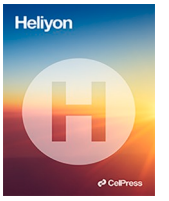
Heliyon 10 (2024) e36707

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

The promising approach of 3D bioprinting for diabetic foot ulcer treatment: A concise review of recent developments

Mattia Biondo [a], Laura Tomasello [b], Carla Giordano [b], Giorgio Arnaldi [b], Giuseppe Pizzolanti [b,c,*]

[a] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze (building 16), 90128, Palermo, Italy*
[b] *Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE) "G. D'Alessandro", University of Palermo, Piazza delle Cliniche 2, 90127, Palermo, Italy*
[c] *ATeN (Advanced Technologies Network) Center, University of Palermo, Italy*

ARTICLE INFO

Keywords:
Diabetic foot
Foot ulcer
Bioprinting
Regenerative medicine
Wounds and injuries

ABSTRACT

Diabetic foot ulcer (DFU), one of the most significant complications of diabetes, is a condition that causes anatomical and functional alterations of the foot resulting in an important social and economic impact, related to disability and health care costs. Recently, three-dimensional bioprinting - which allows the fabrication of complex and biocompatible structures - has been identified as a promising approach in the field of regenerative medicine to promote the healing of chronic wounds, such as DFU. In this concise review we highlight the most relevant and recent attempts of using 3D bioprinted constructs *in vivo* - both on animals and people - in order to treat non-healing diabetic ulcers and prevent their worsening. Finally, we briefly focus on the future implications of bioprinting, suggesting its forthcoming importance not only for DFU treatment but also for other areas of clinical care.

1. Introduction

Diabetic foot ulcer (DFU) is one of the most relevant complications of diabetes mellitus, especially in males with type 2 diabetes. Its prevalence is estimated to be about 13 % in North America, about 5 % in Europe (5.1 %) and Asia (5.5 %), and above 7 % in Africa, while Oceania has the lowest prevalence (3 %) [1].

This condition has an important social and economic impact because of the disability caused by anatomical and functional alterations and related health care costs: it was estimated by Armstrong et al., in 2017 that diabetes directly costs $237 billion in the USA, of which one-third were attributable to care for diabetic foot disease. Moreover, it was evaluated that over 50 % of DFUs is destined to develop an infection; of these, 17 % will probably require an amputation, with a five-year mortality up to 56 % (compared to 31 % for all reported cancer, including breast and lung cancer, according to American Cancer Society in 2019) [2,3].

DFU is caused by a variety of factors related to diabetes such as neuropathy and arterial insufficiency, that increase the odds of traumas and bacterial infections because of an altered proprioception and a reduced blood perfusion.

The neuropathic and/or ischaemic aetiology of DFU is associated to many factors, that influence the onset of the ulcer and its

* Corresponding author. Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (ProMISE) "G. D'Alessandro", University of Palermo, Piazza delle Cliniche 2, 90127, Palermo, Italy.
E-mail address: giuseppe.pizzolanti@unipa.it (G. Pizzolanti).

https://doi.org/10.1016/j.heliyon.2024.e36707
Received 2 May 2024; Received in revised form 6 August 2024; Accepted 21 August 2024
Available online 23 August 2024

difficult healing. Among these, hyperglycaemia - which contributes to peripheral neuropathy, atherosclerosis, vascular damages with dysfunction of skin and endothelial cells and altered perfusion, impairing wound healing; bacterial diversity present in diabetic skin; altered immune cells function, that promotes a proinflammatory environment difficult to treat. Additionally, there are also other lifestyle factors to consider, such as smoking, crucial in the worsening of the ulcer proinflammatory environment and its difficult healing, and improper foot care or structural feet abnormalities, which can promote traumas, infections or damages to an already compromised skin barrier (Fig. 1) [4,5].

Neuropathy, ischemia other features (such as the depth and the size of the ulcer, the swelling, the local or systemic symptoms related to infections, the risk of amputation and others) were considered in the creation of several classifications of the severity of DFU with relevance for clinical practice or clinical audit. Among the most used classification systems - each one evaluating different variables and utilised for different aims - it is worth to consider the University of Texas Staging System for Diabetic Foot Ulcers (Table 1); the Perfusion, Extent, Depth, Infection, and Sensation (PEDIS) Classification System and Score (Table 2); IWGDF Guidelines on the prevention and management of diabetes-related foot disease (Table 3); SINBAD (Site, Ischemia, Bacterial infection, Area, and Depth) Classification System (Table 4) and the Meggitt-Wagner classification (Table 5) [6–10].

A treatment for DFU has to be chosen according to the severity of the lesions and it can range from a specific antibiotic therapy, along with the debridement of necrotic tissue and a proper wound care, to the amputation of the interested area, in case of severe tissue ischemia and gangrene [4,11].

Although DFU can be prevented if the patients were instructed about correct hygiene habits and personal examination, its prevalence will probably increase in the next few years: ageing population, rising obesity and metabolic syndrome, the expected increase of diabetes incidence can explain this peculiar trend. Indeed, it is estimated that 537 million adults aged 20–79 years are currently living with diabetes (10.5 % of the world's population in this age group) and that the total number is predicted to rise to 643 million by 2030 and to 783 million by 2045. It is also estimated that almost one-in-two adults with diabetes are unaware of their condition [12,13].

In order to underline the present and future impact of diabetes and DFU on health systems, it is relevant to report that recently a meta-analysis set that the risk factors associated with 5-year mortality in patients with DFU were age, peripheral artery disease, chronic kidney disease, end-stage renal disease, amputation, infections and history of cardiovascular disease, establishing the last two as the leading causes of death in patients with DFU and highlighting the importance of a resolute prevention and management of cardiovascular risk factors, which significantly affect diabetics and are one of the first causes of death all over the world [14].

Normally, wound healing is a process mediated by growth factors and cytokines released by different cells activated by the immune response: it could be altered in diabetic people because of chronic inflammation, high glucose microenvironment and altered blood perfusion, making treatment of DFU quite difficult and slow [15,16]. Furthermore, the treatment could be more complicated and ineffective because of quality of life factors, which could influence the adherence to therapies and medical advices (e.g. social life,

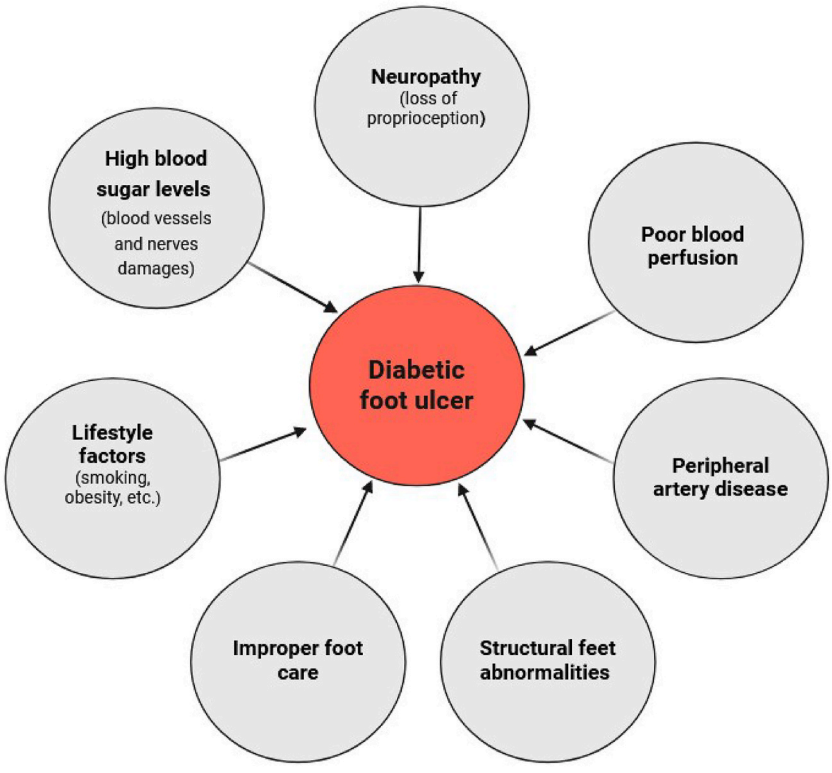


Fig. 1. Overview on main risk factors for diabetic foot ulcer onset.

Table 1
University of Texas staging system for diabetic foot ulcers.

	GRADE			
STAGE	**0**	**1**	**2**	**3**
A	Preulcerative or postulcerative lesions without skin break	Superficial wound (tendon, capsule, bone not involved)	Wound penetrating to tendon or capsule	Wound penetrating to bone or joint
B	Infection	Infection	Infection	Infection
C	Ischemia	Ischemia	Ischemia	Ischemia
D	Infection and ischemia	Infection and ischemia	Infection and ischemia	Infection and ischemia

Adapted from Monteiro-Soares et al., Diabetic foot ulcer classifications: A critical review. *Diabetes Metabolism Res*. 2020.

Table 2
PEDIS classification.

	GRADE			
	1	2	3	4
Perfusion	No PAD	PAD, but not CLI	CLI	–
Extent	Intact skin	<1 cm^2	1–3 cm^2	>3 cm^2
Depth	Superficial ulceration	Deep ulcer involving subcutaneous tissues (e.g. fascia or muscle)	Deep ulcer penetrating to bone or joint	–
Infection	No infection	Involving skin and subcutaneous tissues	Locally severe inflammation (abscess, fascitis)	Systemic infection
Sensation	No evidence of sensory neuropathy	Presence of sensory neuropathy	–	–

Adapted from Monteiro-Soares et al., Diabetic foot ulcer classifications: A critical review. *Diabetes Metabolism Res*. 2020. PAD = Peripheral Artery Disease; CLI = Critical Limb Ischemia.

Table 3
IDSA/IWGDF classification system.

Clinical manifestations	Infection severity
Wound lacking purulence or any manifestations of inflammation.	Uninfected
Presence of $\geq$2 manifestations of inflammation, but any cellulitis/erythema extends $\leq$ 2 cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness.	Mild
Infection (as above) in a patient who is systemically well and metabolically stable but which has $\geq$1 of the following characteristics: cellulitis extending $>$2 cm, lymphangitic streaking, spread beneath the superficial fascia, deep tissue abscess, gangrene, and involvement of muscle, tendon, joint or bone.	Moderate
Infection in a patient with systemic toxicity or metabolic instability.	Severe

Adapted from the IWGDF Guidelines on the classification of foot ulcers in people with diabetes, part of *2023 IWGDF Guidelines on the prevention and management of diabetes-related foot disease.* This classification can be used for classifying a person with an infected ulcer.

Table 4
SINBAD classification system.

Category	Definition	SINBAD score
Site	Forefoot	0
	Midfoot or hindfoot	1
Ischemia	Pedal blood flow intact: at least one pulse palpable	0
	Clinical evidence of reduced pedal blood flow	1
Neuropathy	Intact protective sensation	0
	Lost protective sensation	1
Bacterial Infection	None	0
	Present	1
Area	Ulcer <1 cm^2	0
	Ulcer ≥ 1 cm^2	1
Depth	Ulcer confined to skin and subcutaneous tissue	0
	Ulcer reaching muscle, tendon or deeper	1

Adapted from Ince et al., Use of the SINBAD Classification System and Score in Comparing Outcome of Foot Ulcer Management on Three Continents. *Diabetes Care*. 2008. Considering every item proposed in this classification, a total score between 0 and 6 could be achieved (useful to evaluate lower limb amputation risk).

Table 5
Meggitt-Wagner classification.

Grade	Description
0	Preulcerative or postulcerative lesion completely epithelialized
1	Superficial ulcer, limited to the dermis
2	Ulcer penetrating to tendon, joint or bone (no abscess, no osteomyelitis)
3	Deep ulcer with presence of abscess
4	Forefoot gangrene
5	Extensive foot gangrene (more than two thirds of the foot)

Adapted from Alexiadou K. and Doupis J., Management of Diabetic Foot Ulcers. *Diabetes Ther*. 2012.

anxiety and depression) [17,18].

Thus, nowadays there is a great effort in exploring new ways of treating DFU, including the application of innovative skin substitutes, the systemic infusions of stem cells or the use of topical gels: the main objective is the achievement of a faster healing of ulcers, that could contribute also to an important savings in terms of health care and to a reduction of amputations [19].

In this concise review we want to summarise the possibilities related to a new topical and non-invasive (or minimally invasive) treatment for DFU based on three-dimensional bioprinting, a very promising research field because of its experimental results and initial clinical achievements in replicating tissue three-dimensional structures, such as bone, muscles and skin [20,21]. In the future, 3D bioprinting could bring to an innovative therapeutic approach capable of preserving foot anatomy and promoting its self-regeneration, limiting the social and economic impact of DFU.

2. Methods

In order to select the sources that allow a general and effective recap of the state of the art about 3D bioprinting and its functioning and its relationship with DFU treatment, we utilised *PubMed* as our main research tool. In particular, In particular, we looked up for the keywords "3D bioprinting" OR "bioink" OR "printed" OR "bioprinting" AND "diabetic foot ulcer"; and "3D bioprinting" OR "bioink" OR "printed" OR "bioprinting" AND "diabetic wounds". The search string "3D bioprinting and diabetic foot ulcer" was also used to identify a list of related articles on *Connected Papers*, an AI-powered academic database. These research tools allowed us to detect a network of the most recent papers specifically related to the use of 3D bioprinting in chronic wounds healing, and to ease a reasoned selection, favouring papers referring explicitly to DFU or considering diabetic wounds as one of their application fields.

In both cases, we selected papers based on their recency (specifically, a period between 2019 and January 2024 was considered for the papers regarding bioprinting practical applications for regenerative aims), and the presence of *in vitro* or *in vivo* tests that could be relevant in the demonstration of bioprinting treatment advantages. We tried to fully cite, giving them special relevance in this review, the latest attempts (from 2021) of using 3D printing for DFU healing in clinical care: in this circumstance, we took into consideration also case reports and case series.

As mentioned in our PRISMA flow chart (Supplementary File 1), we screened an amount of 233 articles (duplicates not comprised), excluding a total of 212 papers due to their publication date falling outside of the above indicated; the lack of accessible full text papers; and the presence of reviews or papers not focusing explicitly on bioprinting and DFU or chronic wounds healing.

The included 21 articles are cited in paragraphs 4.1 and 4.2, in which we concisely summarise the latest development in treating DFU with 3D bioprinting, reporting especially *in vivo* tests.

3. Key concepts on 3D bioprinting

Biofabrication is a term referred to the creation of tissue constructs with a hierarchical architecture: it includes different techniques - such as freeze-drying or electrospinning - that allow the creation of three-dimensional scaffolds for cell cultures. Biofabrication has also included 3D bioprinting techniques, characterised by the use of different kinds of cells and materials to obtain high reproducibility and organisation imitating the architecture of tissues and cellular microenvironment (including the tumoral ones) [22,23].

Table 6
Main bioprinting methods and their functioning.

Bioprinting methods	Functioning	Bioinks characteristics
Extrusion-based	Mechanical, pneumatic or solenoid dispenser systems deposit bioinks in a continuous form of filaments	Shear thinning bioinks
Droplet/inkjet	Generation of bioink droplets by thermal, acoustic or electrical stimulation	Low viscosity bioinks
Laser-assisted and vat photopolymerisation	UV light sources (such as lasers or digital light projectors) allow to print structures by a photopolymerisation principle, acting on light-sensitive resins	Photopolymer resins

Sources: Dey M, Ozbolat IT. 3D bioprinting of cells, tissues and organs. *Sci Rep*. 2020 - Li W et al., Stereolithography apparatus and digital light processing-based 3D bioprinting for tissue fabrication. *iScience*. 2023 - Xu X et al., Vat photopolymerisation 3D printing for advanced drug delivery and medical device applications. *Journal of Controlled Release*. 2021.

Bioprinting was defined in 2010 as *"the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organisation in order to produce bio-engineered structures serving in regenerative medicine, pharmacokinetic and basic cell biology studies"* [24] and included a wide range of printing methods (Table 6) such as extrusion-based bioprinting, droplet/inkjet bioprinting, laser-assisted bioprinting and vat photopolymerisation-based techniques (including stereolithography, SLA, and digital light processing, DLP) [25–27]. It is important to highlight that this categorisation could appear slightly different among different authors, according to their backgrounds and their literature references. Moreover, nowadays researchers have tried to combine different types of 3D printing techniques (hybrid manufacturing systems), with interesting practical implications, also in the biomedical field [28].

Generally, it is possible to identify two main approaches to replicate tissues structures: bioprinting cells in a scaffold matrix to support cell proliferation and growth, and bioprinting cells without using a scaffold structure [29]; in any case, all main bioprinting techniques are based on computer aided design (CAD) and computer aided manufacturing (CAM) systems, that allow precise programming processes and the creation of the 3D structures. Moreover, it is possible to select different kinds of material to print, the so called bioinks, according to the purpose of the process, the cells that are eventually involved in the printing and the characteristics required for the final product and its application. However, there could be limitations due to the lack of appropriate bioinks, which have to meet also the requirements in terms of structural characteristics and in guaranteeing the proper bioactivity of cell types: indeed, an ideal bioink should possess specific features such as proper mechanical, rheological, chemical, and biological characteristics, including biocompatibility and the ability to allow cellular oxygenation and intercellular connections and communications for regenerative aims. Additionally, it should meet the needs for a large scale production, involving also a standardisation of its composition. A series of different materials are available for the realisation of ~~a~~ bioinks, both natural and synthetic, such as hyaluronic acid, collagen, silk, gellan gum, agarose, cell aggregates, polycaprolactone (PCL), polyethylene glycol (PEG), and so on. For each of these materials different results were recorded, in terms of cell viability and proliferation as well as functionality of final structures, which are the main criteria in the evaluation of the quality and functionality of a bioink [22,30,31].

4. 3D bioprinting and DFU

3D bioprinting is a promising field for DFU treatment because of the possibility of creating three-dimensional structures starting from a refined digital modelling - thanks to different CAD/CAM systems - based on patients' anatomy and the specific characteristics of the wound. Moreover, the digital calculation and modelling systems - which constitute the basis for the final product structure - are in constant evolution and improvement, even in the specific field of DFU healing. For instance, we can cite the recent presentation of the AiD Regen system, combining 2D semantic segmentation with 3D reconstruction in order to print accurate 3D wound models, with minimal effort for the clinicians or other professionals who want to provide a personalised and adequate treatment based on the specific features of the DFUs [32]. 3D bioprinting has been used both for promoting tissue regeneration - using biocompatible materials - and for building tools consisting of particular polymers capable of preventing the onset or worsening of the lesions.

In the following pages we summarise some of the main remarkable attempts to promote a different approach in diabetes therapy using 3D bioprinting, especially *in vivo*, both on animals and humans. At the moment none of them is largely utilised on patients, except for some experiences in specialised hospitals or facilities.

4.1. Bioprinting for DFU - animal tests

3D bioprinting has proven to be effective in many *in vivo* experiments involving wound animal models, suggesting a great potential in the treatment of lesions such as DFUs.

One of the major applications in this field is related to the fabrication of skin substitutes.

For instance, Baltazar et al. developed a new kind of skin substitute using 3D bioprinting. The point of the study was to improve the efficacy of traditional skin substitutes, such as Apligraf (used to treat chronic wounds, including those related to diabetes), promoting dermal vascular networks, important for a permanent engraftment: the researchers used a first bioink containing human foreskin dermal fibroblasts, human endothelial cells derived from cord blood human endothelial colony-forming cells, and human placental pericytes suspended in rat tail type I collagen, in order to form a dermis. The creation of this structure was followed by the printing of an epidermis with a second bioink containing human foreskin keratinocytes. *In vitro*, this skin substitute proved to create microvascular networks, and a subsequent implantation test on immunodeficient mice confirmed this thesis demonstrating that 3D printed multilayered vascularized human skin grafts can potentially overcome the limitations of graft survival of traditional avascular skin substitutes [33]. The chosen bioprinter was BIO X (by CELLINK), an extrusion-based printer recently used for the ideation of a protocol to create neural tissues [34]. Similarly, Jin et al. created a full-thickness skin structure, consisting of a 1.8 mm dermal layer and a 0.2 mm epidermal layer, thanks to a extrusion-based bioprinter and an acellular dermal matrix-based and gelatin methacrylamide-based bioinks. The 3D structure had the capacity of maintaining cell viability *in vitro* and *in vivo* (mice models) and of promoting wound healing [35].

Significant results were obtained also with other methods such as *in situ* bioprinting. Albanna et al. demonstrated the efficacy of a skin substitute printed layering human fibroblasts and keratinocytes in a hydrogel carrier directly on full-thickness mice wounds and porcine wounds models through an inkjet bioprinter and an integrated imaging technology (in order to mimic wounds characteristics such as size and depth). In this study wounds treated using *in situ* skin bioprinting demonstrated faster wound closure compared to the other animal control groups [36].

Moreover, it is worth to cite the experiment of Zhou et al., who created in 2020 a functional living skin with a new method involving

a digital light based 3D printing technique and a new biomimetic ink composed of gelatin methacrylate, N-(2-aminoethyl)-4-(4-(hydroxymethyl)-2-methoxy-5-nitrosophenoxy) butanamide-linked hyaluronic acid and lithium phenyl-2,4,6-trimethylbenzoylphosphinate, adequate for photopolymerisation and with good biocompatibility, tissue adhesion and mechanical properties. This method also includes in the bioink the allocation of clusters of human fibroblasts and human umbilical vein endothelial cells, in order to form the skin substitute, which demonstrated to promote dermal regeneration also *in vivo* (rats and pigs wound models) [37].

Recently, other authors proposed different kinds of printed skin substitutes, characterised by the loading of bioactive substances with peculiar biological effects (e.g. improvement of tissue regeneration or decrease of inflammatory cytokines). We have considered relevant the experience of Zhao and colleagues, who proposed a method of *in situ* 3D bioprinting preparing human platelet-rich plasma (PRP)-integrated alginate-gelatin composite hydrogel bioinks and evaluating their biological effects *in vitro* and *in vivo* (mouse model). The researchers used an extrusion-based printer and embedded the printed structures with dermal fibroblasts and epidermal stem cells. The study showed that the integration of PRP (also known for its regenerative effects) in the *in situ* bioprinting process accelerated the high-quality wound closure and modulated the inflammation [38]. A similar attempt of constructing 3D skin substitutes integrating PRP (without an *in vivo* model) was performed by Del Amo and colleagues, who also explored the possibility of using bioinks including platelet-poor plasma (PPP), demonstrating that both PRP and PPP could provide complementary molecules with multifunctional roles in wound healing [39,40]. More recently, specifically with the intention of healing wounds induced in type 2 diabetes rats, Huang et al. developed mouse PRP-loaded bioactive multilayer core-shell fibrous hydrogels, inoculated with fibroblasts L929 cells, thanks to a flow assisted dynamic physical cross-linked coaxial microfluidic 3D bioprinting technique (in which the formation of core-shell fibres is based on a coaxial microfluidic spinning technique). The constructs exhibited excellent water absorption and retention properties, antibacterial effects and good biocompatibility. Moreover, these constructs allow a sustained and gradual release of PRP growth factors, and were characterised by the capacity of reducing inflammation, improving the growth of granulation tissues, stimulating angiogenesis and promoting wound healing in diabetic rats [41].

Other interesting attempts of including in bioprinted products some natural and bioactive substances comprehend the experience of Xia and colleagues. They created curcumin-incorporated gelatin methacryloyl hydrogel scaffolds, encapsulating also adipose-derived stem cells, with an extrusion-based bioprinter (Bio-Architect Pro; Regenovo, China). That was the first study to demonstrate the efficacy in diabetic wound healing of curcumin-loaded scaffolds including adipose-derived stem cells: specifically, the researchers showed that this kind of 3D bioprinted scaffolds maintain good biocompatibility and promote tissue repair in diabetic wounds by the decrease of reactive oxygen species generation and stem cells apoptosis (the experiments were conducted on nude mice models with skin defects imitating diabetic cutaneous lesions) [42]. Another relevant attempt was made by Kim et al., who created 3D-printed bioactive dressings for diabetic wounds, utilising Dr. INVIVO 4D6 printer (ROKIT Healthcare, Korea). They incorporated DNA from salmon sperm and DNA-induced biosilica into 3D-printed alginate hydrogel dressings, which provided appropriate porosity, facilitating the absorption of exudates and blood at wound sites, and mechanical properties, protecting the wounds from deformations that can occur during wound healing. The authors demonstrated the efficacy of these dressings on diabetic mice models, highlighting that both the DNA and biomineralised silica acted as nano-therapeutics, enhancing the biological activities of the 3D-printed dressings (e.g. those related to angiogenesis or anti-inflammatory activity) [43].

Finally, we want to highlight some experiences which show the therapeutic relevance of using stem cells and their metabolic products (such as extracellular vesicles) if they are included in bioprinted constructs. Recently, Manso and colleagues described the production of 3D bioprinted biocuratives containing human mesenchymal stem cells associated with a hydrogel matrix for healing skin wounds in type 1 diabetes mice, evidencing that this treatment accelerated wound healing and improved skin collagen deposition [44]. Furthermore, Zhong et al. highlight the issue of stabilising exosomes in scaffolds in order to make the most of their regenerative properties in treating DFU. They utilised decellularised pig skin to obtain a decellularised matrix (dECM). Secondly, quaternised chitosan (Qcs) was made soluble in water and Gel-dECM-Qcs bioink was prepared by adding acellular matrix and quaternised chitosan with temperature sensitive gelatin (Gel) as carrier. So, the researchers fabricated scaffolds with an extrusion-based 3D bioprinter and analysed their physicochemical properties, biocompatibility and antimicrobial capacity, with positive results. Moreover, they demonstrate the scaffolds ability to stabilise exosome loading, suggesting its eventual use in treating DFU [45]. With similar intentions Letizia Ferroni et al. have created - using an INVIVO printer - some methacrylated hyaluronic acid patches in order to deliver extracellular vesicles derived from mesenchymal cells cultured in a bioreactor. This kind of approach has demonstrated an improvement of wounds epithelialisation, angiogenesis and innervation in diabetic mice with pressure ulcers [46].

3D bioprinting could also play a key role in realising customised dressing for atypical wounds in shape and size, thanks to the fabrication of three-dimensional structures with geometric complexity. Some authors ideated a bioprintable hydrogel derived from a combination of biodegradable polyurethane and gelatine. Thanks to an extrusion-based printing technique, they printed it in a planar and curvilinear manner, in order to obtain different constructs and explore possible advantages in treating irregular wounds. After *in vivo* tests with normal and diabetic rats, they showed that the curvilinear-bioprinted hydrogel had better structural integrity compared to the planar-bioprinted one and that the irregular rat skin wounds treated with curvilinear-bioprinted hydrogel loaded with rat cells (such as fibroblasts, endothelial progenitor cells, and keratinocytes) demonstrated full repair at 28 days, showing the possibility of great advantages for this kind of customised structures [47].

4.2. Bioprinting for DFU - relevant applications in clinical care

One of the most frequent and recent clinical experience regarding the use of bioprinting for DFU is related to the treatment with Autologous Minimally Manipulated Homologous Adipose Tissue (AMHAT), which some authors refers to as Minimally Manipulated

Extracellular Matrix (MA-ECM) derived from adipose tissue: it is a technique that imply the harvesting of patient's fat (generally 15–20 mL of fat from abdomen, under local or general anaesthesia) in order to print an autologous dermal substitute for healing the ulcer. The fat, whose regenerative potential is well-known thanks to its growth factors and stem cells [48], is filtered and then dispensed through a printing system called Dr. INVIVO, by ROCKIT Healthcare [49]: this approach guarantees a minimal manipulation of the adipose tissue from the beginning of the process until the last phase of printing (Fig. 2). Before the printing process, a preliminary study of the ulcer with a 3D scanner and computerised calculations allows to adapt the final product to specific wound features.

Armstrong et al. performed a pilot study to propose a customised solution for DFU with the AMHAT approach. Ten patients were included in the study, each of them with non-healing DFU present for more than 4 weeks and refractory to standard of care therapies. The ulcers correspond to University of Texas Grade 1–3A or Wagner grade 1 or 2. After the harvesting of patients' fat and the application of the dermal substitute on the clean wound, weekly visits were conducted. The complete epithelialisation of the wound up to 12 weeks (primary outcome) happens in 60 % of the patients examined. Moreover, wound closure was achieved in an average of 49.1 days and no adverse events were observed [50]. Ahmet et al. performed another clinical prospective study, enrolling twenty diabetic patients with DFU a demonstrating the AHMAT beneficial role in healing the ulcers. The primary endpoint was a reduction in the size of DFU at weekly evaluations after treatment with AHMAT for 12 weeks, and the secondary endpoints were the epithelialisation rate of DFU and the amount of granulation of the wound bed. INVIVO bioprinter, along with preliminary digital study of the wound, was used, in order to create a patch with the same shape of the DFU. All but one of the wounds were completely epithelialized at the ninth week. The mean wound areas decreased significantly at weekly assessments for the first 7 weeks of treatment and the mean time to the complete closure of the wounds was 32.20 $\pm$ 23.862 days. Only three mild subcutaneous bleeding occur, without any relevant adverse events [51].

A randomised controlled monocentric clinical trial based on the same printing method was performed by Rajesh Kesavan and colleagues, with an initial enrolment of 40 patients assigned to two different groups (later, 7 were lost to follow up or excluded): the test group receives a treatment with a patch derived from their fat through Dr. INVIVO printer, and the other one receives standard wound cares. Subjects in the test group displayed complete wound healing and re-epithelialisation at the wound site after about 4 weeks, while in the control group only 50 % of the patients achieved wound healing after a period of 12 weeks, with a slower and less efficient healing process [52].

In addition, we deemed relevant the experience of Mohd Yazid Bajuri et al. and the case report by Hyeon Min Yoon and Woo Jin Song. The first paper apply the same 3D-AHMAT printing technique, but explores the possibility of using a fibrin gel to stabilise the graft and favour the healing process in a single arm study: the researchers reported positive results, with 7 out of 10 patients showing a complete healing of DFU within 12 weeks without adverse events [53]. The second paper is based on the experience of a plastic surgery unit treating a 78 years old woman, with a non-healing DFU and multiple comorbidities, who was recommended for the amputation of her foot because of necrosis and worsening osteomyelitis. The patient refused the procedure and flap reconstruction for the wound was considered impossible because of her poor vascular condition. So, the surgeons decide to apply an AHMAT graft, obtaining a great success: at the 6 weeks postoperative follow-up the wound showcased a 50 % reduction in wound size, with the presence of healthy granulation tissue and without sign of infection, suggesting that AHMAT graft could be considered as a solution to DFU also in complex clinical cases [54].

A different modality of 3D bioprinting in DFU treatment related to clinical care can be found in the case series of Cassano et al., in which the authors printed a polycaprolactone gauze. It was used to treat non-healed DFUs of a 67 years old woman and of a 78 years old man, carrying out periodic dressing changes. In the first case a 70 % reduction in wound size and an improvement in the wound fundus were found over a period of 109 days (with complete epithelialisation in 4 months), while in the second one complete healing of

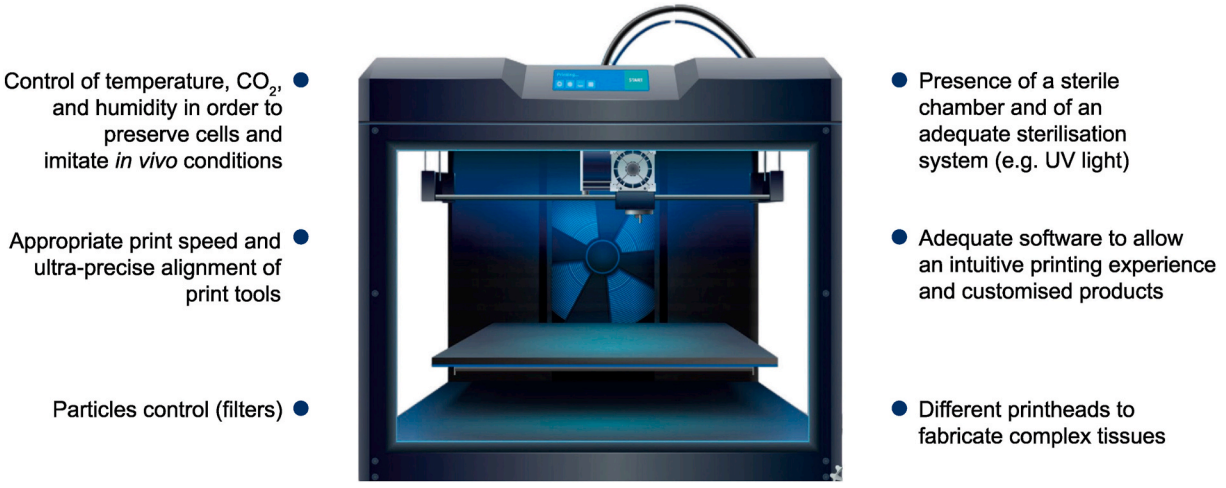


Fig. 2. Essential characteristics of a bioprinter adequate to produce human tissues for clinical use (e.g. Dr INVIVO). *Credits: bioprinter image by Freepik.*

the ulcers happened 10 weeks after the first application. Accordingly, in this case series the 3D printed polycaprolactone gauze showed to be effective for these two non-healing DFU [55].

To elucidate the variety and the complexity of experiences on bioprinting applied to chronic ulcers and DFU, in terms of bioprinted constructs, the *in vivo* tests to prove their biocompatibility and the different mechanisms of wound healing promotion, we have summarised some key information in the table provided as Supplementary File 2.

Moreover, in Table 7 every main strategy of 3D bioprinting has been illustrated and commented, highlighting main advantages and limitations especially related to their clinical applications. We focused on extrusion-based bioprinting because it is the most used methodology in the field of DFU and chronic wounds treatment, both in animal models and in clinical experiences.

4.3. Other significant applications of printing technologies to prevent and cure DFU

We want to cite the use of 3D printing techniques with aims that differ from the creation of bioconstructs directly applicable on wounds for tissue repair. In particular, we want to highlight two recent examples related to DFU prevention and to topical drug delivery for diabetic lesions. For instance, it is worth to cite the experience of Kumar et al., who created 3D printed customised insoles in thermoplastic polyurethane and Kelvin lattice, specifically thought to the prevention of DFU in diabetic patients, providing optimal combination of reduced plantar pressure and more balanced weight distribution [56].

Another relevant example is the production of polycaprolactone scaffolds for drug delivery, obtained with an extrusion-based printer and specifically ideated for diabetic wound healing as published by Glover and colleagues in 2023: in this study, which is based on *in vitro* tests, the researchers created different kinds of scaffolds (five different forms) loaded with levofloxacin, analysing mechanical and drug release properties, showing other possible applications of bioprinting technology in treating DFU [57].

5. Discussion

During the last years 3D bioprinting was a restless research field: as already explained in this concise review, numerous and different modalities of applications and techniques could be found in literature. Focusing on DFU treatment, we have examined only a limited part of a vast range of eventual uses of bioprinting because this kind of biofabrication comprehends a large and billionaire market and could be applied not only in the biomedical field, but also for a lot of industrial aims (for instance, in the food industry) [58].

A great number of new printing methods and techniques are continuously developed, making bioprinting today a complex subject to master technically and professionally. We concentrate our efforts in selecting and summarising different approaches to the treatment of chronic wounds, specifically the diabetic ones. However, we are expecting that other different bioprinting-based approaches will increase in the next few years, and so the possible clinical applications of related constructs. The recent development of the *in situ* printing will be one of the main topics which it is worth paying attention to, because it could be revolutionary not only in the field of DFU, but also generally for the future of surgery and clinical practice. Although nowadays there are a lot of limitations regarding the extensive and systematic use of 3D bioprinting in clinical practice, we could imagine a future application for creating more complex and functional structures such as 3D organs for transplantation [59].

3D bioprinted constructs are one of the most promising treatments in the field of DFU, combining the effectiveness of biocompatible substances, the lenitive capabilities of various biocuratives and the healing effects related to the administrations of different cytotypes, already shown in cell therapy, another promising field of tissue regeneration that has been explored over the last decade.

As illustrated in this concise review, one of the advantages in the use of bioprinting techniques can be the administration of different kinds of cell lines which can improve wound healing. The role of cells in wound healing, fundamental in reaching the promising result of bioprinting, has been confirmed by many attempts of using cell therapy, in which certain kind of cells (such as keratinocytes, fibroblasts or stem cells) are administered with different methods to treat ulcers (e.g. topical administration directly on DFU or intramuscular injection). For example, stem cells, especially mesenchymal stem cells from adipose tissue and bone marrow - which allow autologous collection, avoiding concerns as immune rejection and ethical issues about embryonic stem cells - have been used in many studies regarding cell therapy for DFU, demonstrating the reduction of prolonged inflammation stage and of hypertrophic scarring *in vivo*, the promotion of collagen I deposition and angiogenesis. Moreover, these cells can differentiate into other cytotypes such as keratinocytes and endothelial cells.

However, the lack of consensus on the most effective cytotype to use, the dosage and way of administration, the limited results and the necessity of more clinical trials limit the usage of cell-based therapy, similarly to 3D bioprinting. In the future, it would be desirable to explore new applications in clinical practice of both approaches, evaluating their long-term efficacy, currently not entirely evaluable [60–62].

In conclusion, 3D bioprinting has a vast field of applications: specifically for DFU, we have cited some of the most relevant and interesting attempts to find new ways of treating diabetic wounds and apply them in clinical care. Although further studies are required to prove the efficacy of the different researches and to achieve the routinary use of 3D bioprinted constructs in the DFU treatment, we think that this important amount of scientific studies will bring very soon to the reduction of the impact of this even more frequent condition.

Data availability statement

No data was used for the research described in the article.

Table 7
Comparison among the main bioprinting strategies: highlights of their limitations, advantages and possible improvement in the field of DFU treatment.

Bioprinting strategy	Limitations	Advantages	Possible improvements -Future outcomes
Extrusion bioprinting - animal tests	1. Using human cells often requires an immunodeficient model (immune rejection); 2. Tests on animal models have not a clinical validation and do not reproduce the complexity of certain patients (e.g. compromised perfusion); 3. Extrusion methods require bioinks with a certain viscosity and a careful regulation of shear stress on cells with *in situ* printing.	1. Extrusion bioprinting is one of the most used techniques to create grafts, allowing to explore a great number of biomaterials, cells, biocuratives and customisations; 2. Extrusion bioprinting has been *used also* for *in situ* printing experiments; 3. Animal tests allow a good characterisation of molecular mechanisms of wound healing (cell tracking, histological analysis, etc.).	1. Focus on the problem of immune rejection; 2. Creation of models more adherent to certain clinical issues in order to refine printing techniques; 3. Improvement of shifting from the animal model experiments to clinical studies.
Extrusion bioprinting - clinical tests	1. There are limited clinical trials providing a control group to verify the real advantage of 3D bioprinting in DFU treatment; 2. Absence of clinical application of *in situ* printing for DFUs; 3. Less characterisation of molecular mechanism of wound healing in favour of clinical outcomes (such as the time of wound closure); 4. The harvest of autologous tissue could be limited in certain patients.	1. The successful printing of autologous cells allows to avoid immune rejection; 2. The combination of bioprinting and 3D imaging allows to explore grafts customisation; 3. When AHMAT constructs have been used, they have proved a great regenerative potential.	1. More clinical studies comparing new 3D constructs and standard cares; 2. An improvement of printing methods to overcome some complex clinical issues (limited possibility of harvesting autologous tissue or compromised recipient bed for certain patients) and to refine *in situ* printing techniques.
Inkjet bioprinting – laser-assisted and vat photopolymerisation bioprinting	The are limited applications or clinical experiences regarding DFU treatment.	Some studies with animal models suggest the possibility of using these techniques to promote wound healing creating complex and refined constructs.	Necessity to improve the experiments with these techniques in the field of DFU to explore more applications (new structural features, eventual advantages in the clinical field, etc).

DFU = diabetic foot ulcer; AHMAT = Autologous Minimally Manipulated Homologous Adipose Tissue.

CRediT authorship contribution statement

Mattia Biondo: Writing – original draft, Visualization, Conceptualization. **Laura Tomasello:** Writing – review & editing. **Carla Giordano:** Supervision. **Giorgio Arnaldi:** Supervision. **Giuseppe Pizzolanti:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was promoted by NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 “HEAL ITALIA” to IQ CUP: B53C22004000006.

Mattia Biondo is a PhD Student for the PhD Course “*Technologies and Science for Human Health*” (University of Palermo - Department of Biological, Chemical and Pharmaceutical Sciences and Technologies).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36707.

References

[1] P. Zhang, J. Lu, Y. Jing, S. Tang, D. Zhu, Y. Bi, Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis, Ann. Med. 49 (2017) 106–116, https://doi.org/10.1080/07853890.2016.1231932.

[2] D.G. Armstrong, M.A. Swerdlow, A.A. Armstrong, M.S. Conte, W.V. Padula, S.A. Bus, Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer, J. Foot Ankle Res. 13 (2020) 16, https://doi.org/10.1186/s13047-020-00383-2.

[3] American Cancer Society, Cancer Facts & Figures 2019, American Cancer Society, Atlanta, 2019. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2019.html. last accessed on March 2024.

[4] J.L. Burgess, W.A. Wyant, B. Abdo Abujamra, R.S. Kirsner, I. Jozic, Diabetic wound-healing science, Medicina 57 (2021) 1072, https://doi.org/10.3390/medicina57101072.

[5] K. McDermott, M. Fang, A.J.M. Boulton, E. Selvin, C.W. Hicks, Etiology, epidemiology, and disparities in the burden of diabetic foot ulcers, Diabetes Care 46 (2023) 209–221, https://doi.org/10.2337/dci22-0043.

[6] M. Monteiro-Soares, E.J. Boyko, W. Jeffcoate, J.L. Mills, D. Russell, S. Morbach, F. Game, Diabetic foot ulcer classifications: a critical review, Diabetes Metabolism Res 36 (2020) e3272, https://doi.org/10.1002/dmrr.3272.

[7] O. Niță, L.I. Arhire, L. Mihalache, A.D. Popa, G. Niță, A. Gherasim, M. Graur, Evaluating classification systems of diabetic foot ulcer severity: a 12-year retrospective study on factors impacting survival, Healthcare 11 (2023) 2077, https://doi.org/10.3390/healthcare11142077.

[8] Guidelines on the classification of foot ulcers in people with diabetes, part of 2023 IWGDF Guidelines on the prevention and management of diabetes-related foot disease (https://iwgdfguidelines.org/guidelines-2023/all-guidelines-2023/), last accessed on March 2024.

[9] P. Ince, Z.G. Abbas, J.K. Lutale, A. Basit, S.M. Ali, F. Chohan, S. Morbach, J. Mollenberg, F.L. Game, W.J. Jeffcoate, Use of the SINBAD classification system and score in comparing outcome of foot ulcer management on three continents, Diabetes Care 31 (2008) 964–967, https://doi.org/10.2337/dc07-2367.

[10] K. Alexiadou, J. Doupis, Management of diabetic foot ulcers, Diabetes Ther 3 (2012) 4, https://doi.org/10.1007/s13300-012-0004-9.

[11] W. Marston, J. Tang, R.S. Kirsner, W. Ennis, Wound healing society 2015 update on guidelines for venous ulcers, Wound Repair Regen. 24 (2016) 136–144, https://doi.org/10.1111/wrr.12394.

[12] D.F. Bandyk, The diabetic foot: pathophysiology, evaluation, and treatment, Semin. Vasc. Surg. 31 (2018) 43–48, https://doi.org/10.1053/j.semvascsurg.2019.02.001.

[13] International Diabetes Federation Atlas, tenth ed., 2021. https://diabetesatlas.org/atlas/tenth-edition/. last accessed on February 2024.

[14] L. Chen, S. Sun, Y. Gao, X. Ran, Global mortality of diabetic foot ulcer: a systematic review and meta-analysis of observational studies, Diabetes Obes. Metabol. 25 (2023) 36–45, https://doi.org/10.1111/dom.14840.

[15] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (2008) 314–321, https://doi.org/10.1038/nature07039.

[16] H. Deng, B. Li, Q. Shen, C. Zhang, L. Kuang, R. Chen, S. Wang, Z. Ma, G. Li, Mechanisms of diabetic foot ulceration: a review, J. Diabetes 15 (2023) 299–312, https://doi.org/10.1111/1753-0407.13372.

[17] M. Polikandrioti, G. Vasilopoulos, I. Koutelekos, G. Panoutsopoulos, G. Gerogianni, F. Babatsikou, A. Zartaloudi, G. Toulia, Quality of life in diabetic foot ulcer: associated factors and the impact of anxiety/depression and adherence to self-care, Int. J. Low. Extrem. Wounds 19 (2020) 165–179, https://doi.org/10.1177/1534734619900415.

[18] A. Ahmad, M. Abujbara, H. Jaddou, N.A. Younes, K. Ajlouni, Anxiety and depression among adult patients with diabetic foot: prevalence and associated factors, J. Clin. Med. Res. 10 (2018) 411–418, https://doi.org/10.14740/jocmr3352w.

[19] A. Jodheea-Jutton, S. Hindocha, A. Bhaw-Luximon, Health economics of diabetic foot ulcer and recent trends to accelerate treatment, Foot 52 (2022) 101909, https://doi.org/10.1016/j.foot.2022.101909.

[20] T. Potyondy, J.A. Uquillas, P.J. Tebon, B. Byambaa, A. Hasan, M. Tavafoghi, H. Mary, G.E. Aninwene, I. Pountos, A. Khademhosseini, N. Ashammakhi, Recent advances in 3D bioprinting of musculoskeletal tissues, Biofabrication 13 (2021) 022001, https://doi.org/10.1088/1758-5090/abc8de.

[21] M.S. Kang, J. Jang, H.J. Jo, W.-H. Kim, B. Kim, H.-J. Chun, D. Lim, D.-W. Han, Advances and innovations of 3D bioprinting skin, Biomolecules 13 (2022) 55, https://doi.org/10.3390/biom13010055.

[22] P.S. Gungor-Ozkerim, I. Inci, Y.S. Zhang, A. Khademhosseini, M.R. Dokmeci, Bioinks for 3D bioprinting: an overview, Biomater. Sci. 6 (2018) 915–946, https://doi.org/10.1039/C7BM00765E.

[23] P. Shukla, S. Yeleswarapu, M.A. Heinrich, J. Prakash, F. Pati, Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling, Biofabrication 14 (2022) 032002, https://doi.org/10.1088/1758-5090/ac6d11.

[24] F. Guillemot, V. Mironov, M. Nakamura, Bioprinting is coming of age: report from the international conference on bioprinting and biofabrication in bordeaux (3B'09), Biofabrication 2 (2010) 010201, https://doi.org/10.1088/1758-5082/2/1/010201.

[25] M. Dey, I.T. Ozbolat, 3D bioprinting of cells, tissues and organs, Sci. Rep. 10 (2020) 14023, https://doi.org/10.1038/s41598-020-70086-y s41598-020-70086-y.

[26] W. Li, M. Wang, H. Ma, F.A. Chapa-Villarreal, A.O. Lobo, Y.S. Zhang, Stereolithography apparatus and digital light processing-based 3D bioprinting for tissue fabrication, iScience 26 (2023) 106039, https://doi.org/10.1016/j.isci.2023.106039.

[27] X. Xu, A. Awad, P. Robles-Martinez, S. Gaisford, A. Goyanes, A.W. Basit, Vat photopolymerization 3D printing for advanced drug delivery and medical device applications, J. Contr. Release 329 (2021) 743–757, https://doi.org/10.1016/j.jconrel.2020.10.008.

[28] F. Liu, R. Quan, C. Vyas, E. Aslan, Hybrid biomanufacturing systems applied in tissue regeneration, IJB 9 (2022) 646, https://doi.org/10.18063/ijb.v9i1.646.

[29] I.T. Ozbolat, Scaffold-based or scaffold-free bioprinting: competing or complementing approaches? J. Nanotechnol. Eng. Med. 6 (2015) 024701 https://doi.org/10.1115/1.4030414.

[30] M. Hospodiuk, M. Dey, D. Sosnoski, I.T. Ozbolat, The bioink: a comprehensive review on bioprintable materials, Biotechnol. Adv. 35 (2017) 217–239, https://doi.org/10.1016/j.biotechadv.2016.12.006.

[31] J. Li, M. Chen, X. Fan, H. Zhou, Recent advances in bioprinting techniques: approaches, applications and future prospects, J. Transl. Med. 14 (2016) 271, https://doi.org/10.1186/s12967-016-1028-0.

[32] H.J. Chae, S. Lee, H. Son, S. Han, T. Lim, Generating 3D bio-printable patches using wound segmentation and reconstruction to treat diabetic foot ulcers, in: 2022 IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR), 2022, pp. 2529–2539, https://doi.org/10.1109/CVPR52688.2022.00257.

[33] T. Baltazar, J. Merola, C. Catarino, C.B. Xie, N.C. Kirkiles-Smith, V. Lee, S. Hotta, G. Dai, X. Xu, F.C. Ferreira, W.M. Saltzman, J.S. Pober, P. Karande, Three dimensional bioprinting of a vascularized and perfusable skin graft using human keratinocytes, fibroblasts, pericytes, and endothelial cells, Tissue Eng. 26 (2020) 227–238, https://doi.org/10.1089/ten.tea.2019.0201.

[34] J. Chrenek, R. Kirsch, K. Scheck, S.M. Willerth, Protocol for printing 3D neural tissues using the BIO X equipped with a pneumatic printhead, STAR Protocols 3 (2022) 101348, https://doi.org/10.1016/j.xpro.2022.101348.

[35] R. Jin, Y. Cui, H. Chen, Z. Zhang, T. Weng, S. Xia, M. Yu, W. Zhang, J. Shao, M. Yang, C. Han, X. Wang, Three-dimensional bioprinting of a full-thickness functional skin model using acellular dermal matrix and gelatin methacrylamide bioink, Acta Biomater. 131 (2021) 248–261, https://doi.org/10.1016/j.actbio.2021.07.012.

[36] M. Albanna, K.W. Binder, S.V. Murphy, J. Kim, S.A. Qasem, W. Zhao, J. Tan, I.B. El-Amin, D.D. Dice, J. Marco, J. Green, T. Xu, A. Skardal, J.H. Holmes, J. D. Jackson, A. Atala, J.J. Yoo, In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds, Sci. Rep. 9 (2019) 1856, https://doi.org/10.1038/s41598-018-38366-w.

[37] F. Zhou, Y. Hong, R. Liang, X. Zhang, Y. Liao, D. Jiang, J. Zhang, Z. Sheng, C. Xie, Z. Peng, X. Zhuang, V. Bunpetch, Y. Zou, W. Huang, Q. Zhang, E.V. Alakpa, S. Zhang, H. Ouyang, Rapid printing of bio-inspired 3D tissue constructs for skin regeneration, Biomaterials 258 (2020) 120287, https://doi.org/10.1016/j.biomaterials.2020.120287.

[38] M. Zhao, J. Wang, J. Zhang, J. Huang, L. Luo, Y. Yang, K. Shen, T. Jiao, Y. Jia, W. Lian, J. Li, Y. Wang, Q. Lian, D. Hu, Functionalizing multi-component bioink with platelet-rich plasma for customized in-situ bilayer bioprinting for wound healing, Materials Today Bio 16 (2022) 100334, https://doi.org/10.1016/j.mtbio.2022.100334.

[39] C. Del Amo, X. Fernández-San Argimiro, M. Cascajo-Castresana, A. Perez-Valle, I. Madarieta, B. Olalde, I. Andia, Wound-microenvironment engineering through advanced-dressing bioprinting, IJMS 23 (2022) 2836, https://doi.org/10.3390/ijms23052836.

[40] C. Del Amo, A. Perez-Valle, M. Perez-Garrastachu, I. Jauregui, N. Andollo, J. Arluzea, P. Guerrero, K. De La Caba, I. Andia, Plasma-based bioinks for extrusion bioprinting of advanced dressings, Biomedicines 9 (2021) 1023, https://doi.org/10.3390/biomedicines9081023.

[41] Q. Huang, T. Wu, Y. Guo, L. Wang, X. Yu, B. Zhu, L. Fan, J.H. Xin, H. Yu, Platelet-rich plasma-loaded bioactive chitosan@sodium alginate@gelatin shell-core fibrous hydrogels with enhanced sustained release of growth factors for diabetic foot ulcer healing, Int. J. Biol. Macromol. 234 (2023) 123722, https://doi.org/10.1016/j.ijbiomac.2023.123722.
[42] S. Xia, T. Weng, R. Jin, M. Yang, M. Yu, W. Zhang, X. Wang, C. Han, Curcumin-incorporated 3D bioprinting gelatin methacryloyl hydrogel reduces reactive oxygen species-induced adipose-derived stem cell apoptosis and improves implanting survival in diabetic wounds, Burns & Trauma 10 (2022) tkac001, https://doi.org/10.1093/burnst/tkac001.
[43] N. Kim, H. Lee, G. Han, M. Kang, S. Park, D.E. Kim, M. Lee, M. Kim, Y. Na, S. Oh, S. Bang, T. Jang, H. Kim, J. Park, S.R. Shin, H. Jung, 3D-Printed functional hydrogel by DNA-induced biomineralization for accelerated diabetic wound healing, Adv. Sci. 10 (2023) 2300816, https://doi.org/10.1002/advs.202300816.
[44] G.M.D.C. Manso, J. Elias-Oliveira, J.B. Guimarães, Í.S. Pereira, V.F. Rodrigues, B. Burger, D.M.C. Fantacini, L.E.B. De Souza, H.G. Rodrigues, V.L.D. Bonato, J. S. Silva, S.G. Ramos, R.C. Tostes, A.O. Manfiolli, C. Caliari-Oliveira, D. Carlos, Xenogeneic mesenchymal stem cell biocurative improves skin wounds healing in diabetic mice by increasing mast cells and the regenerative profile, Regenerative Therapy 22 (2023) 79–89, https://doi.org/10.1016/j.reth.2022.12.006.
[45] Y. Zhong, H. Ma, Y. Lu, L. Cao, Y.Y. Cheng, X. Tang, H. Sun, K. Song, Investigation on repairing diabetic foot ulcer based on 3D bio-printing Gel/dECM/Qcs composite scaffolds, Tissue Cell 85 (2023) 102213, https://doi.org/10.1016/j.tice.2023.102213.
[46] L. Ferroni, U. D'Amora, C. Gardin, S. Leo, L. Dalla Paola, E. Tremoli, A. Giuliani, L. Calzà, A. Ronca, L. Ambrosio, B. Zavan, Stem cell-derived small extracellular vesicles embedded into methacrylated hyaluronic acid wound dressings accelerate wound repair in a pressure model of diabetic ulcer, J. Nanobiotechnol. 21 (2023) 469, https://doi.org/10.1186/s12951-023-02202-9.
[47] S. Wu, N. Dai, C. Liao, L. Kang, Y. Tseng, S. Hsu, Planar-/Curvilinear-Bioprinted tri-cell-laden hydrogel for healing irregular chronic wounds, Adv. Healthcare Mater. 11 (2022) 2201021, https://doi.org/10.1002/adhm.202201021.
[48] S.S. Sharath, J. Ramu, S.V. Nair, S. Iyer, U. Mony, J. Rangasamy, Human adipose tissue derivatives as a potent native biomaterial for tissue regenerative therapies, Tissue Eng Regen Med 17 (2020) 123–140, https://doi.org/10.1007/s13770-019-00230-x.
[49] J. Kim, 2155-PUB: diabetic foot ulcer regeneration platform based on 4D bioprinting technology, Diabetes 69 (2020) 2155–PUB, https://doi.org/10.2337/db20-2155-PUB.
[50] D.G. Armstrong, S.G. Harris, Z. Rasor, C.M. Zelen, J. Kim, M. Swerdlow, A.L. Isaac, Autologous minimally manipulated homologous adipose tissue (AMHAT) for treatment of nonhealing diabetic foot ulcers, Plastic and Reconstructive Surgery - Global Open 10 (2022) e4588, https://doi.org/10.1097/GOX.0000000000004588.
[51] A. Yastı, A. Akgun, A. Surel, J. Kim, M. Akın, Graft of 3D bioprinted autologous minimally manipulated homologous adipose tissue for the treatment of diabetic foot ulcer, Wounds 35 (2023) e22–e28, https://doi.org/10.25270/wnds/21136.
[52] R. Kesavan, C. Sheela Sasikumar, V.B. Narayanamurthy, A. Rajagopalan, J. Kim, Management of diabetic foot ulcer with MA–ECM (minimally manipulated autologous extracellular matrix) using 3D bioprinting technology – an innovative approach, Int. J. Low. Extrem. Wounds (2021) 153473462110456, https://doi.org/10.1177/15347346211045625.
[53] M.Y. Bajuri, J. Kim, Y. Yu, M.S. Shahul Hameed, New paradigm in diabetic foot ulcer grafting techniques using 3D-bioprinted autologous minimally manipulated homologous adipose tissue (3D-AMHAT) with fibrin gel acting as a biodegradable scaffold, Gels 9 (2023) 66, https://doi.org/10.3390/gels9010066.
[54] H. Yoon, W. Song, Using 3D-bioprinted autologous minimally manipulated homologous adipose tissue for limb salvage in the treatment of diabetic foot ulcer, Arch Plast Surg (2024), https://doi.org/10.1055/a-2263-7957 a-2263-7957.
[55] V.M. Cassano, C. Parra, G. Bonino, A.D. Carlo, 3D printing dressing in treatment of diabetic ulcer: a case-series, J Diabet Res Rev Rep (2022) 1–4, https://doi.org/10.47363/JDRR/2022(4)157.
[56] R. Kumar, S.K. Sarangi, 3D Printed customized diabetic foot insoles with architecture designed lattice structures – a case study, Biomed, Phys. Eng. Express 10 (2024) 015019, https://doi.org/10.1088/2057-1976/ad1732.
[57] K. Glover, E. Mathew, G. Pitzanti, E. Magee, D.A. Lamprou, 3D bioprinted scaffolds for diabetic wound-healing applications, Drug Deliv. and Transl. Res. 13 (2023) 2096–2109, https://doi.org/10.1007/s13346-022-01115-8.
[58] A. Elemoso, G. Shalunov, Y.M. Balakhovsky, A. Yu Ostrovskiy, Y.D. Khesuani, 3D bioprinting: the roller coaster ride to commercialization, IJB 6 (2020) 301, https://doi.org/10.18063/ijb.v6i3.301.
[59] T.H. Jovic, E.J. Combellack, Z.M. Jessop, I.S. Whitaker, 3D bioprinting and the future of surgery, Front. Surg. 7 (2020), https://doi.org/10.3389/fsurg.2020.609836.
[60] O.A. Krasilnikova, D.S. Baranovskii, A.V. Lyundup, P.V. Shegay, A.D. Kaprin, I.D. Klabukov, Stem and somatic cell monotherapy for the treatment of diabetic foot ulcers: review of clinical studies and mechanisms of action, Stem Cell Rev and Rep 18 (2022) 1974–1985, https://doi.org/10.1007/s12015-022-10379-z.
[61] J.M. Raja, M.A. Maturana, S. Kayali, A. Khouzam, N. Efeovbokhan, Diabetic foot ulcer: a comprehensive review of pathophysiology and management modalities, World J Clin Cases 11 (2023) 1684–1693, https://doi.org/10.12998/wjcc.v11.i8.1684.
[62] N.V. Maksimova, A.V. Michenko, O.A. Krasilnikova, I.D. Klabukov, I.Yu Gadaev, M.E. Krasheninnikov, P.A. Belkov, A.V. Lyundup, Mesenchymal stromal cells therapy alone does not lead to the complete restoration of the skin parameters in diabetic foot patients within a 3-year follow-up period, Bioimpacts (2021) 1, https://doi.org/10.34172/bi.2021.22167.