 3D-printed skin patch containing adipose-derived stem cells (ASCs) and endothelial progenitor cells (EPCs) into decellularized porcine skin ECM was once as soon as mentioned to enhance re-epithelialization [137]. These findings show the applicability regarding 3D-bioprinted cellular constructs in wound recovery by targeting the troubles such as re-vascularization to improve the long-term survival of skin substitutes. While extra studies and optimization will be required, the utilizes concerning 3D-bioprinting to create highly complex cellular constructs with relevant cell types at unique densities and particular spatial distribution will become an essential useful resource to generate novel and targeted DFU treatment.

2.2 In Situ Bioprinting

In situ bioprinting capitalizes of the drop-on-demand inkjet technology to print cell-laden bioinks directly onto body sites with the use concerning handheld devices or robotic automated systems. This approach of bioprinting be able to keep exploited according to request targeted skin cell concentration over wounds to pace upon DFU healing by means of cell–cell signalling, as a viable alternative to use the skin substitutes. In a proof-of-concept study, a portable cartridge-based inkjet printer combined with a laser scanner was used to print suspensions of human fibroblasts and keratinocytes at high densities in fibrin-collagen bioink to full-thickness excisional wounds in experimental mice [138]. The printed skin constructs on the wounds had been rendered according to preserve excessive cellularity, and the arrival of human cells do lie into the tissues for up to 6 weeks post-printing. Wounds treated by direct in situ skin bioprinting had been performed in conformity with heal faster compared to untreated and acellular controls, including the form of organized dermis and epidermal sublayers [138].

The utilizes regarding stem cells was additionally evaluated for within situ wound healing. Skardal et al. used a robotic inkjet bioprinter in conformity with amniotic fluid-derived stem cells (AFSC)s then BM-MSC-laden fibrin-collagen bioink directly onto full-thickness pores and skin wounds in mice [129]. Compared to the gel-only control, mice treated together with AFSCs and BM-MSCs presented greater wound closure and re-epithelialization. In a further experiment to modulate paracrine activities at wound site, the authors afterwards printed AFSCs-containing, heparin-conjugated hyaluronic acid hydrogels directly onto a full-thickness wound. The hydrogel was executed according to sequester AFSC-secreted cytokines of situ, prolonging the paracrine activity and led to more fast closure and vascularization of the full thickness wounds.

A main obtain on in situ bioprinting is the capability to layer the cell-laden bioinks in accordance to the specific topography of the wound, thus, this technique is specifically useful for adaptation in conformity with the remedy wounds with irregular topography. Co-development regarding cell-based with similarly optimization regarding in situ bioprinting methods choice stay key in accordance with producing higher therapeutic preferences for DFU patients. (Table 7) outlines the modern advances within 3D bioprinting procedures in bettering the substantial and purposes concerning wound management.

Table 7. Summary of 3D bioprinting approaches to aid in wound healing.

Bioprinting Devices	Biomaterials for Printing and Construct Applications	Outcomes
In vitro 1.Robotic, inkjet motor-driven device	Bioink: Fibrin and collagen Cells: Neonatal human dermal fibroblast, human dermal microvascular endothelial cells and neonatal human epidermal keratinocytes Application: Full-thickness skin excision (1.7 cm × 1.7 cm) on athymic nude mice	Bioprinted skin graft showed accelerated wound healing with 17% improvement in wound contraction and formation of microvasculature [135].

Bioprinting Devices	Biomaterials for Printing and Construct Applications	Outcomes
2.Robotic, inkjet and extrusion device (in-house built)	Bioink: Decellularized extracellular matrix of porcine skin tissue Cells: Adipose-derived stem cells (ASCs) + endothelial progenitor cells (EPCs) Application: 10 mm biopsy punch full-thickness excisional wound on BALB/c A-nu/nu	Bioprinted skin patch remarkably enhanced neovascularization as well as wound closure and re-epithelization. Cell-printed dECM patch also exhibited better-wound healing activity compared to only ASC/EPCs mixture [137].
3.Robotic, extrusion pressure-driven device	Bioink: Rat tail type I collagen Cells (dermis): human foreskin dermal fibroblasts, human endothelial cells derived from cord blood endothelial colony-forming cells and placental pericytes (PCs) Cells (epidermis): Human foreskin keratinocytes Application: Excised dorsal mouse skin	Skin substitutes formulated with human ECs and PCs contained vascular structures2-weeks post-engraftment and a higher degree of epidermal organization. Grafts containingPCs in addition to ECsappeared to evoke a more extensive antigenic host response [136].
1,Bioink: Fibrin and collagen Cells: Human fibroblasts and keratinocytes	Bioprinted constructs promoted accelerated wound closure with fully formed and organized dermis and epidermis.	

Bioprinting Devices	Biomaterials for Printing and Construct Applications	Outcomes
Application: Full-thickness excisional skin defect (3 × 2.5 cm) on female outbred nu/nu mice	Fibroblasts and keratinocytes remained 6 weeks post engraftment [128].	
2.Robotic, inkjet pressure-driven device (Three-axes movement system with pressure-driven nozzles and an optical sensor)	Bioink: Fibrin and collagen Cells: Amniotic fluid-derived stem cells (AFS) and Bone marrow-derived mesenchymal stem cells (MSC) Application: Full-thickness skin wound (2.0 × 2.0 cm) on nu/nu mice.	Wounds treated with AFS and MSC cells had faster-wound closure, re-epithelialization and better neovascularization than n cell-free controls [138].
3.Robotic, inkjet pressure-driven device (Three-axes movement system with pressure-driven nozzles and swappable hydrogel cartridges in-line with back-	Bioink: Heparin-conjugated hyaluronic acid (HA) and thiolated HA Cells: Amniotic fluid-derived stem (AFS) cells Application: Full-thickness skin wound (2.0 × 2.0 cm) on nu/nu mice.	HA-HP hydrogel supports the extended release of AFS-secreted cytokines by heparin-associated sequestration that improved wound closure, re-epithelialization, and vascularization [128].

Bioprinting Devices	Biomaterials for Printing and Construct Applications	Outcomes
pressure and print nozzle)		

3. Future Directions of 3D Bioprinting Strategies in Diabetic Wound Repair

Moving forward, it is estimated up to expectation 3D bioprinting could apply a complementary position in the development of advanced wound scaffolds or skin grafts that enhance the healing process and restore the skin's structure and function in the diabetic wound. Here, we highlight partial feasible avenues about innovation in which 3D bioprinting ought to stay applied to aid DFU management.

Development regarding Novel Biocompatible ECM-Based Hydrogels as much Bioinks

The utilizes of magnificent biocompatible ECM-based hydrogels to create the native-like ECM-based microenvironment in printed skin constructs is quintessential to tailor these skin grafts for DFU treatment. At present, repeatedly biocompatible ECM-based hydrogels, both natural and synthetic, operate no longer simply recapitulate the bioactivity of native ECM due to absence of biochemical factors. Hence, such is vital in accordance with enhance modern bioink supplies to that amount match the mechanical, rheological, and biological properties of the target tissues.

There are a few techniques in conformity for developing novel bioinks for the DFU-specific bioprinted skin substitutes. One technique being decellularized ECM (dECM)-based bioinks, derived from decellularized tissues of interest consist of the required growth factors and cytokines to cue cellular growth and differentiation [139,140]. Apart from dECM, materials that respond to exterior stimuli can additionally can be used as bioinks. These materials may undergo conformational modifications after special physiological triggers and the microenvironment, making them favourable for tissue regeneration and cure delivery. An example regarding stimulus is the cell traction force, which has been exploited in conformity with flourish vascular community construction in bioprinted tissues and wound restore [141,142]. As chronic wounds are predominantly more alkaline (5.4–7.4) than healthy skin (4.7–5.75) due to defective ECM and the availability of microbes, pH-responsive bioinks may additionally remain good because bettering wound restoration [143]. For instance, a pH-responsive hydrogel has been developed to release sequestered vascular endothelial growth factor (VEGF) at pH 7.4 instead of pH 5 and 6 [144]. Such bio-responsive bioinks execute remain tailored to recreate the most optimal microenvironment to develop removal into DFUs.

4.Personalized Treatment

4.1-Three-dimensional printing

Permits the improvement of medicine by means of tailoring to patient-specific anatomical factors and disease profiles. Examples about personalized tissues by 3D bioprinting include cartilage, bone, as well as cardiac patches. In comparison to commercially skin substitutes, 3D bioprinting skin grafts permits customization about the measure and shape of constructs to fit the patient's exclusive wound topology, thereby construction secure complete wound coverage and better aesthetics post-healing [145]. This attribute is elegantly illustrated via in situ bioprinting, the fabric is preserved onto the wound site to achieve complete coverage [128,129]. However, at present, in situ bioprinting is totally plausible for short wounds (< 3 × 3cm). For wounds with larger surface areas, cellular proliferation, tissue maturation and vasculature formation inside the bioprinted skin bring together choice remain crucial in conformity to maintain the graft viability in vivo [146]. This be able potentially remain done with the aid of capability about first printing skin construct in vitro, the region a bring together about a particular size, geometry and cellular composition is fabricated primarily based on a 3D scan of grafting region on the patient before transplanting onto the wound. In addition, personalized drug-laden wound dressings or skin grafts could be manufactured by 3D bioprinting in imitation of enable the regimen over precise dosages and combinations regarding drug compounds, such as anti-inflammatory drugs, to with the wound sites in accordance with address patient-specific therapeutic regimens [147].

4.2 -Infusion concerning Bioactives in Acellular 3D-Printed Wound Dressings

In addition to proper 3D-bioprinting capabilities into which tissue-like mimetics are printed using cell-laden bioinks, 3D printing strategies be able additionally keep utilized among the fabrication over better therapeutic products for DFU management. For example, the facility and precision of 3D printing strategies have been capitalized upon to incorporate bioactive compounds into wound dressings tailor for DFU-specific treatments to enhance theirs anti-microbial and pro-healing properties. Recent characteristics include wound dressings that were 3D-printed using of biodegradable polydimethylsiloxane infused together with silicon oil and silver nanoparticles. These dressings proven surprisingly excessive quality antibacterial and extended re-epithelialization and granulation tissue structure so utilized to a full-thickness wound inoculated with S. aureus or E. coli in animal models [138].

Re-epithelialization, angiogenesis, and dermal remodelling during wound healing are stimulated by bioactive molecules such as secreted proteins, growth factors, and cytokines synthesized and secreted by physiologically healthy cells. In DFUs, the secretion and the synthesis of these biomolecules are altered due in conformity with impaired cell functions in response to the high-glucose microenvironment.

To enhance the commercially bioartificial dermal template and scaffolds for DFU wound management, growth factors do remain included into the products [148,149,150,151]. For example, the inclusion and shipping concerning VEGF in 3D-bioprinted scaffolds has been shown increasing the proliferation of endothelial cells and vascularization into vivo [150,151]. These effects display the main about the makes use of concerning bioprinted wound scaffolds containing VEGF or other growth factors to restore blood supply to the wound site to facilitate healing. The unique spatial incorporation of bioactive molecules within novel biocompatible ECM by using 3D bioprinting technologies be able keep further exploited to incorporate temporal release and control of the bioactive molecules to further tailor these dressings for DFU treatment.

III. Conclusion

The demand for DFU wound administration is expected to increase following the rising incidence of DM patients. It is quintessential to develop DFU-specific therapeutic tools to enhance contemporary DFU wound management facilitate the development concerning novel and prolonged wound healing strategies. Further co-development of 3D-bioprinting applied sciences and promising novel approaches, such as personalized drug-coated dressings and stem cell-based therapeutics so the increasing demands do no longer over-strain the confined healthcare resources. Compelling evidence demonstrated that the integration concerning a various 3D bioprinting strategies with present wound treatments, will remain the address specific pathophysiological the challenges that DFUs present.

Conflict Of Interest

All authors declare no conflicts of interest.

Authors Contribution

Authors have equally participated and shared every item of the work.

Abbreviation

Pes cavus: is a descriptive term for a foot morphology characterized by high arch of the foot that does not flatten with Weight bearing.

PAD: peripheral arterial disorder.

(DFI): diabetic foot infection.

(LOPS): loss of protective sensation.

(PKC): protein kinase C.

(ROS): reactive oxygen species.

NADPH: nicotinamide adenine dinucleotide phosphate.

(ABI): Ankle-Brachial Index.

(TPs): toe pressures.

(MAC): medial arterial calcification (MAC).

(TBI): toe: brachial pressure index.

(TcPO2): Transcutaneous oxygen tension.

(SPP): Skin perfusion pressure.

(OM): osteomyelitis.

(DFI): diabetic foot infection.

(PBT): probe to bone test.

IWDGF: *International Working Group on the Diabetic Foot.*

TMA: Trans metatarsal Amputation

(MSCs): Mesenchymal stem cells.

(BM-MSCs): bone-marrow-derived MSCs.

(ASCs): adipose-derived stem cells.

(EPCs): endothelial progenitor cells.

(AFSC)s: amniotic fluid-derived stem cells.

(MMP-2): matrix metalloproteinase-2.

(MMP-9): Matrix metallopeptidase 9.

(TIMPs): tissue inhibitors of metalloproteinase.

(STSG): Split-thickness skin grafting.

(HSE): human skin equivalent.

REFERENCES

[1] Zhou, Bin, et al. "Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants." *The Lancet*387.10027 (2016): 1513-1530.

[2] Guariguata, Leonor, et al. "Global estimates of diabetes prevalence for 2013 and projections for 2035." *Diabetes research and clinical practice* 103.2 (2014): 137-149.

[3] Bakker, K., et al. "The 2015 IWGDF guidance documents on prevention and management of foot problems in diabetes: development of an evidence-based global consensus." *Diabetes/metabolism research and reviews* 32 (2016): 2-6.

[4] Singh, Nalini, David G. Armstrong, and Benjamin A. Lipsky. "Preventing foot ulcers in patients with diabetes." *Jama*293.2 (2005): 217-228.

[5] Armstrong, David G., Andrew JM Boulton, and Sicco A. Bus. "Diabetic foot ulcers and their recurrence." *New England Journal of Medicine* 376.24 (2017): 2367-2375.

[6] Rice, J. Bradford, et al. "Burden of diabetic foot ulcers for medicare and private insurers." *Diabetes care* 37.3 (2014): 651-658.

[7] Driver, Vickie R., et al. "The costs of diabetic foot: the economic case for the limb salvage team." *Journal of vascular surgery* 52.3 (2010): 17S-22S.

[8] Ragnarson Tennvall, Gunnel, and Jan Apelqvist. "Health-economic consequences of diabetic foot lesions." *Clinical Infectious Diseases* 39. Supplement_2 (2004): S132-S139.

[9] Kerr, M., G. Rayman, and W. J. Jeffcoate. "Cost of diabetic foot disease to the National Health Service in England." *Diabetic medicine* 31.12 (2014): 1498-1504.

[10] Nussbaum, Samuel R., et al. "An economic evaluation of the impact, cost, and Medicare policy implications of chronic nonhealing wounds." *Value in Health* 21.1 (2018): 27-32.

[11] Petrakis, Ioannis, et al. "Losing a foot versus losing a dollar; a systematic review of cost studies in diabetic foot complications." *Expert review of pharmacoeconomics & outcomes research* 17.2 (2017): 165-180. Boulton, Andrew JM, et al. "The global burden of diabetic foot disease." *The Lancet* 366.9498 (2005): 1719-1724.

[12] Walsh, J. W., et al. "Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom." *Diabetic Medicine* 33.11 (2016): 1493-1498.

[13] Apelqvist, Jan, and Jan Larsson. "What is the most effective way to reduce incidence of amputation in the diabetic foot?" *Diabetes/metabolism research and reviews* 16. S1 (2000): S75-S83.

[14] Lepäntalo, Mauri, et al. "Chapter V: diabetic foot." *European Journal of Vascular and Endovascular Surgery* 42 (2011): S60-S74.

[15] Lavery, Lawrence A., et al. "Impact of chronic kidney disease on survival after amputation in individuals with diabetes." *Diabetes care* 33.11 (2010): 2365-2369.

[16] Ghanassia, Edouard, et al. "Long-term outcome and disability of diabetic patients hospitalized for diabetic foot ulcers: a 6.5-year follow-up study." *Diabetes care* 31.7 (2008): 1288-1292.

[17] Prompers, L., et al. "Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study." *Diabetologia* 51.5 (2008): 747-755.

[18] Nabuurs-Franssen, M. H., et al. "Health-related quality of life of diabetic foot ulcer patients and their caregivers." *Diabetologia* 48.9 (2005): 1906-1910.

[19] Nabuurs-Franssen, M. H., et al. "Health-related quality of life of diabetic foot ulcer patients and their caregivers." *Diabetologia* 48.9 (2005): 1906-1910.

[20] Feldman, Eva L., et al. "New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain." *Neuron*93.6 (2017): 1296-1313.

[21] Lazzarini, P. A., et al. "Diabetes-related lower-extremity complications are a leading cause of the global burden of disability." *Diabetic Medicine* 35.9 (2018): 1297-1299.

[22] Zhang, Yuqi, et al. "Global disability burdens of diabetes-related lower-extremity complications in 1990 and 2016." *Diabetes Care* 43.5 (2020): 964-974.

[23] Greene, Douglas A., et al. "Diabetic neuropathy." *Annual review of medicine* 41.1 (1990): 303-317.

[24] Vinik, A. I., and Anahit Mehrabyan. "Diabetic neuropathies." *Medical Clinics* 88.4 (2004): 947-999.

[25] Madae'en, Saba, et al. "Diabetes knowledge, medication adherence, and glycaemic control among diabetic patients: A cross-sectional study in Jordan." *Journal of Applied Pharmaceutical Science* 10.04 (2020): 041-046.

[26] Kim, Bhumsoo, and Eva L. Feldman. "Insulin resistance in the nervous system." *Trends in Endocrinology & Metabolism* 23.3 (2012): 133-141.

[27] Domenech-Estévez, Enric, et al. "Distribution of monocarboxylate transporters in the peripheral nervous system suggests putative roles in lactate shuttling and myelination." *Journal of Neuroscience* 35.10 (2015): 4151-4156.

[28] Fünfschilling, Ursula, et al. "Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity." *Nature*485.7399 (2012): 517-521.

[29] Pop-Busui, Rodica, et al. "Diabetic neuropathy: a position statement by the American Diabetes Association." *Diabetes care* 40.1 (2017): 136-154.

[30] Callaghan, Brian C., et al. "Enhanced glucose control for preventing and treating diabetic neuropathy." *Cochrane database of systematic reviews* 6 (2012).

[31] Dinh, Thanh L., and Aristidis Veves. "A review of the mechanisms implicated in the pathogenesis of the diabetic foot." *The international journal of lower extremity wounds*4.3 (2005): 154-159.

[32] Van Schie, Carine HM, et al. "Muscle weakness and foot deformities in diabetes: relationship to neuropathy and foot ulceration in Caucasian diabetic men." *Diabetes care* 27.7 (2004): 1668-1673.

[33] Mueller, Michael J., et al. "Insensitivity, limited joint mobility, and plantar ulcers in patients with diabetes mellitus." *Physical Therapy* 69.6 (1989): 453-459.

[34] Fernando, Malindu Eranga, et al. "Gait parameters of people with diabetes-related neuropathic plantar foot ulcers." *Clinical Biomechanics* 37 (2016): 98-107.

[35] Fernando, Malindu E., et al. "Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up." *PloS one* 12.8 (2017): e0181916.

[36] Tapp, Robyn J., et al. "Association of glucose metabolism, smoking and cardiovascular risk factors with incident peripheral arterial disease: the DESIR study." *Atherosclerosis* 190.1 (2007): 84-89.

[37] Jude, Edward B., et al. "Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome." *Diabetes care* 24.8 (2001): 1433-1437.

[38] Paneni, Francesco, et al. "Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I." *European heart journal* 34.31 (2013): 2436-2443.

[39] Prompers, L., et al. "High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study." *Diabetologia* 50.1 (2007): 18-25.

[40] Hinchliffe RJ, Brownrigg JR, Andros G, Apelqvist J, Boyko EJ, Fitridge R, et al. Effectiveness of revascularization of the ulcerated foot in patients with diabetes and peripheral artery disease: a systematic review. Diabetes Metab Res Rev. 2016;32(Suppl 1):136–44.

[41] Meloni, Marco, et al. "Characteristics and Outcome for Persons with Diabetic Foot Ulcer and No-Option Critical Limb Ischemia." *Journal of Clinical Medicine* 9.11 (2020): 3745.

[42] Forsythe, R. O., J. Brownrigg, and R. J. Hinchliffe. "Peripheral arterial disease and revascularization of the diabetic foot." *Diabetes, Obesity and Metabolism* 17.5 (2015): 435-444.

[43] Forsythe, R. O., J. Brownrigg, and R. J. Hinchliffe. "Peripheral arterial disease and revascularization of the diabetic foot." *Diabetes, Obesity and Metabolism* 17.5 (2015): 435-444.

[44] Hinchliffe, R. J., et al. "IWGDF guidance on the diagnosis, prognosis and management of peripheral artery disease in patients with foot ulcers in diabetes." *Diabetes/metabolism research and reviews* 32 (2016): 37-44.

[45] Mills Sr, Joseph L. "Update and validation of the Society for Vascular Surgery wound, ischemia, and foot infection threatened limb classification system." *Seminars in vascular surgery*. Vol. 27. No. 1. WB Saunders, 2014.

[46] Zhan, Luke X., et al. "The Society for Vascular Surgery lower extremity threatened limb classification system based on Wound, Ischemia, and foot Infection (WIfI) correlates with risk of major amputation and time to wound healing." *Journal of vascular surgery* 61.4 (2015): 939-944.

[47] Boulton, Andrew James Michael, et al. "Diagnosis and management of diabetic foot infections." (2020).

[48] Carter, Stefan A. "Indirect systolic pressures and pulse waves in arterial occlusive disease of the lower extremities." *Circulation* 37.4 (1968): 624-637.

[49] Ankle Brachial Index Collaboration. "Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis." *JAMA: the journal of the American Medical Association*300.2 (2008): 197.

[50] Newman, Anne B., et al. "Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group." *Circulation* 88.3 (1993): 837-845.

[51] Gerhard-Herman, Marie D., et al. "2016 AHA/ACC guideline on the management of patients with lower extremity peripheral artery disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines." *Journal of the American College of Cardiology* 69.11 (2017): e71-e126.

[52] Halliday, Alison, and Jeroen J. Bax. "The 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS)." *European Journal of Vascular and Endovascular Surgery* 55.3 (2018): 301-302.

[53] Everhart, J. E., et al. "Medial arterial calcification and its association with mortality and complications of diabetes." *Diabetologia* 31.1 (1988): 16-23.

[54] Potier, L., et al. "Use and utility of ankle brachial index in patients with diabetes." *European Journal of Vascular and Endovascular Surgery* 41.1 (2011): 110-116.

[55] Hyun, Suzanne, et al. "Ankle-brachial index, toe-brachial index, and cardiovascular mortality in persons with and without diabetes mellitus." *Journal of vascular surgery* 60.2 (2014): 390-395.

[56] Young, M. J., et al. "Medial arterial calcification in the feet of diabetic patients and matched non-diabetic control subjects." *Diabetologia* 36.7 (1993): 615-621.

[57] Brooks, B., et al. "TBI or not TBI: that is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients?" *Diabetic Medicine* 18.7 (2001): 528-532.

[58] Widmer, Lukas W., et al. "Reliability and repeatability of toe pressures measured with laser Doppler and portable and stationary photoplethysmography devices." *Annals of vascular surgery* 26.3 (2012): 404-410.

[59] Andersen, Charles A. "Non-invasive assessment of lower-extremity hemodynamic in individuals with diabetes mellitus." *Journal of the American Podiatric Medical Association* 100.5 (2010): 406-411.

[60] Williams, Dean T., Patricia Price, and Keith G. Harding. "The influence of diabetes and lower limb arterial disease on cutaneous foot perfusion." *Journal of vascular surgery* 44.4 (2006): 770-775.

[61] Alavi, Afsaneh, et al. "Audible handheld Doppler ultrasound determines reliable and inexpensive exclusion of significant peripheral arterial disease." *Vascular* 23.6 (2015): 622-629.

[62] Quigley, F. G., and I. B. Faris. "Transcutaneous oxygen tension measurements in the assessment of limb ischaemia." *Clinical Physiology* 11.4 (1991): 315-320.

[63] Quigley, F. G., and I. B. Faris. "Transcutaneous oxygen tension measurements in the assessment of limb ischaemia." *Clinical Physiology* 11.4 (1991): 315-320.

[64] Brownrigg, J. R. W., et al. "Performance of prognostic markers in the prediction of wound healing or amputation among patients with foot ulcers in diabetes: a systematic review." *Diabetes/metabolism research and reviews* 32 (2016): 128-135.

[65] Schaper, N. C., et al. "IWGDF practical guidelines on the prevention and management of diabetic foot disease." *Diabetes Metabl Res Rev* (2019).

[66] Lavery, Lawrence A., et al. "Probe-to-bone test for diagnosing diabetic foot osteomyelitis: reliable or relic?" *Diabetes care* 30.2 (2007): 270-274.

[67] Grayson, M. Lindsay, et al. "Probing to bone in infected pedal ulcers: a clinical sign of underlying osteomyelitis in diabetic patients." *Jama* 273.9 (1995): 721-723.

[68] Hwang, Jessica L., and Roy E. Weiss. "Steroid-induced diabetes: a clinical and molecular approach to understanding and treatment." *Diabetes/metabolism research and reviews* 30.2 (2014): 96-102.

[69] Hwang, Jessica L., and Roy E. Weiss. "Steroid-induced diabetes: a clinical and molecular approach to understanding and treatment." *Diabetes/metabolism research and reviews* 30.2 (2014): 96-102.

[70] Wagner Jr, F. William. "The dysvascular foot: a system for diagnosis and treatment." *Foot & ankle* 2.2 (1981): 64-122.

[71] Lavery, Lawrence A., et al. "Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort." *Diabetes care* 26.5 (2003): 1435-1438.

[72] Armstrong, David G., Lawrence A. Lavery, and Lawrence B. Harkless. "Validation of a diabetic wound classification system: the contribution of depth, infection, and ischemia to risk of amputation." *Diabetes care* 21.5 (1998): 855-859.

[73] Lavery, Lawrence A., David G. Armstrong, and Lawrence B. Harkless. "Classification of diabetic foot wounds." *The Journal of Foot and Ankle Surgery* 35.6 (1996): 528-531.

[74] Ince, Paul, et al. "Use of the SINBAD classification system and score in comparing outcome of foot ulcer management on three continents." *Diabetes care* 31.5 (2008): 964-967.

[75] Mills Sr, Joseph L., et al. "The society for vascular surgery lower extremity threatened limb classification system: risk stratification based on wound, ischemia, and foot infection (WIfI)." *Journal of vascular surgery* 59.1 (2014): 220-234.

[76] Conte, Michael S., et al. "Implementing global chronic limb-threatening ischemia guidelines in clinical practice: Utility of the Society for Vascular Surgery Threatened Limb Classification System (WIfI)." *Journal of Vascular Surgery* 72.4 (2020): 1451-1452.

[77] Darling, Jeremy D., et al. "Predictive ability of the Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system after first-time lower extremity revascularizations." *Journal of vascular surgery* 65.3 (2017): 695-704.

[78] Darling, Jeremy D., et al. "Predictive ability of the Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system following infrapopliteal endovascular interventions for critical limb ischemia." *Journal of vascular surgery* 64.3 (2016): 616-622.

[79] Causey, Marlin W., et al. "Society for Vascular Surgery limb stage and patient risk correlate with outcomes in an amputation prevention program." *Journal of vascular surgery* 63.6 (2016): 1563-1573.

[80] Beropoulis, Efthymios, et al. "Validation of the Wound, Ischemia, foot Infection (WIfI) classification system in nondiabetic patients treated by endovascular means for critical limb ischemia." *Journal of vascular surgery* 64.1 (2016): 95-103.

[81] Ward, Robert, et al. "Outcomes of critical limb ischemia in an urban, safety net hospital population with high WIfI amputation scores." *Annals of vascular surgery* 38 (2017): 84-89.

[82] Hicks, Caitlin W., et al. "The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system correlates with cost of care for diabetic foot ulcers treated in a multidisciplinary setting." *Journal of vascular surgery* 67.5 (2018): 1455-1462.

[83] Hicks, Caitlin W., et al. "The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIfI) classification system correlates with cost of care for diabetic foot ulcers treated in a multidisciplinary setting." *Journal of vascular surgery* 67.5 (2018): 1455-1462.

[84] Shah, Syed Ahmed, et al. "Biopolymer-based biomaterials for accelerated diabetic wound healing: A critical review." *International journal of biological macromolecules* 139 (2019): 975-993.

[85] Paul, Esther Jemima, and B. Padmapriya. "A pragmatic review on the property, role and significance of polymers in treating diabetic foot ulcer." *Materials Today: Proceedings* 23 (2020): 91-99.

[86] Gonzalez, Santiago R., Keith G. Wolter, and James C. Yuen. "Infectious Complications Associated with the Use of Integra: A Systematic Review of the Literature." *Plastic and Reconstructive Surgery Global Open* 8.7 (2020).

[87] Dalla Paola, Luca, et al. "Limb salvage in diabetic patients with no-option critical limb ischemia: outcomes of a specialized center experience." *Diabetic foot & ankle* 10.1 (2019): 1696012.

[88] Tsai, Sue, and Pere Santamaria. "MHC class II polymorphisms, autoreactive T-cells, and autoimmunity." *Frontiers in immunology*4 (2013): 321.

[89] McCrudden, Maelíosa TC, et al. "The host defence peptide LL-37 is susceptible to proteolytic degradation by wound fluid isolated from foot ulcers of diabetic patients." *International Journal of Peptide Research and Therapeutics* 20.4 (2014): 457-464.

[90] Lipsky, Benjamin A., Kenneth J. Holroyd, and Michael Zasloff. "Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream." *Clinical infectious diseases* 47.12 (2008): 1537-1545.

[91] McCartan, Brant, and Thanh Dinh. "The use of split-thickness skin grafts on diabetic foot ulcerations: a literature review." *Plastic surgery international* 2012 (2012).

[92] Brem, Harold, et al. "Healing of diabetic foot ulcers and pressure ulcers with human skin equivalent: a new paradigm in wound healing." *Archives of surgery* 135.6 (2000): 627-634.

[93] Streit, M., and L. R. Braathen. "Apligraf–a living human skin equivalent for the treatment of chronic wounds." *The International journal of artificial organs* 23.12 (2000): 831-833.

[94] Dinh, Thanh L., and Aristidis Veves. "The efficacy of Apligraf in the treatment of diabetic foot ulcers." *Plastic and reconstructive surgery* 117.7S (2006): 152S-157S.

[95] Kaur, Amtoj, et al. "Functional skin grafts: Where biomaterials meet stem cells." *Stem cells international* 2019 (2019).

[96] Falanga, Vincent, et al. "Wounding of bioengineered skin: cellular and molecular aspects after injury." *Journal of investigative dermatology* 119.3 (2002): 653-660.

[97] Hu, Shasa, et al. "Evaluation of Apligraf® persistence and basement membrane restoration in donor site wounds: a pilot study." *Wound repair and regeneration* 14.4 (2006): 427-433.

[98] Griffiths, M., et al. "Survival of Apligraf in acute human wounds." *Tissue engineering* 10.7-8 (2004): 1180-1195.

[99] Cao, Yue, et al. "Mesenchymal stem cells improve healing of diabetic foot ulcer." *Journal of diabetes research* 2017 (2017).

[100] Higashiyama, Reiichi, et al. "Differential contribution of dermal resident and bone marrow–derived cells to collagen production during wound healing and fibrogenesis in mice." *Journal of Investigative Dermatology* 131.2 (2011): 529-536.

[101] Wu, Yaojiong, et al. "Bone marrow-derived stem cells in wound healing: a review." *Wound Repair and Regeneration* 15 (2007): S18-S26.

[102] Deng, Weimin, et al. "Engrafted bone marrow-derived Flk-1+ mesenchymal stem cells regenerate skin tissue." *Tissue engineering* 11.1-2 (2005): 110-119.

[103] FFathke, C., et al. "Contribution of bone marrowderived cells to skin: collagen deposition and wound repair." *Stem Cells* 22 (2004): 812-822.

[104] Cooley, Heather, and Rapichan Phurisamban. *The cost of alternative water supply and efficiency options in California*. Oakland, CA: Pacific Institute, 2016.

[105] Procházka, Václav, et al. "Cell therapy, a new standard in management of chronic critical limb ischemia and foot ulcer." *Cell transplantation* 19.11 (2010): 1413-1424.

[106] Amin, Ali H., et al. "Modified multipotent stromal cells with epidermal growth factor restore vasculogenesis and blood flow in ischemic hind-limb of type II diabetic mice." *Laboratory Investigation* 90.7 (2010): 985-996.

[107] 106-Lu, Debin, et al. "Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: a double-blind, randomized, controlled trial." *Diabetes research and clinical practice* 92.1 (2011): 26-36.

[108] Amann, Berthold, et al. "Autologous bone marrow cell transplantation increases leg perfusion and reduces amputations in patients with advanced critical limb ischemia due to peripheral artery disease." *Cell transplantation* 18.3 (2009): 371- 380.

[109] Ján, V., et al. "Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot." *Neuroendocrinology Letters* 27 (2006): 2.

[110] Lopes, Lara, et al. "Stem cell therapy for diabetic foot ulcers: a review of preclinical and clinical research." *Stem cell research & therapy* 9.1 (2018): 1-16.

[111] Sasaki, Mikako, et al. "Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type." *the Journal of immunology* 180.4 (2008): 2581-2587.

[112] Otero-Viñas, Marta, and Vincent Falanga. "Mesenchymal stem cells in chronic wounds: the spectrum from basic to advanced therapy." *Advances in Wound Care* 5.4 (2016): 149-163.

[113] Huang, Yi-Zhou, et al. "Mesenchymal stem cells for chronic wound healing: current status of preclinical and clinical studies." *Tissue Engineering Part B: Reviews* 26.6 (2020): 555-570.

[114] Veves, Aristidis, Peter Sheehan, and Hau T. Pham. "A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers." *Archives of surgery* 137.7 (2002): 822-827.

[115] Kong, Lingzhi, et al. "Bioactive injectable hydrogels containing desferrioxamine and bioglass for diabetic wound healing." *ACS applied materials & interfaces* 10.36 (2018): 30103-30114.

[116] Gorustovich, A., J. Roether, and A. R. Boccaccini. "Effect of bioactive glasses on angiogenesis." (2010): 199-207.

[117] Osborne, C. S., and P. Schmid. "Epidermal–dermal interactions regulate gelatinase activity in Apligraf®, a tissue-engineered human skin equivalent." *British Journal of Dermatology* 146.1 (2002): 26-31.

[118] McColgan, Maureen, Ali Foster, and Mike Edmonds. "Dermagraft in the treatment of diabetic foot ulcers." *Diabetic Foot* 1 (1998): 75-78.

[119] Kim, Sung-Whan, et al. "Amniotic mesenchymal stem cells enhance wound healing in diabetic NOD/SCID mice through high angiogenic and engraftment capabilities." *PloS one* 7.7 (2012): e41105.

[120] Li, Xiao-Yan, et al. "Treatment of foot disease in patients with type 2 diabetes mellitus using human umbilical cord blood mesenchymal stem cells: response and correction of immunological anomalies." *Current Pharmaceutical Design*19.27 (2013): 4893-4899.

[121] Kogelenberg, Sylvia van, et al. "Three-dimensional printing and cell therapy for wound repair." *Advances in wound care* 7.5 (2018): 145-156.

[122] Tabriz, Atabak Ghanizadeh, Dennis Douroumis, and Joshua Boateng. "3D printed scaffolds for wound healing and tissue regeneration." *Therapeutic Dressings and Wound Healing Applications* (2020): 385-398.

[123] Saunders, Rachel Elizabeth, and Brian Derby. "Inkjet printing biomaterials for tissue engineering: bioprinting." *International Materials Reviews* 59.8 (2014): 430-448.

[124] Angelopoulos, Ioannis, et al. "Engineering inkjet bioprinting processes toward translational therapies." *Biotechnology and bioengineering* 117.1 (2020): 272-284.

[125] Murphy, Sean V., and Anthony Atala. "3D bioprinting of tissues and organs." *Nature biotechnology* 32.8 (2014): 773-785.

[126] Hennink, Wim E., and Cornelus F. van Nostrum. "Novel crosslinking methods to design hydrogels." *Advanced drug delivery reviews* 64 (2012): 223-236.

[127] Xu, Tao, et al. "Inkjet printing of viable mammalian cells." *Biomaterials* 26.1 (2005): 93-99.

[128] Albanna, Mohammed, et al. "In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds." *Scientific reports* 9.1 (2019): 1-15.

[129] Skardal, Aleksander, et al. "A tunable hydrogel system for long-term release of cell-secreted cytokines and bioprinted in situ wound cell delivery." *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 105.7 (2017): 1986-2000.

[130] 129-Velasco, Diego, et al. "3D human skin bioprinting: A view from the bio side." *Journal of 3D printing in medicine* (2018): 141-162.

[131] Chang, Robert, Jae Nam, and Wei Sun. "Effects of dispensing pressure and nozzle diameter on cell survival from solid

[132] freeform fabrication–based direct cell writing." *Tissue Engineering Part A*14.1 (2008): 41-48.

[133] Ghibaudo, Cristian. *Design of a 3D printed nanocellulose based moisturizer for wound dressing applications*. Diss. Politecnico di Torino, 2018.

[134] -Bohandy, J., B. F. Kim, and F. J. Adrian. "Metal deposition from a supported metal film using an excimer laser." *Journal of Applied Physics* 60.4 (1986): 1538-1539.

[135] -Morales, M., et al. "Laser-Induced Forward Transfer Techniques and Applications." *Advances in Laser Materials Processing* (2018): 339-379.

[136] Yanez, Maria, et al. "In vivo assessment of printed microvasculature in a bilayer skin graft to treat full-thickness wounds." *Tissue Engineering Part A* 21.1-2 (2015): 224-233.

[137] Baltazar, Tânia, et al. "Three dimensional bioprinting of a vascularized and perfusable skin graft using human keratinocytes, fibroblasts, pericytes, and endothelial cells." *Tissue Engineering Part A* 26.5-6 (2020): 227-238.

[138] Kim, Byoung Soo, et al. "3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: a step towards advanced skin tissue engineering." *Biomaterials* 168 (2018): 38-53

[139] Skardal, Aleksander, et al. "Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds." *Stem cells translational medicine* 1.11 (2012): 792-802.

[140] Hiller, Thomas, et al. "Generation of a 3D liver model comprising human extracellular matrix in an alginate/gelatin-based bioink by extrusion bioprinting for infection and transduction studies." *International journal of molecular sciences* 19.10 (2018): 3129.

[141] Pati, Falguni, et al. "Biomimetic 3D tissue printing for soft tissue regeneration." *Biomaterials* 62 (2015): 164-175.

[142] Zhang, Guangliang, et al. "ECM concentration and cell-mediated traction forces play a role in vascular network

[143] WOUND, FORCES DRIVING EPITHELIAL. "A research roundup of recent papers relevant to wound care." *Plast Reconstr Surg*133.5: 1178-83.

[144] Jones, Eleri M., Christine A. Cochrane, and Steven L. Percival. "The effect of pH on the extracellular matrix and biofilms." *Advances in wound care* 4.7 (2015): 431-439.

[145] Garbern, Jessica C., Allan S. Hoffman, and Patrick S. Stayton. "Injectable pH-and temperature-responsive poly (N-isopropylacrylamide-co-propylacrylic acid) copolymers for delivery of angiogenic growth factors." *Biomacromolecules* 11.7 (2010): 1833-1839.

[146] Varkey, Mathew, et al. "Skin bioprinting: the future of burn wound reconstruction?" *Burns & trauma* 7 (2019).

[147] Chouhan, Dimple, et al. "Emerging and innovative approaches for wound healing and skin regeneration: Current status and advances." *Biomaterials* 216 (2019): 119267.

[148] Liang, Kun, et al. "3D printing of a wearable personalized oral delivery device: A first-in-human study." *Science advances* 4.5 (2018): eaat2544.

[149] Poldervaart, Michelle T., et al. "Prolonged presence of VEGF promotes vascularization in 3D bioprinted scaffolds with defined architecture." *Journal of controlled release* 184 (2014): 58-66.

[150] Pascucci, Luisa, et al. "Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumour growth: a new approach for drug delivery." *Journal of Controlled Release* 192 (2014): 262-270.

[151] Park, Ju Young, et al. "3D printing technology to control BMP-2 and VEGF delivery spatially and temporally to promote large-volume bone regeneration." *Journal of Materials Chemistry B*3.27 (2015): 5415-5425.

[152] Masaeli, Elahe, et al. "Tissue engineering of retina through high resolution 3-dimensional inkjet bioprinting." *Biofabrication* 12.2 (2020): 025006.

[153] Masaeli, Elahe, et al. "Tissue engineering of retina through high resolution 3-dimensional inkjet bioprinting." *Biofabrication* 12.2 (2020): 025006.