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REVIEW ARTICLE

Novel Advances in 3-D Printing Technology in Drug Delivery

3D Printing as a Technological Strategy for the Personalized Treatment of Wound Healing

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Abstract

Wound healing is a dynamic process which involves stages of hemostasis, inflammation, proliferation and remodeling. Any error in this process results in abnormal wound healing, generating financial burdens for health systems and even affecting the physical and mental health of the patient. Traditional dressings do not meet the complexities of ideal treatment in all types of wounds. For this reason, in the last decades, different materials for drug delivery and for the treatment of wounds have been proposed reaching novel level of standards, such as 3D printing techniques. The use of natural or synthetic polymers, and the correct design of these printed products loaded with cells and/or combined with active compounds, can generate an effective system for the treatment of wounds, improving the healing process and generating customized dressings according to the patient needs. This manuscript provides a comprehensive review of different types of 3D printing techniques, as well as its use in wound healing and its different stages, including the advantages and limitations of additive manufacturing and future perspectives.

Keywords additive manufacturing · drug delivery · wound healing · 3D printing · technology

Introduction

Taking into account the surface area, the skin is the largest organ in the human body. It is the first defense of the organism against external aggressors, which protects internal tissues from mechanical aggression, ultraviolet radiation, high temperatures, and infections [1]. This makes it one of the most vulnerable tissue and highly susceptible to injury. Globally, the advanced wound care market could reach $18.7 billion by 2027, with a Compound Annual Growth Rate of 6.6% (2020–2027) [2]. Wounds healing treatment are associated with major economic burdens and to the society, comprising direct costs (medical and health care) and indirect costs (productivity losses, sick leave, and early retirement). The morbidity associated with delayed wound healing imposes an enormous impact on health, both psychologically and economically [1, 3].

The repair of wounds is carried out through continuous and coordinated processes. The normal wound healing process involves four synchronous stages, such as hemostasis, inflammation, proliferation, and extracellular matrix remodeling [3, 4]. Many of the problems caused by some type of injury, especially in chronic wounds, occur during the treatment of the injury either by poor care and/or inadequate procedure, which limits the correct repair of the wound and the restoration of tissue integrity [5]. Patients with diabetes, genetic disorders, advanced age, and also those showing skin burn injuries and skin cancer are prone to alterations in the normal wound healing process which can lead to long-term sequelae [1, 3, 6]. However, the current protocols of intervention do not minimize this unpleasant situation, and often they are only moderately effective.

A strategy to improve wound healing is the use of new forms of dressings, better than conventional ones, which aim to double the healing rate [7]. To facilitate and promote the wound healing process, it is necessary to select appropriate dressings according to the wound site, type, and size. Furthermore, the ideal dressing must provide protection from

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mechanical, chemical, and biological attacks (infections and microorganisms); allow permeability of water and oxygen; controlling wound moisture as well as removing excess exudate; be biocompatible, non-toxic, and biodegradable; and still be provided at an acceptable cost [8–10]. Based on this information, the development of new technologies to accelerate the healing process and improve the treatment of these injuries in an easy, painless way and restore skin integrity are highly encouraged.

In recent years, the development of new techniques and products/materials for wound dressing has entered a new level of standard [11]. Currently, the products available on the market for the treatment of wounds do not meet the needs of the patient, that is, they are not customizable and do not meet the specific conditions of the patient in order to provide the correct dose of the drug [12]. Drug delivery systems obtained by 3D printing have the potential to provide customized and innovative products that will adapt to the patient needs. Therefore, the additive manufacturing has been shown a promising tool in various applications and offers a positive approach in accelerating healing, adapting the mechanical and physical properties of the product, and providing a suitable environment for wound healing [13].

3D printing involves a plurality of techniques such as stereolithography, needle extrusion, laser-assisted, or inkjet printing. For proper printing, the composition of the ink used is fundamental to allow the construction of products with the desired design and activity. These inks must be biocompatible and biodegradable; for this reason, the use of natural and synthetic polymers is recurrent. Throughout this manuscript, we present the stages of the healing process, and the main 3D printers used to print products that can facilitate wound healing. Furthermore, the advantages and limitations of additive manufacturing and also the future perspectives are discussed.

Wound Healing

The skin epidermis is an epithelial tissue that protects the body from the external environment, becoming susceptible to injuries. When the skin suffers some type of aggression injury, for wound repair to occur, synchronization of different cell types is required in sequential steps. Wound healing is divided into four stages: hemostasis, inflammation process, proliferation, and remodeling (Fig. 1) [14]; however, *in vivo*, the stages can overlap due to several anatomical and physiological factors. There are many factors (local or systemic) that can affect wound healing. These factors, when they interfere in one or more phases of the healing process, can lead to inadequate tissue repair.

Hemostasis

Hemostasis marks the first stage of skin healing after an injury, stopping bleeding after vascular damage by activating

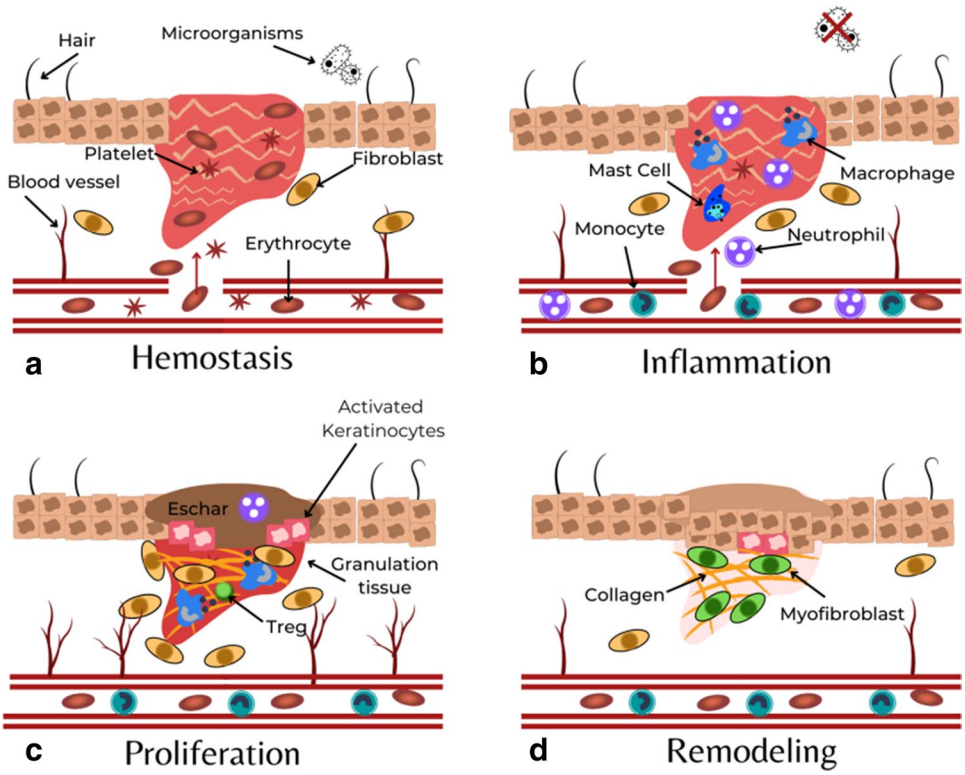

Fig. 1 Cellular aspect in skin wound repair in the normal stages of wound healing. Adapted from Wilkinson and Hardman [15]

platelets. Platelets, involved in homeostasis and coagulation, are activated when they encounter the subendothelial matrix, and with fibrinogen, react together and stop extravasation by forming a platelet plug [1, 15]. Fibrin (clot) restores hemostasis and platelets act by capturing immune cells at the injured site and/or secreting growth factors that stimulate resident skin cells, including fibroblasts and keratinocytes [16, 17]. Moreover, platelets also have another important role in addition to healing. These cells play an important role in the early prevention in the bacterial and viral infections. After sufficient initial formation of the platelet plug, the coagulation cascade leads to the formation of the fibrin mesh that encapsulates and reinforces the thrombus. As soon as a clot is formed sufficiently, the clotting process is stopped immediately, preventing excessive formation of thrombosis (Fig. 1a).

Inflammation

The second stage of wound healing is the inflammatory one. It is characterized as the immediate response to the trauma and begins right after the injury. At this phase, inflammatory-type cells move to the wound and mast cells, connective tissue cells responsible for initiating the inflammatory reaction, release vasoactive substances that cause increased vascular permeability and the wound's defensive response [18]. In the inflammatory phase, exogenous pathogens, damaged or dead cells and tissues are removed from the wound area. In addition, at this stage, an important process takes place, which is the formation of new tissue in wound healing, with inflammatory cells (i.e. lymphocytes, neutrophils, mast cells, dendritic cells, and macrophages) being responsible for producing cytokines, growth factors, and chemokines that prepare the wound for the formation of a tissue of granulation [19, 20] (Fig. 1b).

Proliferation

During the proliferative stage, damaged and necrotic tissues are replaced by fibroblasts and epithelial cells. Normally, the proliferative stage occurs from day 3 to approximately the third week [4, 18, 21, 22]. At this stage, fibroblasts play a critical role, with the function of proliferating and migrating to the wound site to secrete a new extracellular matrix (ECM). From this, the lesion space that was previously open is filled with granulation tissue [23]. The ECM is a structure made up of several types of proteins. For a new ECM to be produced, a variety of matrix proteins (such as hyaluronic acid, collagen, fibronectin, and proteoglycans) are required [24, 25]. After ECM deposition, the process of angiogenesis (responsible for producing new vessels and capillaries) becomes fundamental, since this process restores an adequate blood supply to the newly formed tissue at this stage [26, 27]. This step is marked as the stage that completes the filling of the wound [18] (Fig. 1c).

Remodeling

Finally, the wound healing process ends with the remodeling phase. The elements involved at this phase try to reproduce the normal tissue structure of the skin. The remodeling stage manly occurs from the third week up to one year or even longer [22, 28]. ECM remodeling, in the normal healing process, occurs throughout the injury response process, starting with a deposition of a fibrin matrix and ending with a type 1 collagen-rich scar [28–30]. Later, fibroblasts of granulation tissue die by apoptosis or differentiate into myofibroblasts. These myofibroblasts are fibroblasts with contractile capacity that respond to agonists and exhibit characteristics similar to smooth muscle cells and carry out the wound contraction process [21] (Fig. 1d). Even though processes involved in wound repair start almost instantly, not all wounds go through this cascade and often, some scars do not fully replicate the original characteristics of uninjured tissue.

Conventional Treatment and New Drug Delivery Systems

Wound care is one of the health concerns that affect millions of people around the world, and if not treated properly, it can lead to complications that are exacerbated in cases of diabetes or infections [31–33]. In the treatment of wounds, the popular and traditional treatments are gauze, cotton, plasters, and bandages. Basically, they act as wound protectors, preventing contamination by pathogens and other environmental hazards. However, its use may compromise adequate healing, as the materials used in the manufacture of gauze, cotton, and bandages absorb exudate and adhere to the wound; and if not removed with care, they cause more damage to the wound and cause a lot of pain to the patient.

Several therapies used in wound healing comprise different factors such as practices, type of product and injury, and diverse knowledge of a particular culture or country. Among these diverse therapies are the uses of living beings (e.g. leeches, bees, fishes) [34–37] and natural compounds such as plants and minerals [38–41]. It is important therefore finding an optimal treatment. However, many factors contribute to the difficulty of finding a good drug and/or wound dressing, due to the variety of wound types (e.g. exuding, dry, acute, or chronic wounds) and the fact that there is no single effective dressing for all types of wounds. In addition, the market does not have any dressing that acts specifically at any stage of the wound healing process.

Therefore, developing an innovative product for the treatment of wounds has become a necessity, leading the scientific community to study new treatments and also improve

the effectiveness of current therapies. Among the new drug delivery systems (DDSs) used for wound healing are lipid-based drug delivery system, natural and synthetic polymeric systems (e.g. hydrocolloid, hydrogel dressings), emulgels, silicon microparticles, foams, silver and gold nanoparticles, among many others [42–48]. Although these dressings based on biocompatible natural or synthetic polymers and others do not have the disadvantages of conventional dressings, as they have a more flexible and breathable design, these systems do not allow individualized dosing and form. Furthermore, the conventional dressings such as gauze, cotton, and bandages do not have the characteristic of having biological activity, while advanced dressings such as 3D printed are designed to induce therapeutic action due to their composition and ability to modify the release of bioactive components.

For this reason, studies have been carried out to develop new DDSs in order to release bioactive compounds in a controlled and personalized way, respecting the patient's needs. These mentioned characteristics can be obtained by 3D printing. It is known that patients who have undergone ineffective wound care suffer from the consequences of this therapy, carrying with them hypertrophic scars, skin deformities, and even loss of movement. For this reason, when a treatment using 3D printing is suggested for the patient, in which the wound closes quickly, with a guarantee of avoiding infections and visits to treatment centers, protecting the skin, keeping the microenvironment moist and personalizing the treatment with adequate dosages, patients choose for adhering to treatment regardless of price. And yet, for the health system, fast and effective treatment brings savings to public and private services by avoiding expenses with insurance transport to treatment centers, costs with caregivers, absence from work, among others.

In addition, technology based on 3D printing is increasing significantly in all manufacturing sectors, due to its ability to increase production efficiency with reduced cost and with low numbers of defects; as well, with the significant advantage of flexibility and the ability to customize and create a wide variety of simple to complex geometries.

An Overview of Additive Manufacturing

Approximately three decades ago, 3D printing was introduced to the market impacting several industrial fields and offers the possibility to create different shapes and models instantly and at an affordable price [13]. In recent decades, drug development focused on patient well-being has received considerable attention. 3D printing has become a great candidate for this purpose and has gained wide interest in the pharmaceutical technology field. Recently, in 2015, the first drug produced using a 3D printer (3DP) was approved by the United States Food and Drug Administration (FDA) and now is marketed. The drug called Spritam (Levetiracetam) is being manufactured by Aprecia Pharmaceuticals [49].

Compared to conventional manufacturing technologies, 3D printing has proven to be a flexible and highly versatile technique capable of producing modifiable medications suitable for the individual and specific needs of patients [50–55] (Fig. 2). As a result, 3D printing provides tools capable of developing personalized and unique medicines with effective delivery systems, resulting in a revolutionary advance in healthcare.

Basically, a 3DP aims to manufacture 3D dressings or medicines from previously developed digital models by depositing layer-by-layer of printed materials over and over until the designed object is formed, using the computer-aided design (CAD) software or 3D Scanners [56–59]. The 3D positioning system of a 3DP corresponds to the overall operation of its x, y, and z axes. Depending upon the 3DP, only one axis will be movable, or two, or even all three. The x and y axes correspond to the lateral movement of the 3DP and the z axis corresponds to the vertical movement.

Different types of 3DP technologies have been developed over the years and are currently available for several applications. In the pharmaceutical field, the 3DP commonly used are laser-based system (Fig. 3a), inkjet-based systems (Fig. 3b), and extrusion-based 3D printing system (Fig. 3c) [60–62]. To choose the appropriate 3DP and to avoid negative influences on the final print object, it is essential and recommended to check the printer's resolution, biocompatibility of the materials to be printed, adequate temperature, nozzle output volume, cost-effectiveness, among others [63–65]. The number of techniques used for 3D printing has increased in recent decades and each technique has its particularities. Among them is the biomaterial suitable for printing [66–88]. With the ideal biomaterial, different designs can be realized depending on their purpose (Table I).

3D printing, so far, has proved to be an effective tool in the precise fabrication of various pharmaceutical systems or devices. Experts predict that 3D printing will revolutionize the pharmaceutical industry, reducing the "one-size-fits-all" treatment approach to personalization, meeting individual needs and ensuring the development of high-quality dosage forms with different standards of drug delivery release (Fig. 2) [89–91]. To obtain a high quality of dosage form product with adequate stability, safety, and efficacy, certain printing parameters must be properly checked and optimized, such as print speed, print bed or nozzle temperature, fill density, and pressure. In addition, a thorough knowledge of the physicochemical characteristics and release profile of polymers and active pharmaceutical ingredients (APIs) are essential to produce 3D-printed objects. As the use of 3D printing evolves, business models involving selling

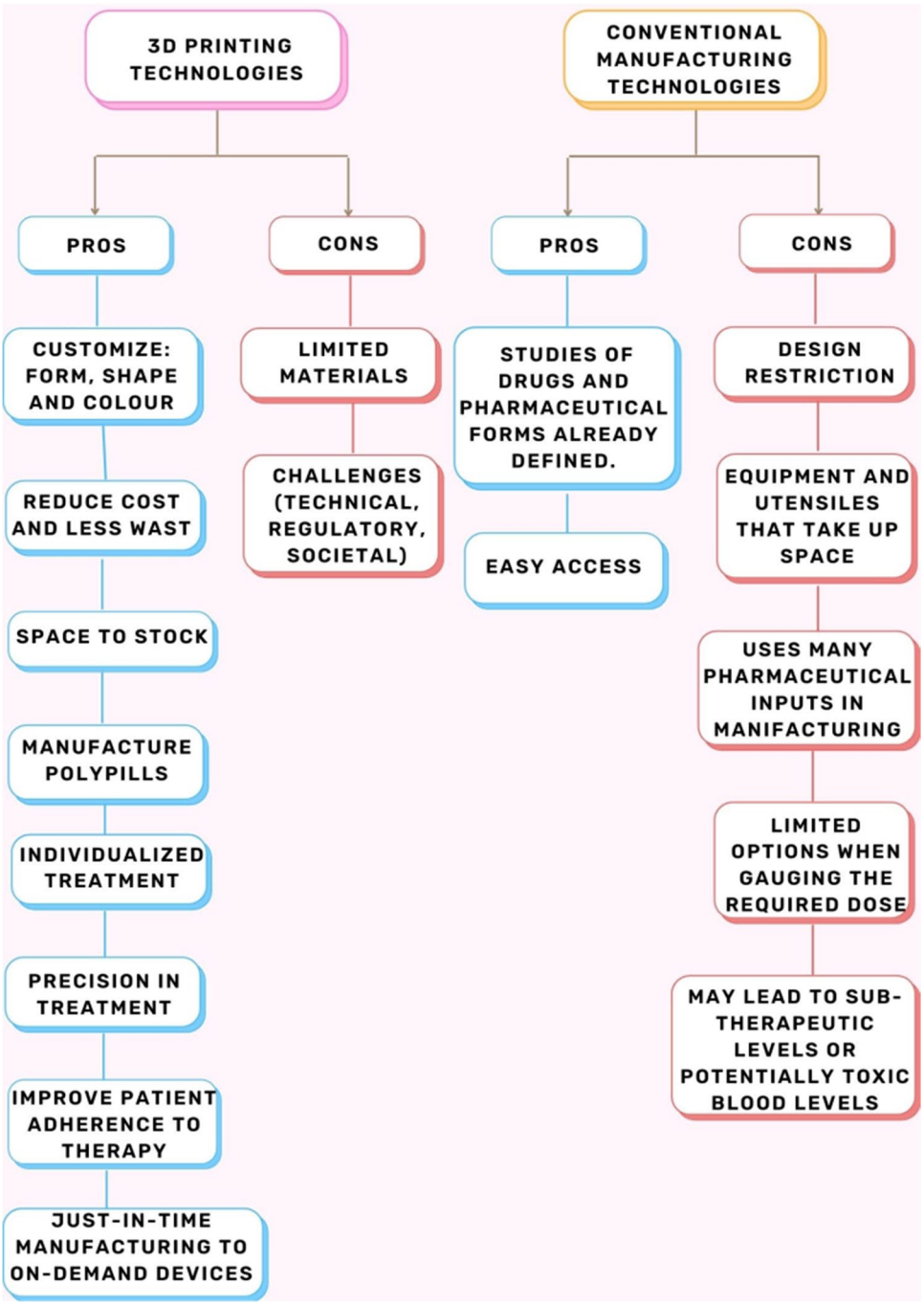

Fig. 2 Advantages and disadvantages of 3D printing and the conventional manufacturing technologies in pharmaceutical industry

3D-printed products will become viable. This type of therapy will be suitable, as with 3D printing it is possible to print devices with drug delivery, dosage, and/or custom geometry. There is currently no regulatory guideline that directs the correct way to manufacture these 3D-printed drugs [92]. As soon as regulatory guideline and quality control systems begin to be used, permission to use the 3D printer closer to the patient will appear on the market, such as in pharmacies and hospitals. This will allow quality-by-design printing of pharmaceutical forms, with high quality dosage forms.

Biomaterials

The inks or bioinks are essential for the fabrication of the material and depending on the final object to be 3D printed and the specific application; these inks or bioinks can be crosslinked or stabilized during or after printing. For printing and bioprinting of pharmaceutical materials, the lack of biologically safe materials has led researchers to develop new formulations to be used as inks and bioinks.

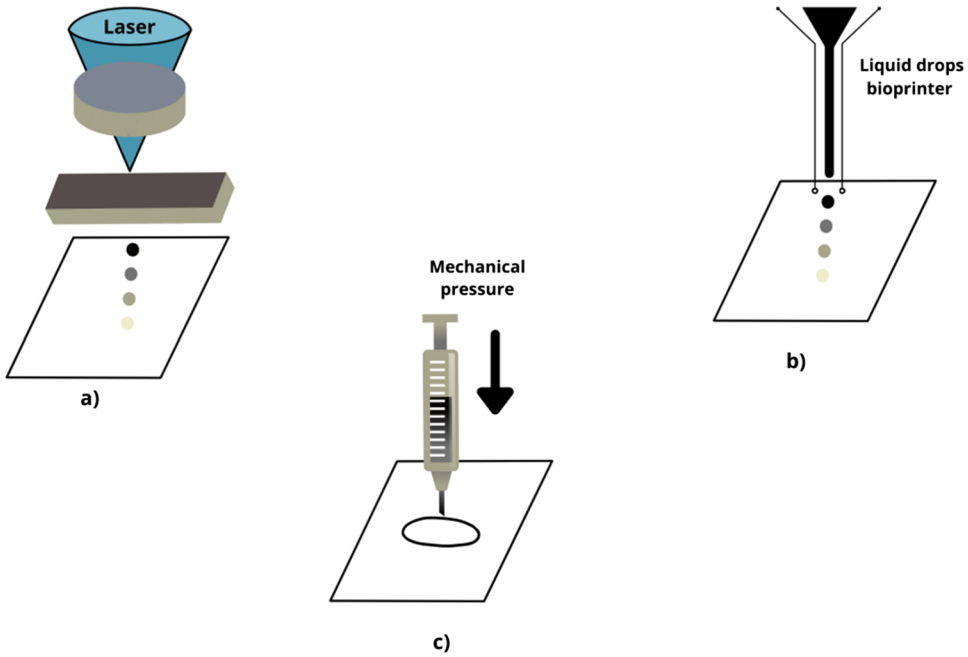

Fig. 3 Different types of printing techniques: **a** laser-based system; **b** printed-based inkjet systems; and **c** extrusion-based 3D printing system

In wound healing, the use of 3D bioprinting promises to favor faster wound closure. The term bioprinting emerged inspired by 3D printing, which uses printers that deposit bioprintable and biologically compatible materials along with cells known as bioinks. In addition to what was mentioned above, for the manufacture of the desired dosage form, other parameters must be evaluated for the selection of the appropriate 3DP such as the APIs, the biomaterial composition, the desired geometry, the appropriate rheological properties, and the drug release pattern [62, 93]. For printable materials, a viscoelastic behavior is recommended to facilitate and improve the ability of bioprinting. With the search for sustainable materials, the use of natural biomaterials in 3D printing shows attractive features for advanced wound treatment, such as biocompatibility between the material and the wound, biodegradation of the printed product, and low or non-toxicity. In addition, dressings made by 3D printing technologies keep the microenvironment moist and oxygenated, and are able to minimize infections by microorganisms [94].

A large number of hydrogels based on natural polymers such as alginates (ALG), hyaluronic acid (HA), collagens, gelatins, chitosan, and cellulose have been used as printable materials in the treatment of wound healing [72, 82, 95–105]. To facilitate and improve the resolution of 3D printing objects, synthetic biopolymers can be incorporated together with natural biopolymers and APIs [88, 106–113]. Table II summarizes the characteristics of the biopolymers and characteristics of the inks most commonly used in bioprinting/3D printing. The selection of these materials and also the selection of the cell source to be used have a direct impact on the desired response. In wound healing, it is important that this printed material ensures a suitable microenvironment, favoring normal skin cell proliferation and cell migration. Thus, the diversity of biopolymers and APIs that can be incorporated make this technology a promising approach in the construction of several DDS systems such as scaffold, biofilm, microneedles, artificial skins, among others, which will assist in wound healing.

The flexibility presented by the 3D printer and bioprinter shows its efficiency in wound healing treatment. With the software used, it is possible to individualize the treatment, either by changing the heads (which will allow printing/bioprinting of different materials) or by customizing the shape of the object with different designs according to the desired architecture. This technology is able to manage a large extent of the wound surface with the desired material and create any structure with better esthetic properties. In addition, it is capable of reducing the waste of medicines, hospital waste and depending on the inputs used; it is capable of producing an ecologically correct product without risks of contamination.

3D Printer/Bioprinter in Wound Healing

The use of 3D printing in wound healing and skin regeneration has paved the way for advancement in the development of therapeutic approaches. The versatility of this technology offers high resolution and accurate printing with high spatial resolution. Furthermore, 3D cell printing can be used for wound healing assays to study the possible

Table I Types of synthetic and natural materials used to print different devices on various types of 3D printers in wound healing

3D Printer	Materials	Design	References
Laser-based system	Methacrylated hyaluronic acid (MeHA) + gelatin methacryloyl (GelMA)	Scaffolds	[66]
	Methacrylate-based Formlabs Clear photoresin®	Microneedle	[67]
	Water-soluble methacrylatedpoly(ethylene glycol-co-depsipeptide)	Tissue engineering grafts	[78]
	Gelatin methacrylamide(GelMA) and collagen(Col)doped with tyrosinase(Ty)	Tissue engineering	[82]
	Polycaprolactone (PCL) + polyethylene glycol (PEG)	Elastic 3D-printed scaffold	[83]
Inkjet-based system	Gelatinous bioink composed of fibrin-collagen, mixed in a thrombin solution	*In situ* printing	[84]
	Type I collagen + fibroblasts, endothelial cells, derived from cord blood human endothelial colony-forming cells and human placental pericytes	Tissue engineering grafts	[85]
	Sodium alginate + collagen	Tissue engineering	[86]
Extrusion-based 3D printing systems			
Fused deposition method (FDM)	Poly-lactic acid (PLA) + Hyaluronic acid (HA), copper carbon dots (Cu-CDs), rosmarinic acid, and chitosan hydrogel	Scaffolds	[87]
	Poly-lactic acid (PLA) + minerals particles	Scaffolds	[88]
	Chitosan + D-(+) raffinose pentahydrate	Scaffolds	[68]
	Poly (ethylene glycol) diacrylate (PEGDA) + Gellan gum	Tissue engineering	[69]
	Lignin, Poly(lactic acid) (PLA) and castor oil + curcumine	Scaffolds	[70]
	Poly-lactic acid (PLA)	Microneedle	[71]
Pressure-assisted microsyringe (PAM)	Pectin + Chitosan and cyclodextrin complexes with propolis extract	Patches	[72]
	Methylcellulose + Alginate crosslinked in gallium solution	Tissue engineering	[73]
	Carboxymethylcellulose (CMC) and electrospun CMC based nano + Lidocaine and diclofenac sodium	Scaffold	[74]
	Methylcellulose, ALG and poly(N-isopropylacrylamide)	Biofilm	[75]
	Nanocellulose hydrogel crosslinked with Ca2+ and 1,4-butanediol diglycidyl ether	Scaffold	[76]
	Cellulose nanofibrils + 2,2,6,6-tetramethylpiperidine-1-oxyl radical and gelatin methacrylate	Scaffold	[79]
	Bovine gelatin + very-low-viscosity alginate and 2% w/v fibrinogen	Tissue engineering	[77]

mechanisms involved in wound repair, as well as in the investigation of potential therapeutic drugs and treatments to enhance wound healing. These *in vitro* assays allow a quick, economic, and ethical alternative to animal models [114]. Currently, large companies, such as L'Oreal, are entering into research collaboration agreements with other companies to produce 3D-printed skin models for testing cosmetic products [115].

In recent years, this technology has evolved and become increasingly sophisticated, making it possible to print dressings, artificial skin and organs, showing that 3D printing has an enormous potential in tissue engineering, regenerative medicine and the development of new pharmaceutical dosage forms. As 3D printing progresses, new challenges arise, mainly in terms of resolution, speed, and print reproducibility, which are resolved according to the type of 3D printer used and its purpose. Recently, 3D printing approaches have been introduced for wound healing, as seen below.

Stereolithography (SLA)

It is based on the hardening of liquid resin by photo-polymerization using ultraviolet light (UV). In this process, the laser focus falls on a given depth of the surface of the material (liquid photo-polymerizable resins) and, through photo-polymerization, solidifies the area covered by the laser focus [116, 117]. The configuration can be bottom-up, where the UV source is located below the SLA 3DP and the moving platform above, or top-down, where the UV source is above and the platform is below [90]. Each manufacturer of this type of 3DP uses a different strategy to carry out this process. However, basically, the printed product is obtained

Table II Characteristics of polymers and inks used in 3D bioprinting or printing

Polymer type	Biomaterial	Characteristics	Printing ink	References
Natural	Alginate	Alginate is a linear polymer derived from the cell wall of brown algae and the capsule of some microorganisms. It is biocompatible, biodegradable, commercially inexpensive and non-toxic. In addition, it is a negatively charged, water-soluble polysaccharide that supports cell growth.	With its ECM-like structure, low extraction cost and ease of gelation processes, alginate-based hydrogels have been studied for the manufacture of bioinks for various applications such as wound healing and tissue regeneration.	[97, 98]
	Collagen	Collagen is the main component of ECM and plays a structural and functional role. It is a natural, biocompatible, biodegradable polymer, which promotes cell adhesion, proliferation, and migration. It is safe for the host and does not cause serious adverse reactions.	It can be printed at low temperatures and forms a solidified gel at body temperature. The printed material has high porosity, high absorption capacity and low immunogenicity, favoring cell adhesion.	[82, 100]
	Chitosan	Chitosan has good biocompatibility, biodegradability and is non-toxic. Chitosan, derived from chitin, is a polysaccharide derived from marine crustaceans.	It has a linear structure and to form a hydrogel its matrix must be neutralized with a solution of NaOH.	[101]
	Cellulose	Cellulose is the most abundant water-insoluble polysaccharide in nature. It is biocompatible, biodegradable, and non-toxic.	In printing inks, cellulose is used as an adjuvant material that provides good bioadhesion and good mechanical properties. Due to its mechanical and physico-chemical properties, cellulose is an alternative for several applications, especially in tissue engineering, skin regeneration, and wound healing.	[102]
	Gelatin	Gelatin is obtained by partial hydrolysis of collagen. Gelatin is biodegradable, biocompatible, low cost, and non-toxic.	Gelatin forms a high quality thermosensitive hydrogel and has been highlighted for its beneficial effects on adhesion, migration, and cell proliferation in healing processes. Its good biodegradation is great for scaffolding, as its degradation facilitates the deposition and production of a new ECM. To improve its stability and hardness in 3D printing, crosslinking is recommended.	[103, 104]
	Xanthan gum	Xanthan gum is a high molecular weight heteropolysaccharide derived from microorganisms. It is biocompatible, low cost, and easy to handle.	Used as a thickener, suspender, and emulsifier, it became of interest in the manufacture of 3D scaffold.	[104, 105]
	Hyaluronic acid (HA)	Biocompatibility, biodegradability, bioresorption, high viscosity and mechanical stability.	To be used as a printable bioink, its mechanical and rheological properties need to be modified. For this, HA is chemically modified by mixing with other types of polymers. It is able to retain moisture and carry out cell proliferation. It is a biomaterial capable of producing non-adhesive, non-thrombogenic, and non-immunogenic scaffolds.	[95, 96]
	Pectin	Pectin is a natural polysaccharide derived primarily from the peel of citrus fruits. It has good biocompatibility, biodegradability, with good gelling properties.	The linear chain of D-galacturonic acid units linked to α- [1, 4] of pectin enables it to be methylated. Depending on the degree of methylation, they are classified as high methoxyl pectin (above 50%) and low methoxyl pectin (below 50%), these values impact the functional properties of the hydrogel formed.	[72, 97]

Table II (continued)

Polymer type	Biomaterial	Characteristics	Printing ink	References
Synthetic	Pluronic F-127®	Pluronic, known as poloxamer 407, is a block copolymer consisting of two blocks of poly (ethylene oxide) (PEO) and a central block of poly (propylene oxide). It is a thermoresponsible polymer with inverse thermo-gelling property. It has favorable properties such as non-toxicity, biocompatibility, and biodegradability.	The FDA has approved it for use in humans and it has been used extensively in formulations for drug delivery and controlled release. Due to the reversible rheological and thermogelling properties, it makes your ink biocompatible for 3D printing.	[80, 81]
	Polycaprolactone (PCL)	Polycaprolactone (PCL) is a biodegradable and biocompatible polyester material.	PCL has attracted attention in studies on scaffolding and bone tissue engineering. It allows fabrication of complex biomimetic and patient-specific structures.	[106, 107]
	Polyethylene glycol (PEG)	PEG is a synthetic material that has been widely used due to its hydrophilicity and biocompatibility. Its ability to be chemically adapted makes it a good material for 3D printing.	For 3D printing, this synthetic polymer provides biomedical and biotechnological applicability. Poly (ethylene glycol) diacrylate (PEGDA) is one of the best materials to use in stereolithographic 3D printing for biological applications because it is UV light curable.	[108, 109]
	Polyglycolic acid (PGA)	y-PGA is a water-soluble polymer obtained by in natura microbial fermentation. It is non-toxic, biodegradable, and biocompatible.	For 3D printing, its water retention and absorption capacity favor its use in wound healing products.	[110]
	polylactic-co-glycolic acid (PLGA)	Poly-lactic-co-glycolic acid (PLGA) is an FDA-approved, biocompatible, biodegradable synthetic polymer. The degradation of PLGA supports a controlled release of the drug at desirable doses.	PLGA has good characteristics for 3D printing, as it is a good drug delivery carrier and as scaffolds for tissue engineering.	[111]
	Polylactic acid (PLA)	Polylactic acid (PLA) is obtained from bio-based starches such as wheat, sugar cane, corn and beet. It has been widely used as a scaffold due to its ability to degrade by hydrolysis and enzymatic action. For this reason it is biodegradable and biocompatible.	Considering its properties, PLA is being researched as scaffolds for tissue engineering applications.	[88, 112]

after the light-curing process. The first layer is scanned by the laser in the x and y axes; and photopolymerized. This light-cured layer is coupled to a platform that moves along the z axis according to the previously defined geometry. Finally, the following layers go through the same process and are coupled to each other until obtaining the desired format.

Although it has advantages, SLA has a limited application in the pharmaceutical field, because they have few polymers suitable for pharmaceutical use (Table III). Based on fact that the hyaluronic acid (HA) (a native ECM derivative) provides an anti-inflammatory response and cell signaling, Rakin and team successfully developed a bioink useful for SLA 3D bioprinting using methacrylated hyaluronic acid (MeHA), laden hydrogel scaffolds [66]. The ability to retain water in the ECM results in important functions of HA in the body, such as protecting cartilage against impacts, filling spaces between organs and lubricating joints. In addition, it participates in important processes in the integrity of the ECM and wound healing, signaling cell adhesion, migration, and proliferation. A scaffold with this molecule contributes to the rapid improvement of wound healing and as it is a biocompatible molecule, rejection and allergic reactions are reduced.

Recently, 3D printing has been shown to be useful in the fabrication of polymeric microneedles (MN), and one of the methods is SLA (Fig. 4). The main challenge found to manufacture MN by the SLA method is the high toxicity of the resins used and the low resolution of the print. These challenges were overcome by developing a biocompatible resin with low toxicity and by looking for strategies that increase the resolution of bioprinting, for which the use of the two-photon polymerization technique was suggested [118]. Microneedles are composed of pointed, microscopic, and sharp structures that break through the dermal barrier to reach the dermal microcirculation. The MN constitutes a minimally invasive DDS that has been used in various types of wound healing treatments [119] such antimicrobial wound treatment [120, 121]; scar repair [122, 123]; and treatment of wound and burns [124]. Nowadays, the MN is the focus of numerous studies by 3D printing methods [67, 71]. Farias and collaborators developed hollow microneedle first reported in literature that can be expandable into applications of wound healing therapies [67] showing the effectiveness of MN 3D printing by the SLA method.

The development of a hydrogel that is biocompatible with the human body brings benefits both for the treatment of wounds and for the treatment of scars. Microneedling is a technique that proposes the stimulation of collagen production in a fast, minimally invasive way, without causing the total de-epithelialization of the skin. These microneedles are able to penetrate unfavorable wound sites and facilitate the transport of the active compound. When printed, it has the benefit of being able to incorporate the active compound into the microneedle matrix in the desired dosage and print the microneedle in the desired size and shape; promoting a personalized treatment to the patient, and in addition, providing fast, reliable, and cost-effective microneedle fabrication in a robust and scalable manner.

Inkjet-Based System

Inkjet system use digitally controlled devices that superimpose tiny ink droplets (usually in picoliters) in a burst (thousands of times in a few seconds) and print the product non-contact on a substrate. This kind of printing technology has developed in recent years; its applications have evolved from two-dimensional (2D) to 3D [117, 125]. To create prints using droplets on demand, the approaches used in these systems are piezoelectric, thermal, and electromagnetic. In addition, to eject ink drops, printers mostly use heat or mechanical compression. Aiming at the application in tissue engineering and regeneration, many researchers choose to use thermal inkjet printing technology compared to piezoelectric printing, as it is more biocompatible with the living system [126].

With the evolution of this technology, it was possible to create a mechanism capable of generating a high pressure inside the droplet formation support, making it possible to print materials with a very high viscosity and even molten materials [90, 117]. By featuring droplet control integrated into compact print heads, inkjet printers are able to accurately print 3D products using a low amount of ink droplets resulting in affordable products with a high level of resolution and throughput (Table III) [117]. In recent years, there has been an increase in materials developed to replace conventional printer inks with technological biomaterials (e.g. cells, polymers, gelatins). With this in mind, appropriate mixtures of drugs and pharmacologically inactive substances (which may be inks formed by biomaterials) are being studied for use in various pharmaceutical products, depositing this mixture in layers on a suitable substrate [90].

Researchers have developed a versatile method to manufacture a complex and heterogeneous 3D tissue using an ink composed of multiple cell types printed simultaneously and have demonstrated the feasibility of fabricating this type of complex heterogeneous tissue [86]. This technology has proved useful for skin bioprinting. Bioprinted skin with 3D technology provides an excellent solution to recover injured tissues and aid the healing process, as specific cells and active compounds that will facilitate healing can be incorporated providing the patient with a personalized treatment. 3D skin printing is able to mimic the skin structure, printing in an ideal shape and size for the patient. In addition, it

Table III Advantages and disadvantages of the types of 3D printer systems

System	3D printing	Advantages	Disadvantages	Reference(s)
[Laser-based system]	Stereolithography	• Low thermal stress involved • High-resolution process • Print high cell densities without affecting cell viability	• Materials must be photo-curable • Post-curing steps necessary • Reduce number of polymers approved for pharmaceutical field • Photosensitive resin are difficult to store due to stability • Materials used can be cytotoxic	[182]
Printed-based inkjet systems	Continuous Inkjet	• Prevents clogging of nozzle	• Wastage of material, low resolution, and expensive	[34, 58, 183]
	Drop on demand	• High precision and low cost and minimizes wastage of material • Thermal - thermal inkjet printers include high print speed, low cost and wide availability	• Thermal - might degrade heat sensitive materials • Cell viability can be affected by thermal and mechanical stress; and acoustic printers • Viscosity of the material: the higher the viscosity, the more force required to eject the drop from the printer nozzle	
Extrusion-based 3D printing system	Fused deposition modeling	• High resolution • Solvent-free process • No post-fabrication steps • Produces mechanically strong dosage forms • Low-cost • Drug uniformity is obtained	• Extruder nozzle heat can degrade material • Polymers must be thermoplastic • Prior preparation of filaments is required • Lack of suitable biocompatible/biodegradable thermoplastic polymer • Thermally labile drugs degradation may occur due to the high processing temperature. • Pre-processing steps of filament making are required	[91, 140, 183, 184]
	Pressure-assisted microsyringe	• Room temperature process • Wide range of printing material • Easy implementation and easy handling • High drug loading is achieve	• Organic solvents are required • Post-fabrication drying is necessary • The rheology of the polymer, can impact the formation of the structure and the printing process • Low print resolution • Drug instability may occur	

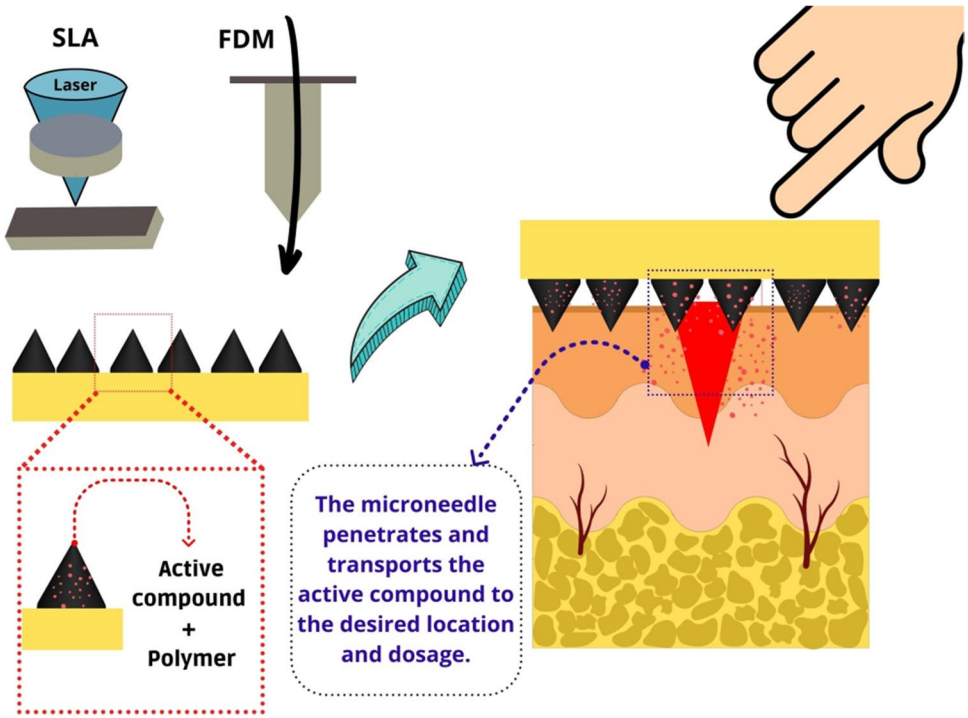

Fig. 4 Overview of SLA and FDM 3D printer used for production of microneedles

minimizes the risks of rejection by the organism after the implantation of this bioprinted material and can delivery drugs according to the patient's genetic profile.

Inkjet-based printing is also being applied *in situ*; the healing agent is applied directly to the wound. The researchers developed a bioink composed of human keratinocytes and fibroblasts and performed the impression of this material on the skin using 3DP. Results demonstrated that the two different types of skin cells can be imprinted directly at wound sites and can mimic normal skin [127, 128], making possible for a wide applicability of this system for different types and sizes of wounds. Cells such as stem cells incorporated into a gelatinous bioink also showed positive results in wound healing when printed *in situ*, showing an effective and rapid closure with increased wound re-epithelialization [84]. Inkjet-based printing favors a quick treatment for patients who have suffered burns, without damaging another region of the patient's body as in grafts and without bringing discomfort to the patient's appearance as in xenografts. Furthermore, it is useful for various skin wounds, due to its ability to adapt dosages and cell lines.

Thinking about an environment different from planet Earth, the wound healing process can undergo some changes. In space, astronauts suffer more from the loss of skin cells than on Earth, during space flight their skin ages faster causing skin injuries. In addition, a thinning and increased sensitivity of the skin has been reported by astronauts, which, combined with delayed wound healing, can increase the tendency to skin infections [129–131]. Some researchers have evaluated 3D-printed materials, under standard gravity conditions, for the use in wound healing processes to the astronauts present in space missions. 3D printings such as drop on demand (inkjet-based system) and extrusion have been studied using different types of bioink [84, 85, 127, 132–134]. In space, 3D bioprinting proved to be effective in wound healing compared to conventional treatments in tissue engineering, presenting advantages due to the ease of application because it is a semi-automatic treatment. Both inkjet-based system and extrusion-based 3D printing systems allow the impression of complex tissue equivalents rich in cellular structure that increase adhesion and vascularization of the wound region, in addition to being a quick and simple process that can be performed during space flight [129].

Note that inkjet printing is a technique that has been used in pharmaceutical applications for the preparation of individualized DDSs enabling the printing of various biomaterials [135]. It is an economical technology where commercial printers can be easily modified; the inkjet printer can offer high speed and good resolution for printed materials. The main disadvantage is the use of bioinks with low cell density, in order to avoid nozzle clogging and maintain cell viability. The use of inkjet printing can be an important alternative to deliver specific doses of drugs to facilitate wound healing or improve the treatment of other dermatological diseases [136–138].

Extrusion-Based 3D Printing System

In this method, the material to be printed is extruded from the automated nozzle (y axis) on a fixed or mobile platform (x axis). It can be obtained by two methods: fused deposition

method (FDM) and pressure-assisted microsyringe (PAM). In FDM, the thermoplastic filaments are melted using a high temperature, extruded through a nozzle, and deposited layer by layer by the pre-defined CAD model [93, 139]; then, the material is solidified and the desired object is formed. PAM presents similarity with FDM. What make it different from FDM is that in PAM, there is no need to melt the material to be printed [60, 140].

3D printing by the FDM method was also used for tissue regeneration by printing system known as scaffolds. In these scaffolds (chitosan), the proliferation of fibroblasts and keratinocyte cells was observed, forming an initial skin-like structure [68]. In this type of 3DP, synthetic polymers can be used, such as poly(lactic acid) (PLA), due to its biodegradable and biocompatible characteristics and due to its high mechanical strength and low coefficient of thermal expansion. It is known that the presence of free radicals and reactive oxygen species is detrimental to wound healing. It also helps the emergence of diseases such as rheumatoid arthritis, atherosclerosis, or cancer. With that in mind, the incorporation of antioxidant substances, such as lignin (LIG), into 3D-printed devices can accelerate wound healing [70]. The PLA played an important role in wound healing by keeping the wound environment moist. As shown in other studies, a moist environment facilitates wound healing, as well as reducing pain and preventing scarring. It was also verified that the PLA/LIG filament printed by FDM was able to design meshes with different designs, being very efficient for use in dressings, meeting the needs of the patient.

The ability of the FDM printer to print various formats makes it possible to print MNs. The fabrication technique for MNs using FDM 3D printing has been developed (Fig. 4). Taking into account that the FDM method presents the challenge of having low resolution of these printers, to overcome this, Luzuriaga and collaborators developed a protocol where they improved the resolution of printed parts through a post-manufacturing chemical etching, showing that the MNs obtained present mechanical strength comparable to conventional MNs. Furthermore, they reported that PLA expansion can be beneficial in carrying small molecules drugs and its degradability in the skin can contribute to obtain a new DDS [71].

3D printing PAM technique is often used, in the pharmaceutical literature, synonymously with semisolid extrusion (SSE) [141]. SSE is a broader term that encompasses different mechanism of material extrusion, such as pneumatic extrusion, mechanical extrusion, and solenoid extrusion [142]. As we will report on both the printing of liquid and semi-solid materials, the term PAM will be used. For printing using the PAM technique, prior knowledge of the physico-chemical properties of the polymers used to manufacture the ink is essential. The viscosity must be optimized for the correct control of droplet extrusion at the time of deposition of the material on the printing table. For this reason, evaluating the rheological properties and processing parameters of materials to be printed by 3D printing based on PAM extrusion are important to obtain the desired dosage form with sufficient physical strength [143]. In this technique, the subsequent processes, after deposition of the material, include drying and solidification of the desired device.

Pain caused by an injury can impair wound healing. To relieve pain during wound care and promote wound healing, the 3D printer makes it possible to print systems (scaffolds, patches) containing anesthetics, anti-inflammatory, and antimicrobial agents [72, 74]. Natural polymers have shown to be efficient in printing systems using PAM. Pectin is a hydrophilic agent who reacts with wound fluid to form a gel capable of promote wound healing by keeping the microenvironment moist, protecting the wound from infectious agents and absorb the exudates produced by it [72]. Furthermore, due to its solubility in water, after being printed, it forms transparent films and presents rapid disintegration in an aqueous medium.

Thermoresponsive hydrogels are a class of polymeric system sensitive to temperature changes. This class of system is being studied for the development of dressings manufactured in 3DP. Hydrogels containing cellulose derivatives, such as nanocellulose and methylcellulose, showed optimal rheological characteristics for extrusion-based printing, due to their viscosity and shear thinning behavior [75, 76]. 3D-printed devices exhibited temperature-responsive transformation behavior; and showed biocompatibility and antimicrobial activity. Furthermore, cell tests with nanocellulose scaffolds did not show cytotoxicity for fibroblasts and also promoted cell proliferation, which are essential factors for fast and effective wound healing [76]. All these factors guarantee the application of this mixture as a new generation of dressings.

Pluronic F-127® (poloxamer 407) is a thermo-responsible polymer with inverse thermosetting property, i.e. at low temperatures, the poloxamer 407 formulation is liquid and as the temperature increases, the formulation forms a gel. This rheological characteristic, of low viscosity at low temperature, allows and makes its ink biocompatible for 3D printing, especially in the PAM technique. Although it has good printing characteristics, its ink does not guarantee long-term cell culture. To circumvent this disadvantage, Muller and collaborators (2015) presented a nanostructuring approach. In the method presented by the researchers, the acrylate was mixed with unmodified Pluronic F127 and it was possible to form a stable gel with good printing properties by means of UV crosslinking. As a result, it was demonstrated that this reticulated network was able to increase cell viability [80]. This ink proved effectiveness and allows the incorporation of other polymers and APIs that will aid in wound healing and scaffold development.

Blood plasma as the main bioink to introduce other cells into the 3D-bioprinted object could form blood vessels, mimicking the skin to be replaced [144]. It was verified that this system showed better activity than animal collagen and could form an artificial skin. For wound healing, this system has advantages due to the possibility of using the patient's own plasma to be treated. Studies to obtain artificial skins manufactured in 3D can be performed using PAM with excellent physical and biological properties [145]. Bioprinted skin has enough characteristics to accelerate wound healing; and reduce contraction and formation of scars. In addition, there is the possibility to print the desired tissue size according to the extent, shape, and location of the wound, catering to all types of wounds.

3D-Printed Materials at Different Stages of Wound Healing

It is noted that the polymers used in 3D printing exhibited good printability and integrity, maintaining the desired shape and self-adhesion to the skin. These 3D prints showed an excellent swelling index and exudate absorption property, which are necessary requirements for a good wound dressing. In addition, care such as mechanical and microbiological protection of the wound during the healing process is important [75, 146, 147]. For these reasons, several studies have been carried out and demonstrated effectiveness in each phase of the wound healing process.

Hemostasis

Hemostatic materials are used to prevent and stop bleeding and hemorrhage. Among these materials, there are traditional wound dressings that are placed on the wound, which favor the absorption of fluids from the site and block the wound from external agents. Removing these materials from the wound is very inconvenient, as the fluid (e.g. blood) is absorbed into this traditional dressing, forming a solid clot that adheres to the wound and causes secondary bleeding and pain. The structure of traditional dressings is not effective in controlling bleeding and promoting regeneration of functional tissues. A good wound dressing must have some advantages such as protecting wounds against the penetration of microorganisms, preventing secondary infections and maintaining a moist microenvironment for wound healing. A dressing with antimicrobial properties, with the ability to adhere to the wound site and stop bleeding is essential. For this, it is known that it is important to use a good printing ink with suitable polymers, an antimicrobial agent and a good printing technique. New candidates for the use of non-traditional dressings are being proposed [148].

New hemostatic systems with elasticity, high fluid absorption rate, and high permeability favor bleeding stagnation and wound healing. These systems, often made from hydrogels, promote sealing of organic tissues, and it is reported that a hydrogel with a 3D structure can influence the wound healing process. When applied over the wound, the product absorbs fluids and expands, occludes the lesion and creates pressure against the tissues, preventing bleeding. Hemostatic microparticles have been widely studied and used in surgery. A 3D structure using a tannic acid and CMC hydrogel was able to promote cell growth and wound repair [149]. Through the synergistic effect of these natural materials, the authors reported that the wound healing process can be regulated in a controlled manner.

3D bioprinting using bioinks offers the desired structural complexity, vital for hemostasis activity and targeted cell proliferation in rapid and controlled wound healing [149–154]. Compared to traditional fibers (gauze and cotton), nanofibers are thinner and softer and have a larger surface area. Furthermore, they allow the incorporation of active compounds and control their release. The 3D-printed nanofiber sponge acquires beneficial properties for wound healing. In addition, they have important characteristics such as the fact that they provide closure of deep wounds and create a favorable 3D microenvironment that enables cell growth, promoting the regeneration of injured tissue.

One of the challenges of 3D printing scaffolds is to develop suitable bioinks with specific desirable characteristics for wound healing. *In vivo* studies revealed that chitosan played a crucial role in the inflammatory phase, while collagen played an important role in the proliferation and maturation phase. After obtaining the scaffolds, aerosols of fibrinogen and thrombin were deposited on the surface of the scaffolds to improve hemostasis and wound healing [150]. An integrative strategy developed using cross-linking and biocomposition resulted in the formation of a compound called HI/DA-Gel. The 3D-printed structure resulted in a print with wettability, thermal stability overcoming the limitations of gelatin and HA; and still, showed adhesive and mechanical properties; been effective in hemostasis and healing of full-thickness dermal wounds [152]. Hemostatic materials containing collagen and chitin are commonly evaluated for their absorption and bioactivity. A "cotton-like" collagen-based biomaterial and chitin was developed and its printout proved to be effective in hemostatic and tissue repair, suggesting the replacement of conventional collagen materials by this new generation of collagen [154].

The interest in the development of new hemostatic systems arises from the need to control bleeding in wounds caused in emergency and surgical situations. In addition, these systems must have mechanical properties and tissue adhesion, biocompatibility, biodegradability and antibacterial effect. Polymeric hydrogels have these properties and

can be improved when 3D printing technology is applied. The improved properties of this printed material are due to the ability to form a porous structure with flexibility in manufacturing, the ability to incorporate active compounds, and the ability to retain large amounts of water or biological fluids. Due to concerns about the potential toxicity of the products, using non-toxic and biodegradable products is important. For this reason, as described throughout the literature, products based on collagen, gelatin, hyaluronic acid, and other 3D-printed hemostatic products can be used as hemostatic dressings. In addition, they can contribute to infection and/or reduced wound healing. Some of these polymers, whether natural or synthetic, have passive interaction properties and stop wound bleeding through the absorption process, while others have active activity in the clotting process through interaction with clotting factors and promote hemostatic mechanisms.

Inflammation

Inflammation is activated immediately in the early stages of wound healing, soon after tissue injury has occurred. The correct initiation and stages of the inflammation process are essential for wound repair. There is increasing evidence that in exacerbated inflammation in which macrophages are involved, scar formation is frequent. In the inflammation phase during the healing process, subsets of macrophages appear and facilitate collagen deposition, and facilitate excess extracellular matrix components, which results in fibrosis. The use of tissue adhesives and traditional dressings delays the healing process, which can lead to chronic inflammation. Based on this, the demand for a minimally invasive therapy is a necessary strategy to reduce scar formation and improve wound healing by inhibiting inflammation.

The incidence of deep injury caused by burns is something of concern. Currently, the most-used treatment is autologous skin graft or xenograft. However, finding availability of healthy skin, and still for a large area, is challenging. The wound caused by a burn injury can induce a state of immunosuppression, causing them to become chronic wounds. Another cause of global concern is wounds caused by complications of diabetes mellitus. Current standard dressings are unsatisfactorily ineffective for treating chronic wounds. Chronic inflammation is the main cause of the long-term incurable nature of chronic wounds. Developing therapies other than conventional ones represent a promising approach.

In a wound, inflammation is a natural step in wound healing. In many cases, antimicrobials are used to prevent an excessive inflammatory response and future infections. Associating an antimicrobial agent in a system containing growth factor is capable of promoting the healing of diabetic wounds and also preventing bacterial infections [155]. Although it is known that inflammation is a fundamental step to eliminate microorganisms and create a favorable environment for healing to occur, it is not known for sure how long inflammation must be present for optimal wound healing. Studies have investigated the role of early inflammation in the healing process [156].

In addition, another promising approach is the use of stem cells. Due to their ability to self-heal and their multilineage potential, designing a new therapeutic system, using stem cells can improve wound healing and promote proper integration of skin replacement. For stem cells to fulfill their role, it is necessary to create a correct delivery system capable of creating an appropriate microenvironment for stem cells to survive and proliferate. 3D-printed systems using stem cells could favor skin regeneration at the wound site and also showed potential anti-inflammatory activity; suggesting their use as advanced dressings in tissue repair and regeneration [157–161].

Among the causes of injuries, there are not only injuries caused by burns and complications of diabetes mellitus. Other agents, whether internal and/or external, can cause injuries that will require good healing. The development of polymeric systems that modulate inflammation can reduce complications in wound closure and reduce the use of anti-inflammatories. An inflammation-modulating biomaterial scaffold was developed using a phosphate-crosslinked PVA polymer, named by the authors as a bio-scaffold for soft tissue repair [162]. This project assumes that if excess pro-inflammatory cytokines are trapped by the bio-scaffold, the inflammatory response can be modulated, reducing post-surgical complications and the use of anti-inflammatory agents. New design strategies have been studied to revolutionize the pharmaceutical market, such as the use of adhesives capable of adhering to the wound site. The adhesion of mesoporous silica nanoparticles to the tissue triggers a good acute inflammatory response that results as a good strategy to accelerate healing and promote the resolution of inflammation [163]. These 3D-printed mesoporous silica nanoparticles form a tissue adhesive at the wound site and with the formation of nanocomposites in wound gaps, injured tissues can be reattached conveniently, resulting in tissue regeneration.

Proliferation

Excessive inflammation, ease of infection at the site, and impaired angiogenesis make wound healing difficult. After the inflammation stage, the next stage is proliferation. This transition from the stage of inflammation to the stage of proliferation occurs through the immunoregulation of macrophage polarization towards the M2 phenotype. It is in this transition that some authors bet on a new type of

bioactive material in the healing of diabetic wounds, using an anti-inflammatory, antioxidant, and antibacterial conductive hydrogel scaffolds enabled for the M2 phenotype. This 3D-printed scaffolds were able to generate a faster process of angiogenesis and diabetic wound repair, through anti-inflammatory activity and polarization of M2 macrophages [164].

It is known that the macrophage is an essential cell to control the initial inflammatory state. Lesions with poor healing are usually associated with disrupted transition from M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophages. For this transition to occur, cytokines and growth factors must be secreted by fibroblasts, which are abundant and fundamental cells during the wound healing process. Unlike stem cells, fibroblasts are easily isolated and their duplication occurs in a short period, facilitating their use. For this reason, a 3D fibroblast-based cell therapy is being suggested as a good alternative in wound healing [165].

Fibroblasts are the main cells involved throughout the wound healing process, which maintain tissue integrity by producing and synthesizing ECM; and this occurs through the production of some proteins, such as collagen and fibronectin. Thus, new drugs and DSS may assist in the correct targeting of these fibroblasts during wound healing. For example, 3D-printed methacrylated gelatin and nanocellulose scaffolds, which improve healing while decreasing fibrosis and scarring, favoring cell proliferation of fibroblasts [76, 145, 166, 167]. These proliferation activities can be improved by incorporating active compounds into printing inks such as HA and growth factors [168].

Studies involving egg whites have shown promise, and were originated from a treatment widely used in antiquity as a poultice for dressings on burns and wound healing [169]. Currently, the search for biodegradable, easily obtainable, and low-cost hydrogels has attracted the industry and pharmaceutical research. Combined with this, finding a platform using this type of hydrogel effective in the healing process of chronic wounds is a good indication in clinical practices. The use of the 3D printer as a platform for obtaining new pharmaceutical products has proven to be innovative. The physical, mechanical, and biological characteristics of the structures obtained by it has been gaining ground in the industry, and we increasingly observe its advantages in wound healing therapy.

Remodeling

Any failure in the normal wound healing process results in abnormal scar formation. During wound remodeling, ECM components undergo constant changes. Collagen III (produced during the proliferative phase) is replaced by type I collagen. In remodeling stage, fibroblast differs into myofibroblast. In this process which is mediated by inflammatory factors and mechanical stimuli, the wound contracts and closes. The excessive activation of myofibroblast results in abnormalities in cell regeneration, which leads researchers to study alternatives to promote tissue regeneration and prevent scarring [170, 171].

Scar formation is the end point of the entire wound repair process. When excessive scarring occurs, it indicates that there is an imbalance between biosynthesis and degradation, mediated by apoptosis and ECM degradation. This dysfunction causes the inflammatory and proliferative phase to be prolonged and persistent, causing reduced remodeling. The exaggerated deposition of collagen fibers is one of the factors that lead to failures in healing. Systems involving piezoelectric responses can be used to simulate and amplify endogenous bioelectricity, allowing healing and preventing scarring [172]. In addition to hydrogels, new systems for 3D printing are being studied, including materials of mineral origin. Due to these positive characteristics, the graphene enables the incorporation of MSCs increasing healing and, as a beneficial consequence, an anti-scarring effect [173].

3D skin printing is a strategy used to provide an effective treatment for larger wounds due to strong shrinkage and scar reduction [145]. Porcine ECM has been used for a long time in wound repair due to its human-like structure. The 3D printing of a system composed of porcine ECM showed encouraging results in the reduction of scar contraction [174]. In some cases, an active component in a dosage form does not have the expected result, but when changing the dosage form, the active starts to present the desired therapeutic function. This often occurs when using ECM for some treatment. With that in mind, the incorporation of this ECM in a hydrogel for later fabrication of a 3D-printed dermal analogue represents a good alternative for the use of ECM in wound healing. This is what happened with the porcine ECM; when they change the dosage form of application of the porcine ECM, their therapeutic characteristics improve, reaching a better therapy. The ability to mimic a cellular environment and possess porosity makes graphene-based materials a good material in the manufacture of 3D-printed products. These studies show us the versatility of 3D printing. Its ability to print different structures indicates its importance for the development of new therapies for several existing active components.

Clinical Case Studies

3D-printed products are known to offer a promising treatment for difficult-to-heal wounds. *In vitro* tests have demonstrated the efficacy and safety of this type of system. For this reason, clinical case studies have been carried out to verify the effectiveness of these systems. Hydrogels as a synthetic biomimetic substitute showed, in a clinical case study, a good percentage of healing, even providing pain relief [175, 176]. 3D-printed products need evaluation in

humans to verify if their microstructure can adhere to the wound site and mimic the microenvironment in a real situation. Its mechanical properties, biocompatibility and biodegradability are also factors that need to be evaluated. For the clinical application of these forms, it is necessary that this printed material has an application facility that generates comfort to the patient. In addition, the condition of the wound should also be checked to assess healing time.

A randomized controlled clinical trial evaluating the feasibility and efficacy of using a 3D-printed polycaprolactone scaffold was performed to assess whether its insertion into fresh extraction sockets would allow normal bone healing and its results were encouraging [177]. The use of 3D printing has been presented as a good alternative in bone healing [178, 179]. The results are so encouraging that in these studies, they found that bone healing can occur from fractures in both smaller and larger bones, because of the bioadhesiveness and biocompatibility of the inks used for 3D printing.

In the case study, it is important to define the patients who will participate in the study. In the study evaluating the 3D-printed PLLA and gelatin scaffold, patients over the age of 18 years and with wounds without signs of serious infection where traditional wound care methods had failed were selected for the study [180]. The printed scaffold was able to adhere to the wound and form a fibrin membrane quickly, and yet, it favored re-epithelialization and wound closure. Other systems are also being studied. Such as 3D antibedsore overlay applied to the wound, which has been shown to be significantly effective in healing [181]. But like any other test, the case study has its disadvantages such as getting a good number of people to the test and also having the ability to get all patients to complete the study. Despite this disadvantage, it is worth mentioning that this type of study is essential for the product to reach the market.

Clearly, the number of patients recruited must be greater to truly assess whether the 3D-printed product is more effective than conventional treatment methods. Case studies evaluating 3D-printed wound healing products, with a larger cohort and a control group, are needed to provide more confident data. Currently, there are few case studies involving 3D printing technology in wound healing. However, given the positive results evaluated *in vitro* tests, where they verified the efficiency of these 3D-printed products, in the near future. We will come across more case studies and the sale of these products will be something not too distant.

Authors' Point of View

Wound healing is a complex process that involves a cascade of molecular pathways that are activated, in an organized way, at the moment of injury in order to restore injured tissue. However, in some cases, wound healing can be affected and compromised by some change or interference in the healing cascade, causing a prolongation of healing phases and/or an exacerbated response injury. In general, the studies demonstrated here showed the application of 3D printing technologies for topical administration in wound healing, providing a customized medicine based on the needs of patients. The idea of using 3D printing in wound healing and tissue engineering revolutionized the field of biomedical engineering. We can see that these 3D printers are capable of printing different sizes, shapes, and pores, helping to treat different types of wounds. 3D-printed products come as a proposal for modern dressings, due to their ability to keep the microenvironment moist and facilitate the healing process, and yet, it does not have the disadvantages of traditional dressings, as they allow for a more flexible and breathable design. Its success for the treatment is also due to the type of ink used for the manufacture of the 3D print and the active compound. Each printer presented its particularities and relevance in the manufacture of 3D-printed products for wound healing. The materials to be printed must present adequate crosslink mechanisms and rheology to facilitate the deposition. In addition, they must be biocompatible and stable to reduce the risk of rejection, allergies, degradation of the formulation and/or cellular degradation.

All 3D-printed systems presented here helped to restore the integrity of the injured site and tissue regeneration, providing effective healing, due to the structuring of the printed material and the hydrogel used. Among the active compounds incorporated in hydrogels based on synthetic and/or natural polymers, there are those with anti-inflammatory, antioxidant, and antimicrobial properties (natural, mineral, and synthetic origin) to accelerate tissue healing, preventing infection by microorganisms and inflammation; with analgesic property to reduce pain and discomfort; and with peptides that, in addition to structuring the printed material, also have activity in cell regeneration. All of them, in some way, will help wound healing in any of its stages (hemostasis, inflammation, proliferation, and remodeling).

In addition, 3D bioprinting allows the printing of skin substitutes with a favorable cellular environment (using the patient's own cells) capable of facilitating oxygenation of the wound site and covering an extensive wound area. It is hoped that in the near future this may facilitate skin grafting, which is a process that has a certain disadvantage when it comes to lesion size and finding donors. 3D printing allows for a more natural approach. By embedding cells in a three-dimensional space, it is possible to model *in vitro* what occurs *in vivo*. This system allows creating a favorable microenvironment and incorporating more than one type of cells. What makes this system more interesting is that different types of cells can be added to different printed structures and they interact with each other. These cells can be added within the printed structure or the cells themselves create their ECM.

Advantage of One 3D Printer Over Another

3D printing technology allows the fabrication of bespoke microstructures with high resolution. There are different types of 3D printers on the market that can be used for different purposes, each with its advantages and disadvantages (Table III) [93, 140, 182–184]. The choice of printer will always depend on the purpose of the product to be printed; and the material and/or active components used to manufacture what is desired. For this reason, it is difficult to say which printer is better than the other. In wound healing, we have several types of treatments presented here in this work, each with its own particularities, especially in terms of the active ingredient and the form of drug release. In tissue engineering, for example, 3D bioprinters must be evaluated according to which cannot print live cells directly onto the structure and that one which can.

The inks or bioinks (synthetic and/or natural polymers) represent one of the most important roles in the final product. They are the ones that maintain the desired shape and help with wound healing. If the wrong choice of ink or bioink is made, the printer will be unable to produce the desired material. In addition, they must be highly biocompatible, biodegradable, and mechanically stable during and after printing. In addition to these parameters, understanding the crosslinkability and rheology is critical in the choice of printer and print nozzle.

Obviously, choosing the ideal printer for wound care is a difficult task. To manufacture a printed product, depending on functionality, the print resolution must be good, as is the case with MN manufacturing. In this work, two forms were presented, one using stereolithography and the other using FDM. So, which one is better? This is a complicated question, but it is a good one because it all depends on the purpose. In the case of using the laser printer, the authors needed a better resolution, as the MN needed to be hollow so that the encapsulated cells could pass through them. In the MN printed by FDM, the printer had to be good to print micrometric MN, but they did not need to be hollow, since when applying these MN they need to penetrate and break to release the active component.

If we are going to analyze cell bioprinting for wound healing, which printer is the most efficient? Again, the answer is it depends on the purpose. The inkjet printer is a good option, as it has a good printing capacity; it is even very useful for cell printing because its printing is based on droplets, but depending on the type of inkjet printer, it can decrease cell viability due to heating. While the PAM printer can affect cell viability due to the use of printing needles, it has the advantage of printhead temperature control. Printed based inkjet technology has been shown to produce a more regulated product, while FDM has shown metering accuracy in its printed products due to the high resolution in these 3DP. The commonly used printing techniques are printed based inkjet and extrusion-based system, probably because they have advantages that outweigh the disadvantages, which make them interesting as they are practical and easy to implement.

Benefits that 3D-Printed Products Bring to the Patient

The wound healing treatments provided nowadays are designed as a simple non-drug dressing and that in some cases this type of treatment worsens the lesion. 3D-printed products have been shown to be effective during all stages of wound healing and can be applied as a new type of treatment. 3D printing has proven to be versatile in printing a variety of secure systems, including *in situ* equivalent tissue and cell printing (Fig. 5). Furthermore, it was able to overcome several limitations presented by traditional dressing, by improving their physical and mechanical properties and promoting an ideal environment for wound healing to occur.

In this sense, the manufacture of 3D-printed products is a promising tool in wound healing, due to its versatility and ability to offer different synthesis methodologies using a wide variety of materials, active compounds and their combinations. This therapeutic approach offers a personalized treatment to the patient capable of accelerating wound healing and protecting against infections, external agents, and excessive inflammation. The 3D printer makes it possible to print patient-friendly multifunctional dressings, as they are systems capable of controlling the release of active compounds.

The main inks and bioinks used are biodegradable hydrogels, based on natural and/or synthetic polymers, which favor the printing of different geometries and sizes, without harming the lesion site and causing pain for the patient. This diversity of the printed product (scaffolds, tissue, patches, biofilm, etc.) promotes individualized therapies for different wounds, by favoring an adequate dosage and promoting tissue regeneration, and yet, they are able to keep the microenvironment moist. Biomimetic materials are effective in mimicking the structure of the skin. In combination with stem cells, polymers, and growth factors, they favor the reduction of wound healing time and consequently, the cost for the patient.

Providing a personalized medicine, 3D-printed materials reduce both costs and waste of time and resources. In addition, it lessens the difficulties associated with physical storage and has positive implications for economic, environmental (less pollution, smoke, and waste), scientific, and political issues. The positive point of additive manufacturing is that the process of a printed product is very simple and has become very widespread, including in homes. Taking into account the patient clinical needs, the type and size of the

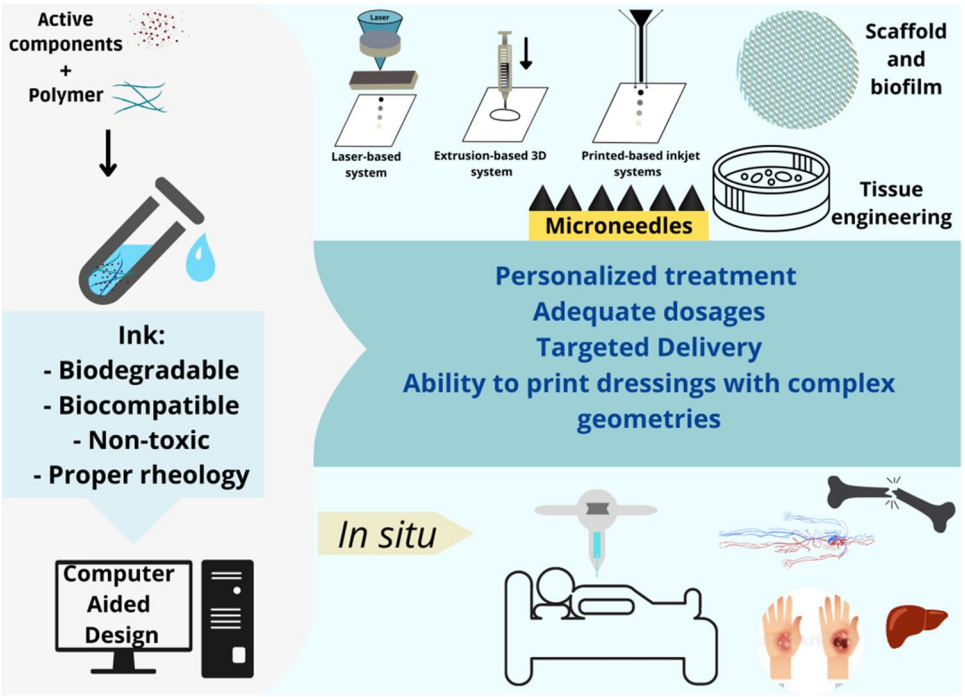

Fig. 5 Schematic representation of 3D printing process, types, and applications

wound, it would be possible to generate more personalized treatments that would demonstrate better clinical efficacy.

Regulatory and Future Perspectives

Traditional drug manufacturing methods are becoming obsolete with the evolution of 3D printing. In light of what has happened during recent years, the COVID-19 pandemic has exponentially increased the use of 3D printers. Its increase was visible mainly in the health area and among professionals, as they saw the need to print equipment necessary to protect the lives of workers and society in general. In addition, 3D printing specialist provided their services to relieve pressure on governments and supply chains [185]. This increase in the production of 3D-printed products has led the FDA to study more about additive manufacturing in different fields. For information about production using additive manufacturing, the FDA provides a "Technical Considerations for Additive Manufactured Medical Devices" and "Guidance for Industry and Food and Drug Administration" [186]. Faced with the wonders of 3D printing, challenges have arisen mainly in terms of intellectual protection [185, 187, 188]. This guidance only gives an initial idea about the technical considerations of 3D printers, and it is clear that some parameters will have to be reflected to ensure the success of your applications, as well as the quality control of the entire process through to the final product.

Nanomaterials are commonly used in additive manufacturing, but the active properties of these materials make risk assessment and even regulation of these materials difficult [189]. Ensuring protection due to the exposure of these materials to humans and the environment is challenging for regulatory authorities. For this reason, regulatory agencies in the USA, Canada, and Europe are, in most cases, requiring additional information from the industries specific to these materials used in 3D printing. Implementing this technology in the market will not be easy; there will be obstacles to be faced by regulatory authorities that will have to define and establish effective strategies, laws, and guidelines to control the manufacture and consumption of these printed medicines. In the same way that conventional medicines have stringent regulatory requirements to be sold, products manufactured by 3D printing must go through the same regulatory process to assure patients of the safety, efficacy, and stability of these products. Efforts are being made to standardize additive manufacturing processes by ISO/TC 261. Furthermore, it aims to standardize the entire printing process chain (from hardware and software to its applications), testing procedures, quality parameters, inputs, environment, health and safety, fundamentals, and vocabularies [190]. While such regulations promote patient safety, they often stand in the way of modern technological advances.

Although customized on-demand products present themselves as a good alternative in wound care, it will hardly compete with the mass production of the pharmaceutical industry. To overcome this limitation, a strategy has been discussed to accelerate large-scale production using 3D printers and is being called 3D printer farms. Basically, it is a set of 3D printers, producing the same product in one

room. In order to be produced on demand in pharmacies, hospitals, and health services, in addition to the regulatory issues that need to be defined, many rounds of discussion on some ethical issues and product stability will have to be carried out.

While regulations for 3D-printed medicines are ongoing, a new DDS strategy is being touted as the new revolution in the additive manufacturing, called 4-D printing (4DP). 4DP originates from a 3D-printed object that transforms into a different structure from the original through an environmental stimulus such as temperature, light, or other factors [191]. This new DDS system employs smart biomaterials in its bioinks capable of responding to cellular stimuli and/or stimuli-responsive materials over time. In recent years, 4DP has attracted attention not only in the medical and pharmaceutical field but also in the engineering field (construction, materials, and robotics) as a new technology in which components can transform into a new form under favorable conditions. As Nam and Pei (2019) reported, the biggest advantage of using 4DP is the size reduction due to the computational fold that can be achieved [192]. Unlike 3D printing, large parts can be 4-D printed due to the ability and ease of bending or even compressing them. It is expected that in the near future this technology will be used in the health area for the manufacture of tissue, valves, and organs. It is a promising technology that mimics the organization and functionality of the place to be treated, but presents challenges to be overcome.

Concluding Remarks

A considerable and increasing number of studies about 3D printing technology for pharmaceutical and biomedical applications have been observed in the last decade. The feasibility and advantages of this technology in the development of novel dosage forms and modified DDS in the treatment of wound healing have also been demonstrated. 3D printing technology can improve wound closure quickly and effectively, due to its ability to offer different manufacturing strategies, through a variety of methods, materials, and printers. However, it is essential to choose an effective material to be printed. Bioink constitutes an interesting direction and exhibits properties capable of accelerating the healing process and providing the best therapeutic approach. Hydrogel systems also can be printed and constitute an important strategy, able to maintain the microstructure of the prevascularized wound and, when used as scaffolds, present self-healing characteristics. Despite few studies carried out, *in situ* bioprinting has shown promise in the regeneration of injured skin, which can be used for printing tissues and/or deliver the drug in a controlled manner. Furthermore, the 3D-printed dosage form provides the personalization and individualization of an effective and comfortable therapy for the patient, presenting an appropriate release profile with personalized pore structure, size, dosage, and shape. Although there are still many challenges for 3D printing of dosage forms, advances in technology, science, and healthcare will undoubtedly drive 3D printing forward and meet the need in wound care each more.

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Declarations

Conflict of Interest The authors declare no competing interests.

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