REVIEW ARTICLE

IJAMB JOURNALAMB.COM
INTERNATIONAL JOURNAL OF ADVANCES IN MEDICAL BIOTECHNOLOGY

Bioprinting for skin: current approaches, technological advancements and the role of artificial intelligence

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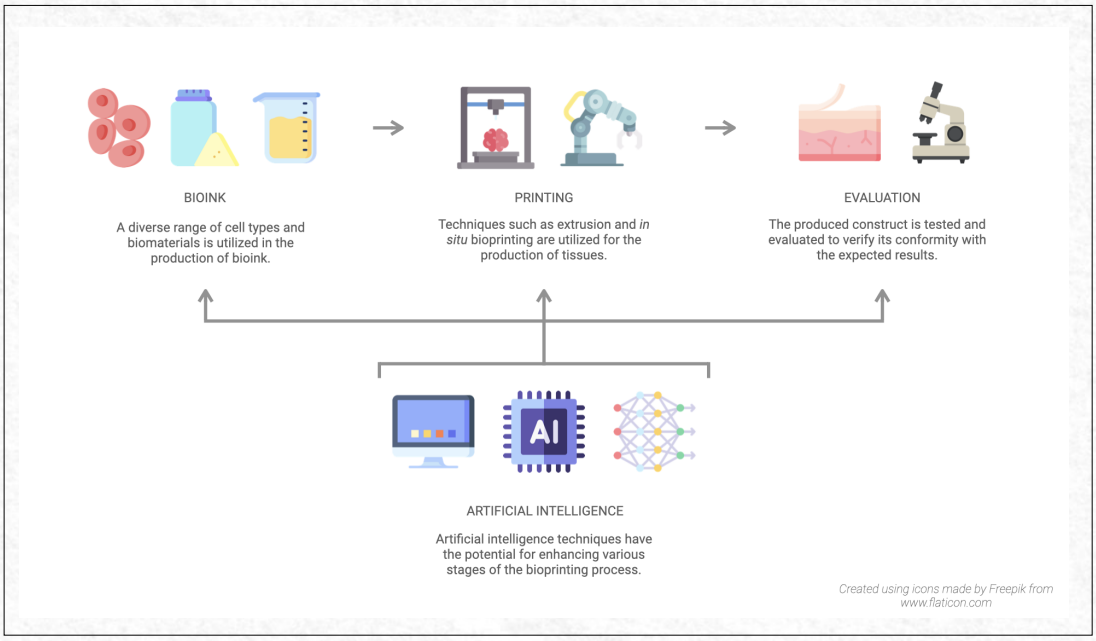
Received: October 2024; Accepted: December 2024.



Abstract: Bioprinting is a technique adapted from 3D printing to create biological constructs, including high-quality skin substitutes. It matches or exceeds the quality of traditional fabrication methods, offering precision, consistency and speed, critical attributes for large-scale production. A variety of materials are used, most of them natural, such as alginate, chitosan and gelatin, with cells incorporated into the bioink. These cells may belong to the replicated tissue or include stem cells that can differentiate into the desired cell types. Bioprinting enables precise placement of the skin's layers: hypodermis, dermis and epidermis, allowing for replication of the skin's complex architecture. Notably, bioprinted skin constructs can closely resemble native tissue, even forming structures like hair follicles and glands as the incorporated cells grow, migrate and differentiate. Artificial intelligence (AI) and machine learning (ML) have recently been applied to enhance efficiency, precision and success. AI tools reduce trial and error by optimizing parameters, bioink composition and quality control. This review explores bioprinting methods, materials and advancements, including in situ bioprinting, the use of robotic devices and the emerging role of artificial intelligence.

Keywords: Biomaterials. Three-dimensional (3D) culture. Regenerative Medicine. Tissue Engineering.

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https://doi.org/10.52466/ijamb.v6i2.135

Introduction

Bioprinting is a promising and rapidly evolving technique capable of assembling high-quality tissue types, including skin substitutes. It is an adaptation of 3D printing to produce biological constructs. It matches the quality of skin constructs built using traditional methods,[1] and even surpasses them, offering advantages in terms of precision, consistency and speed, key factors for mass production. It has been shown not to affect the capacity for cells growth or the biocompatibility with materials.[2] Traditional 3D printing uses a variety of materials, with artificial polymers being the most common; however, when printing biological constructs, and especially skin, natural materials such as alginate, chitosan and gelatin are the most frequently chosen. In bioprinting, cells are incorporated into the ink, which is then referred to as bioink.[3] These cells may be composed of types that belong to the tissue being replicated or not; for example, stem/stromal cells can be used, which differentiate into the desired cell types under the guidance of specific chemical or physical cues. Moreover, sometimes cells are incorporated not only for their structural role in the tissue, but also for additional benefits in supporting graft maturation.

Skin is a complex organ composed of multiple cell types organized in distinct layers. The structure of skin, with its different layers, is shown in Figure 1. It is the largest human organ and serves various functions, including thermoregulation, immune defense and sensory input. One of the goals of bioprinting skin is to replicate its layers, namely the hypodermis, dermis and epidermis (which are themselves further subdivided), to ensure that specialized cells can perform specific tasks that are highly dependent on the skin's intricate architecture. Bioprinting offers the ability to print each layer of the skin individually in a controlled manner using different materials and cell populations for each layer, thus closely mimicking both the cellular and the architectural makeup of real skin.

It has already been demonstrated that when cells and biomaterials are incorporated in the printing process the cells are able to organize themselves into layers similarly to real skin. More impressively,

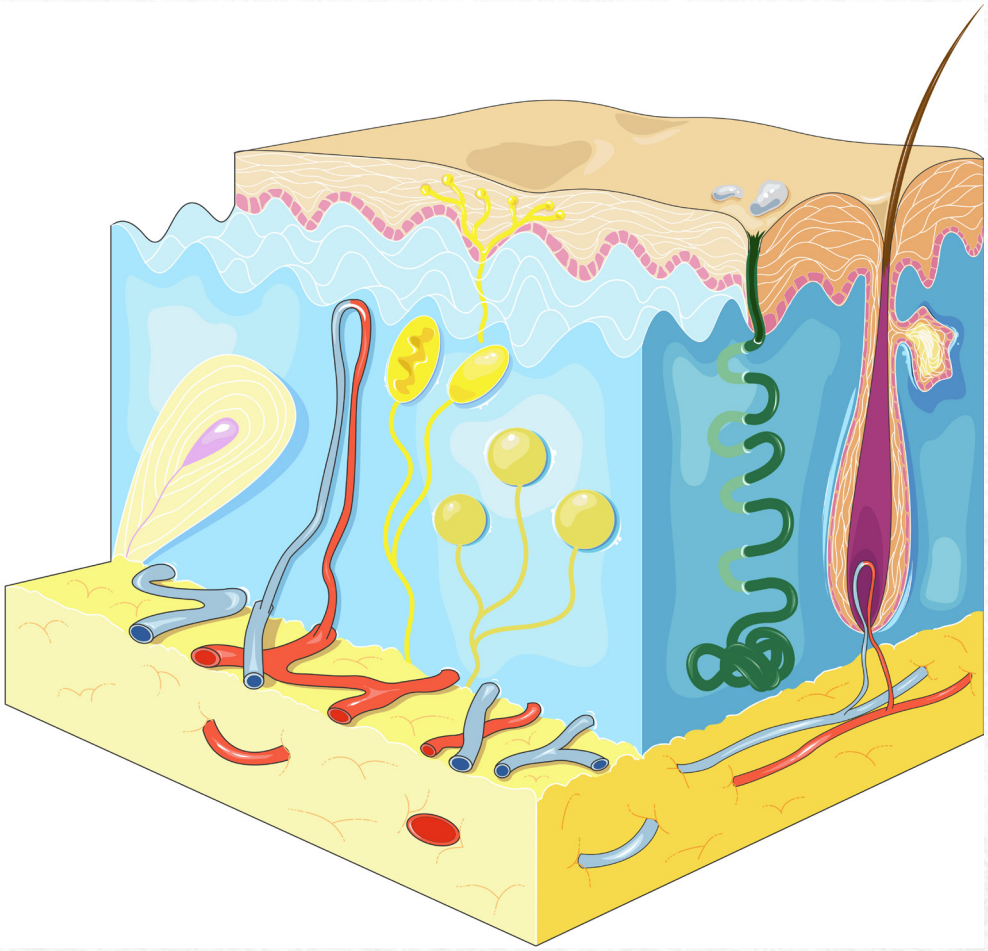

Figure 1 - A representation of skin. Structures shown include a hair follicle, an eccrine sweat gland, cutaneous receptors and the vascular network. Dermis is shown in blue, epidermis is above it, and hypodermis is below it. "Skin icon" by Servier https://smart.servier.com/ licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/

some bioprinted constructs resemble native tissue to the point of forming specialized structures such as hair follicles and sebaceous glands.[4–7] This is possible because the cells in the bioink are alive and dynamic. The final product is not fully realized immediately after the printing process; instead, the cells continue to grow, migrate and differentiate over several days, forming new structures and maturing into their final positions and functions.

Recently, artificial intelligence (AI), particularly its subfield of machine learning (ML), has been integrated into bioprinting as a set of tools aimed at enhancing efficiency, precision and overall success. These AI tools, grounded in statistics and linear algebra, have the capacity for handling complex datasets, including those with non-linear and irregular distributions, developing predictive models, classifying data and offering valuable insights. AI can thus help address the multitude of parameters involved in bioprinting, which often necessitate trial-and-error experiments to have their optimal values determined. By reducing the need for extensive experimentation, AI makes the overall process more efficient. Other applications include fine-tuning bioink composition, ensuring quality control, and even enabling fully automated bioprinting. AI tools can also be used alongside bioprinting for applications such as drug discovery, cancer research and cell classification.

This review explores the literature on bioprinting skin, focusing on the different printing methods, materials and the types of cells incorporated into bioinks. Additionally, it examines recent advances in artificial intelligence and machine learning and their contributions to bioprinting. At the end, there is a selection of the diverse uses of bioprinted skin, which may serve, for example, as a model for basic research or as a transplantable graft.[8] These new technologies are not only improving the printing process itself but are also transforming the field of biofabrication as a whole.

Bioprinting methods

There are various methods used in bioprinting, most of which are adapted from traditional 3D printing. Setting it apart from other biofabrication methods, bioprinting allows for the construction of printed tissue with all the components (materials, cells and additional factors) in a layer-by-layer fashion, eliminating the need for a prior scaffold to be seeded with the appropriate cells at a different time.

The feasibility of printing skin without a scaffold was demonstrated in a 2017 study with the printing of a construct in the shape of a human ear.[9] Researchers employed an extrusion method to print a bioink composed of alginate, gelatin and fibrinogen onto a refrigerated support.

An overview of the process of bioprinting is shown in Figure 2.

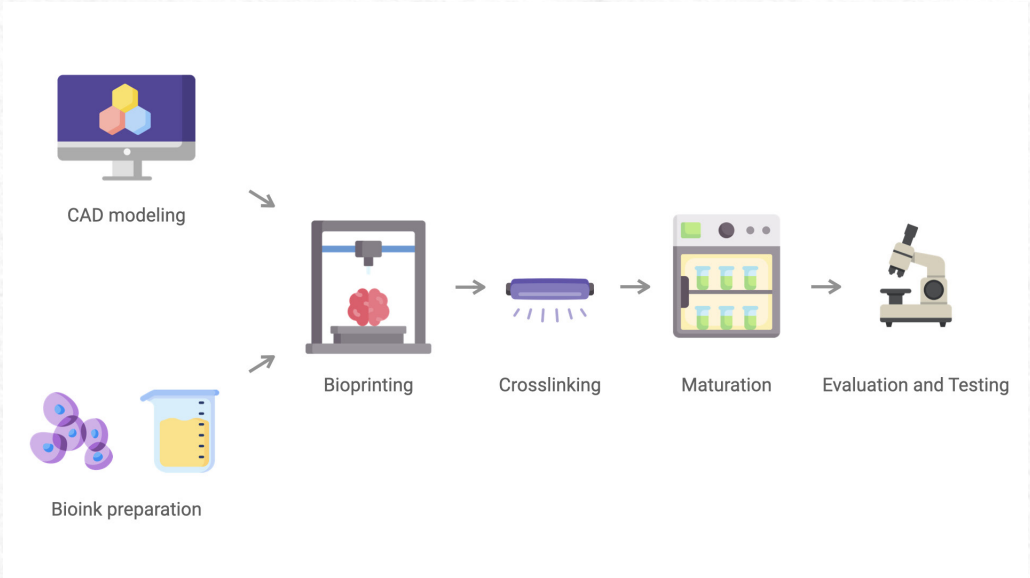

Figure 2 - An overview of bioprinting. First, a computer-aided design (CAD) 3D model is created, and from it, printing instructions are generated for the bioprinter in the form of a G-code. A bioink is prepared by mixing biomaterials and cells. The bioprinter prints using the bioink, following the instructions in the G-code. An optional crosslinking or crosspolymerization step, represented here by an ultraviolet lamp, may follow. The printed construct then undergoes a maturation process, which may last several days. Finally, the construct is evaluated and tested to verify that it has the desired characteristics. Created with icons by Freepik from www.flaticon.com.

Extrusion-based bioprinting

Extrusion is a popular method used in bioprinting and is also one of the most widely employed techniques in 3D printing overall. In this method, the bioink is loaded into a container, such as a cartridge or a syringe, and extruded through a nozzle, often a needle. To propel the bioink through the nozzle, various methods can be applied, including a plunger and air pressure. This versatile technique accommodates a wide range of bioinks, provided they are adequately viscous to be extruded. Precise control over both the extrusion rate and the movement of the nozzle allows for the creation of intricate structures.

Extrusion-based methods have been widely used to bioprint skin constructs. For example, researchers have used extruded bioinks containing fibroblasts and keratinocytes derived from skin biopsies to print bilayer skin substitutes.[10] One study used extrusion bioprinting to build a scaffold of alginate-gelatin-collagen that had a porous structure to promote perfusion of oxygen and nutrients.[11] Another group generated an oriented anisotropic microporous structure.[12] Aligned microarchitectures have an impact on fibroblast-to-myofibroblast transition. The oriented micropores helped with cell spreading and adhesion.

Despite its popularity and versatility, extrusion bioprinting has some limitations. One challenge when bioprinting skin is the difficulty of producing air-exposed cellular monolayers. In traditional extrusion techniques, cells are submerged within the bioink, which may cause an impact on cellular layers. A confluent monolayer of basal keratinocytes, for example, is an important element when mimicking the natural structure of skin. Ongoing advances in extrusion methods are being developed to address this challenge, such as using a sacrificial gelatin, which in one study lead to significant improvements in epidermal differentiation and stratification.[13]

Drop-on-demand

Drop-on-demand bioprinting uses droplets of the bioink to create complex structures layer-by-layer. The size and placement of the droplets can be precisely tuned, allowing for high resolution of the printed construct and controlled cell placement. A microvalve-based printer, for example, may apply pressure to the printing cartridges and print droplets of the biomaterial onto a receiving surface.[14]

One of the main goals of skin bioprinting is to promote the organization of specialized cell populations into functional structures. A two-step bioprinting strategy utilizing the drop-on-demand printing method has been employed to emulate epidermal melanin units, which are epidermal patterns of melanocytes and keratinocytes.[15]

In one study, piezoelectric inkjet bioprinting was used to bioprint distinct keratinocyte subpopulations within a single skin construct to create a model for studying specific clinical conditions, namely atopic dermatitis and ichthyosis vulgaris.[16] When exposed to a voltage pulse, a piezoelectric actuator undergoes deformation, creating mechanical stress, and, consequently, pressure, which ejects droplets of the bioink from the printhead nozzle in a very controlled manner.

One group employed droplet-based bioprinting to print full-thickness grafts for skin defects intraoperatively in a rat model.[7] The bioprinter had three heads, loaded with a dermal bioink, a hypodermal bioink and a crosslinker.

Light-based techniques

Light-based techniques include laser-assisted bioprinting (LaBP) and light-sheet bioprinting.

The LaBP setup consists of two layers of glass, one of them is coated with material able to absorb a laser (e.g. gold) and attached to a layer of biomaterial.[17] The other one is the receiver glass slide that is mounted below it. The laser goes through the upper glass layer and locally evaporates the laser absorbing layer. The vapor pressure propels the biomaterial to the receiving glass slide. Complex structures can be printed on the receiver glass by changing the relative position between the two glass slides.

Light sheet bioprinting projects the light onto a container of biomaterial that has a photoinitiator, a substance that triggers the solidification or polymerization of the material when exposed to light.[18] By changing the position of this light sheet in relation to the biomaterial, the structure is gradually built layer-by-layer. Due to the thinness and precision of the light sheet, this method allows for high resolution bioprinting.

A laser-assisted bioprinted method, similar to the ones described above, was employed to create an *in vitro* skin model in one study.[19] The authors called it four-dimensional due to the addition of time as the fourth dimension, considering here the maturation time of the printed construct an essential part of the process.

Robot-assisted methods and in situ bioprinting

There is a growing interest in developing new, versatile methods of bioprinting which make use of printers with robotic arms, allowing greater range of movements and adaptation to unusual printing surfaces, especially *in vivo*. In addition, a group utilized a robotic system to bioprint pigmented, pre-vascularized dermal-epidermal skin substitutes

as a proof-of-concept for the implementation of an automatized manufacturing process for the production of skin substitutes.[20]

One approach to uneven surfaces is using a stereotactic technique, which allowed researchers to bioprint a skin substitute *in situ* in mice.[21] Skin wounds in the animals were scanned and identified with binocular cameras that informed the path for a robotic arm to deposit a bioink directly onto the wounds.

Others have also investigated *in situ* bioprinting. It was used to print with a hydrogel containing amniotic fluid-derived stem (AFS) cells and bone marrow-derived mesenchymal stem cells (BMMSCs).[22] One group employed an enzyme-free protocol to mechanically extract keratinocytes and fibroblasts from human skin biopsies and produce a bioink for *in situ* printing.[23]

A mobile printer also printed autologous skin cells *in situ*.[24] The cells used were human dermal fibroblasts (HDF) and human epidermal keratinocytes (HEK). Immunohistochemistry determined that the cells were present in the wound area up to 6 weeks after printing in nu/nu mice. A further study used *in situ* bioprinting assisted by a robotic printer, [5] this time using a GelMA hydrogel loaded with epidermal stem cells (Epi-SCs) and skin-derived precursors (SKPs) obtained from 1-3-day-old C57BL/B6 mice.

The examples above show that multiple methods are being tested, with none of them being universally preferred, not just in relation to the printer, but also with the utilized cells and materials.

Other methods of printing

In addition to the more popular methods, and also as a complement to them, innovative approaches are being explored to improve precision, efficiency and functionality; and to enable printing with materials that may not be feasible otherwise.

One such method is suspended layer additive manufacturing, which was used in one study to create a continuous tri-layered implant closely resembling human skin.[25] This method uses a suspension reservoir: a material, typically a fluid gel, that supports each printed layer while being printed. This allows for greater flexibility when choosing bioinks, because they do not need to exhibit high viscosity or rapid curing to support the printed structure as the shape is held in place by the suspension reservoir.

Cryogenic 3D bioprinting using free-form extrusion has also been investigated.[26] A group used a modified extrusion system paired with a cryogenic platform. The technique consists of extruding the material and quickly cooling it to form porous 3D structures.

To create a hybrid biomedical and electronic skin construct, one group employed microfluidic-regulated 3D bioprinting (MRBP).[27] Tri-layer artificial skin patches were built combining a bioprinted layer of polyurethane and bioactive glass (PU-BG) and electrospun layers of polycaprolactone (PCL) and of polyurethane (PU) with polyacrylic acid. A sensing film was also added to the construct. The skin patch was able to sense pressure and, when put on the wrist of a human volunteer, it could detect pulse waves. The tri-layer construct showed faster healing in a mouse model when compared to the controls.

Crosslinking and crosspolymerization

Some bioinks require an additional step after being printed to achieve the necessary rigidity and the desired mechanical properties. Polymerization establishes the chemical bonds necessary for the formation of long polymer chains and, consequently, solidification. Crosslinking links existing polymer chains, also leading to increased rigidity.

One approach involved extruding bioinks containing alginate onto a calcium-containing substrate, which allows gradient secondary crosslinking and a final result that resembles dermal stiffness. [28] Natural polymers such as gelatin and xanthan gum have been crosslinked with glutaraldehyde.[29] One study used a hyaluronic acid crosslinker in a thiol-ene hydrogel composed of a dextran-based backbone.[18]

Another compound that has been used as a crosslinker is genipin, extracted from gardenia fruits.[30] It is biocompatible and has anti-inflammatory and antibacterial effects. Researchers prepared a hydrogel of chitosan, genipin and polyethylene glycol (PEG), laden with human keratinocytes and dermal fibroblasts, that was used to print layers on a basal layer of alginate. Cell viability in 7 days was over 85% for both cell lines. Other studies also used genipin, producing hydrogels of gelatin combined with polyvinyl alcohol.[2,31]

Photopolymerization and photocrosslinking are techniques that use light to provide the energy, via photons, required for the reactions. These techniques allow the bioink to remain in a liquid state while being extruded through the printer nozzle but solidify rapidly once printed and exposed to light of adequate wavelength. This helps overcome challenges related to the viscoelastic properties of bioinks, which must be fluid enough to be extruded but capable of forming solid structures upon printing. An example of this approach is a study in which a photopolymerizable bioink was formulated using GelMA, silk fibroin methacrylate, and photoactivated platelet releasate (PPR).[32] This combination of

components ensured adequate physicochemical and rheological characteristics both for printing and for the formation of a solid structure after polymerization. A research group combined extrusion with a dual-photo source cross-linking technique, enabling the bioink to achieve the desired stiffness soon after extrusion.[33]

Another method for controlling the rigidity of the final construct is enzyme crosslinking, which employs enzymes as catalysts for the reactions.

One study combined both photocrosslinking and enzyme crosslinking to achieve enhanced mechanical properties.[34] Tyrosinase was added to a hydrogel of GelMA and collagen for its ability to cross-link collagen and its bioactivity in regeneration. Another study also combined two different methods of crosslinking and polymerization.[35] Fibrinogen was combined with alginate in a bioink that was enzymatically polymerized by thrombin and ionically crosslinked using calcium. A third example of mixed methods was the coupling of photocrosslinking and thermosensitive crosslinking.[36] A bioink was prepared using GelMA and hyaluronic acid methacryloyl (HAMA) mixed with decellularized extracellular matrix (dECM), whose thermosensitivity allows crosslinking at 37°C.

Maturation

After the bioprinting, it is crucial to provide the appropriate environment for cells within the construct to mature, migrate and, in the case of stem cells, differentiate. Besides the traditional method of cell culturing in medium in a submerged fashion, others may be employed.

Air-liquid interface (ALI) culture has been used to replicate full-thickness skin.[37] In this method, skin constructs mature in both culture medium and air at the same time. Usually, the structure is mounted on a permeable membrane that allows the bottom part to be nourished by the culture medium, while exposing the top layer to air. This method is frequently used for culturing epithelia, such as the epidermis, due to superior differentiation results when compared to submerged cultures.[38]

Defining shape for printing

One of the main advantages of bioprinting is its ability to produce structures with precise shapes. As a result, ensuring that the printer can accurately replicate anatomical shapes is a key aspect of research aimed at improving the technique.

One approach involves the creation of a virtual model of the structure to be printed using medical diagnostic imaging. A research team demonstrated the feasibility of using computed tomography (CT) scans to guide the design of bioprinted skin.[39] They printed a skin equivalent in the shape of a human face using a bioink of hyaluronic acid, glycerol, gelatin and fibrinogen with HEKs and HDFs. By using CT scans or other 3D images, it is possible to print structures that accurately replicate the anatomical features of the intended recipient, potentially improving the integration of the bioprinted tissue with the host and leading to more functional outcomes.

Vascularization

When printing complex tissue such as skin, one of the primary challenges is to achieve good vascularization so that the cells within the construct receive adequate nourishment. It is difficult to ensure that the transplanted structure integrates properly with the recipient's vascular network. One method is by establishing a vascular network in the construct prior to transplantation because grafts often fail to integrate due to the absence of a vascular network within the dermis.

It is possible to bioprint a pre-vascularized dermal layer. One group printed sequential layers using two bioinks, laden with HDFs and human umbilical vein endothelial cells (HUVEC), in an alternating pattern.[32] Immunofluorescence showed the formation of endothelialized microvascular structures in the dermal layer after 14 days. Another approach was used to bioprint a vascularized dermis in a bilayered skin substitute.[40] The bioink contained human fibroblasts, human endothelial cells and human pericytes. The dermis was printed using two layers of the same bioink intercalated by a sterile polyglycolic acid (PGA) mesh, with the goal of improving mechanical properties.

Endothelial cells (ECs) cultivated from cord blood human endothelial colony-forming cells (HECFCs) can be used for this goal, as shown in another study.[41] These cells self-assembled into endothelial networks after being transplanted to immunodeficient mice. Vascular structures were observed 4 weeks after the engraftment. Zielinska and colleagues co-cultured human dermal microvascular endothelial cells (HDMECs) with human fibroblasts, which lead to the formation of a vascular network of capillaries in two weeks of an *in vitro* culture.[42] In a rat model, anastomosis between the capillaries of human origin and those from the animal were observed 1 week after engraftment.

Biomaterials used in bioinks

As bioprinting technology advances, researchers continue to develop and optimize bioinks that combine multiple natural and synthetic components. These materials not only provide the structural framework required for skin regeneration but also actively promote cellular processes such as adhe-

sion, proliferation and differentiation, making them essential for creating functional, long-lasting skin constructs. The published literature shows that a wide variety of materials have been employed to create functional constructs that mimic human skin. The combination of different materials within the same structure can provide specialized support for different cell populations.

Biomaterials

Biomaterials are materials designed to interact with biological systems, playing an essential role in tissue engineering. With a diversity spanning from natural materials and synthetic polymers to complex compounds, biomaterials offer tailored solutions for various therapeutic and regenerative needs.

Synthetic polymers are sometimes used in bioprinting skin. They are usually more resistant than biological materials, and can thus enhance the structural integrity and mechanical strength of the printed structures. One study employed a porous PU layer as a wound dressing material to give structural support to the dermal and epidermal layers printed onto it.[39] Poly(lactic-co-glycolic) acid (PLGA) is another polymer that has been incorporated into the fabrication of skin substitutes.[43] Patterned nanofibers of polymer films have also been integrated into bioprinted scaffolds to guide cell behavior, providing biological cues that aid in tissue development. In the study by Bian et al., the films composed of electrospun nanofibers of a combination of PLGA and GelMA were used with this goal in mind.[44]

Alginate is a biocompatible naturally occurring polymer derived from the cell walls of brown seaweeds. It is a very popular material in bioprinting due to its availability and biochemical and mechanical characteristics, which allow it to form hydrogels and be chemically modified and mixed with many other compounds. Several studies use alginate in the bioink formulation.[45–49] Figure 3 shows a bioprinter in operation using a hydrogel containing alginate and polyethylene glycol (PEG).

Chitosan is a biocompatible polymer derived from chitin, which is found in the exoskeletons of crustaceans. Characteristics such as its antimicrobial activity and capacity to form hydrogels make it a valuable material in bioprinting. As an alginate, it is also chemically modifiable and blends well with other materials, making it a popular choice for the composition of bioinks.[50–52]

Gelatin is derived from collagen, commonly obtained from animal connective tissue. Its resemblance to extracellular matrix components makes it an excellent choice in bioprinting applications. Its versatility is similar to that of

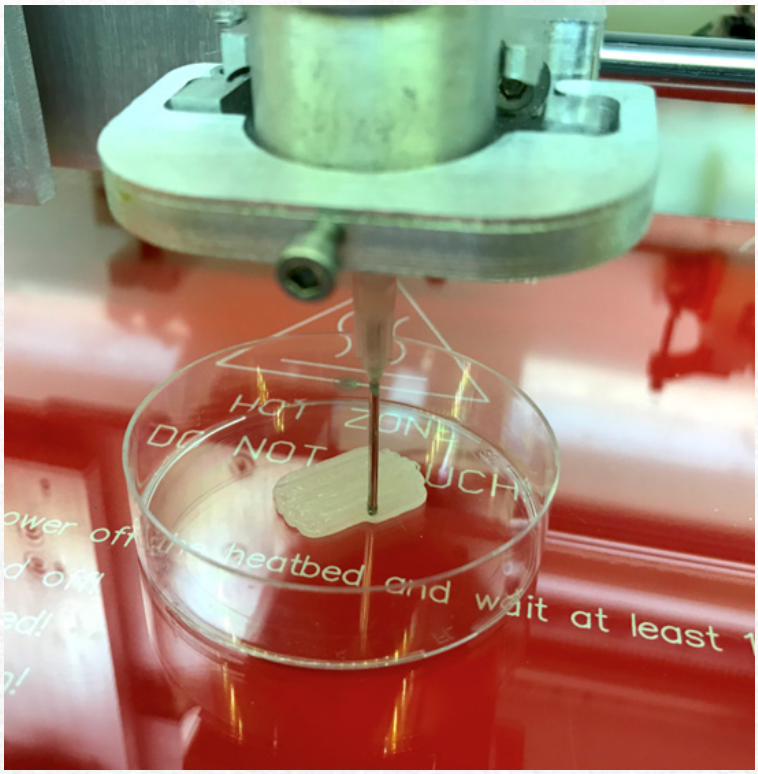

Figure 3 - An extrusion-based bioprinter. The needle extrudes a hydrogel composed of polyethylene glycol (PEG) and alginate into a Petri dish, following a design defined by the researchers. Photograph of the Stem Cell Laboratory, Universidade Federal do Rio Grande do Sul.

alginate and chitosan: it also mixes well with other components and is often subjected to chemical modifications. It provides good support for cell adhesion, proliferation, and differentiation, which explains its high prevalence in bioink formulations. [2,45,46,48–51]

GelMA is a modified gelatin with added methacrylate groups, which allows it to be crosslinked under UV light. It has been widely used as a bioink base due to its biocompatibility, low immunogenicity and the possibility of adjusting physical and chemical properties through different degrees of methacrylation and concentration.[53] Recombinant human type III collagen (rhCol3) has been incorporated into a GelMA bioink to support the bioprinting of skin equivalents.[54] It showed a faster wound healing in a rat model when compared with GelMA alone. GelMA has also been combined with nanocellulose to generate skin constructs with hair follicles and early-stage rete ridge structures.[55] Nanocellulose has also been added to other materials such as gellan gum (GG) and alginate for the fabrication of skin bioinks.[56–58] One study mixed GelMA with dermis-derived decellularized extracellular matrix.[13] GelMA and silk fibroin glycidyl methacrylate (SilMA) were mixed into a bioink that could be photocrosslinked using UV light in one step while combining both materials.[59]

Pectin can also be methacrylated and used to mimic the mechanical properties of the dermal extracellular matrix, presenting cell-adhesive ligands and protease-sensitive domains for tissue development.[60]

Some materials are incorporated into bioinks to promote specific effects during the maturation of the construct. For example, one group added phosphosilicate calcium bioglasses, a type of bioactive glass, to a bioink of alginate and GelMA to stimulate angiogenesis in the printed tissue.[61] Another study developed a salvianolic acid B, alginate and gelatin (SAB-SA-Gel) composite scaffold due to the antioxidant, free-radical scavenging and angiogenic capacities of SAB. These effects were observed when fibroblast-like cells of rat skin (RSI) and HUVECs were cultivated in the scaffolds.[62] In another work, a bi-layered GelMA-gelatin structure, incorporating keratinocytes for the epidermis and fibroblasts with HUVECs for the dermis, was further enhanced with amniotic membrane extract (AME) to promote angiogenesis.[63] The effect of AME in angiogenesis is inconclusive.

Human-derived products including plasma and fibrin are also possible biomaterials for skin biofabrication. A plasma-based bioink has been used to print bilayered skin constructs by combining it with human fibroblasts and keratinocytes to treat burns and other types of wounds.[64] One study used a blend of fibrin and gelatin to produce a "biopaper", a biomimetic hydrogel to serve as a scaffold for a bioink loaded with fibroblasts.[65] Fibrinogen, the precursor of fibrin, was also mixed with gelatin and sodium alginate to produce a hydrogel for bioprinting.[37]

One approach to mimic the extracellular matrix is to create bioinks directly from extracellular matrices, mixing them with hydrogels. For example, one study used microfragmented adipose extracellular matrix (mFAECM) incorporated into a bioink to support skin regeneration.[66] Other studies also used matrices from sources including the adipose tissue and the skin itself.[7,13,67,68] Additionally, decellularized matrices from non-human sources, such as fish and pig skin, have been used, retaining key extracellular matrix components that aid in tissue development.[69,70] One study showed that a fibrinogen hydrogel supplemented with dECM resulted in improved biological, physical and printability properties when compared with unsupplemented hydrogel.[71] One group built scaffolds of dECM, gelatin, quaternized chitosan (QC) and poly(ionic liquid)s (PILs).[72] QC has a modified amino group that confers better antibacterial activity when compared to regular chitosan. PILs are made from the polymerization of ionic liquid monomers; they are biocompatible and have an antibacterial effect.

An interesting addition to bioinks are extracellular vesicles, which are small lipid membrane particles that mediate intercellular communication.[73,74] They are varied and, thus, the composition of the vesicle-loaded bioinks can be tailored to influence diverse local effects.

When creating a bioink, the materials and the cells are usually prepared separately and then mixed. One study developed a novel passive mixing technique to incorporate the cell suspension into highly viscous bioinks.[75] Greater cell viability was observed with the novel mixing method when compared to traditional ones.

Free radical-copolymerization is another unusual technique that was used to produce a skin bioink. Polyethylene oxide (PEO), chitosan and poly(methylmethacrylic acid) (PMMA) were used.[76] The mixture was heated and at 65°C, ammonium persulfate (APS) was added to initiate copolymerization by generating sulfate radicals. After temperature reduction, HDFs were incorporated into the bioink.

Cells

Various types of cells are used in bioprinting skin constructs. Approaches often involve using cell populations derived from the skin, such as keratinocytes and fibroblasts, or stem cells that can differentiate into skin cell types. Different types of cells are

often incorporated into the same skin constructs. One study used six different primary human cells to print three layers of skin.[77]

When using stem cells, one viable source is adipose tissue. Adipose-derived stem cells (ASCs) have been encapsulated in hydrogels to serve as a foundational element in skin regeneration.[28] Another source of stem cells is human amniotic fluid. Amniotic fluid-derived stem (AFS) cells have shown the ability to modulate immune responses. In one study, these cells were suspended in a fibrin-collagen gel, alongside bone marrow-derived mesenchymal stem cells, and printed directly over wound sites, supporting new tissue formation.[22] Another study mixed epidermal stem cells and skin-derived precursors from neonatal mice with Matrigel®, printing them directly onto wounds for skin regeneration.[21] Human platelet lysate (HPL) was added to a bioink with adipose tissue derived mesenchymal stromal cells to potentially increase cell proliferation and tissue healing.[53]

Human endothelial cells, fibroblasts, pericytes and keratinocytes are frequently included in bioinks. One research group isolated and expanded these cells from human skin biopsies, combining them with human collagen type I and human plasma fibronectin to form stratified skin grafts.[40] These grafts exhibited mature epidermal structures in mice models. One study used HDFs in a gelatin-hyaluronan hydrogel, which demonstrated good viability and proliferation.[44] Another study paired a dermal layer with a keratinocyte-laden bioink to form the epidermal layer, which was then exposed to air-liquid interface to promote maturation and stratification.[78]

Other human skin cells, including dermal microvascular endothelial cells, have been bioprinted to create multi-layered skin models, in this case in a fibrinogen-based bioink.[79] Melanocytes have been incorporated into bioinks to produce pigmented bioprinted skin.[15,34,80]

Human foreskin is also a source of cells. One study used both human foreskin dermal fibroblasts and human foreskin keratinocytes for different layers of skin.[41] Primary neonatal keratinocytes and immortalized human keratinocytes have been used in the context of toxicology testing.[81]

HUVEC are sometimes incorporated to promote the formation of a vascular network.[63,68]

Some skin constructs are developed to study specific conditions and mimic pathological patterns in diseased tissue. To achieve this, specially selected cell populations may be used. In one study, researchers printed keratinocyte subpopulations with down-regulated expression of filaggrin, a structural protein, to model atopic dermatitis and ichthyosis vulgaris.[16] Additionally, skin constructs have been developed for cancer research, using bioprinted models of cancerous cells to study tumor growth and treatment responses, such as in the case of squamous cell carcinoma.[82]

Spheroids

The use of spheroids has emerged as a strategy in bioprinting due to their ability to enhance tissue organization and development. Spheroids are compact clusters of cells that, when incorporated into bioinks, can better replicate the complex interactions between different cell types and promote the maturation of tissue.

In one study, researchers produced skin spherical organoids composed of human keratinocytes, fibroblasts and vascular endothelial cells as an initial step for bioprinting.[33] These spheroids were then incorporated into a GelMA hydrogel to form a bioink. Subsequently, in a nude mouse model of full-thickness skin wounds, the bioprinted skin spherical organoids promoted faster healing when compared with pure hydrogels and with hydrogels loaded with cell cultures.

In another study, spheroids containing dermal papilla cells (DPCs), HEKs, human epidermal melanocytes (HEMs) and HUVEC in different combinations were evaluated.[4] DPCs and HUVEC were printed within the dermal layer. Following this, an epidermal layer containing HEKs and HEMs was printed. After maturation, spheroids of DPCs and HUVEC were surrounded by cells that migrated from the epidermal layer, forming hair follicle structures that resembled the native tissue.

Spheroids have also been used to promote the differentiation of hair follicles (HF) alongside sweat glands (SG) within a bioprinted skin construct.[6] HF spheroids were seeded onto bioprinted SG scaffolds made of alginate-gelatin gel and mesenchymal stem cells cultivated in a medium for SG differentiation. The presence of HF spheroids promoted the differentiation of both HF and SG in the construct.

Artificial intelligence and machine learning

AI contributions to printing

Artificial intelligence (AI), particularly its subfield of Machine Learning (ML), encompasses a diverse group of mathematical and computational techniques. Recently, these techniques have been gradually integrated into bioprinting processes to enhance efficiency, precision, parameter optimization and overall outcomes. ML is especially valuable for fine-tuning parameters that traditionally rely on trial-and-error approaches.[83] However, the applications of AI and ML are much more diverse, ranging from relatively simple predictive models to

robotic automation of bioprinting processes.[84] A list of the main ML models is presented in Table 1.

The integration of ML within the domain of bioprinting remains at an early stage, with only a limited number of studies available to date. Preliminary studies, comprised mostly of proof-of-concept demonstrations, are primarily focused on constructs other than skin. Notably, a study employed supervised ML algorithms to test scaffold performance for skin tissue engineering, although it employed electrospun scaffolds rather than bioprinted ones.[85]

The integration of ML within the domain of bioprinting remains at an early stage, with only a limited number of studies available to date. Preliminary studies, comprised mostly of proof-of-concept demonstrations, are primarily focused on constructs other than skin. Notably, a study employed supervised ML algorithms to test scaffold performance for skin tissue engineering, although it employed electrospun scaffolds rather than bioprinted ones.[85] Despite the lack of studies specifically focused on skin constructs, the procedural framework for bioprinting is generally consistent across tissue types. The advancements discussed henceforth highlight the prospect of leveraging ML to enhance various bioprinting processes, potentially benefiting applications tailored to skin bioprinting.

Bioprinting parameters, for example, have been optimized with ML using data collected from published literature.[86] A process traditionally dependent on trial-and-error, parameter optimization was demonstrated in a study that used different AI models in the production of 6-thioguanine (6-TG) loaded PLGA microparticles for bioprinting.[83] Compared to the traditional design of experiments (DoE) methods, AI models showed superior performance by predicting key formulation parameters, thus increasing efficiency. ML techniques, like support vector machines (SVMs), can assist in selecting printing parameters. In one study, an SVM model was employed to reduce the need for extensive experimentation and improve the printability of pluronic hydrogels.[87]

One group developed an AI-based model to improve digital light processing-based bioprinting.[88] This method faces challenges due to light scattering caused by the cells in the bioink, which can disrupt photopolymerization. The developed AI model used data from trial prints to learn and help compensate for these scattering effects, leading to improved consistency and quality in the printed constructs.

Hierarchical machine learning (HML) frameworks have been employed to improve printing fidelity with an alginate hydrogel.[89] This model includes domain knowledge information about physicochemical relationships. Strategies like Gaussian process modeling have been applied to evaluate nozzle geometries that have an impact on shear stress and, consequently, cell viability.[90] A computational fluid dynamics (CFD) model was used to calculate shear stress, thus showing how the coupling of different numerical models can improve prediction capacity. In another example, a learning-based cell injection control (LCIC) model that combined CFD and a multilayer perceptron (MLP) network was used with piezoelectric drop-on-demand printing to eliminate satellite droplets, a common issue with this method.[91]

One of the most interesting applications of artificial intelligence is computer vision (CV). It

Table 1 - Popular ML models. Supervised models are trained using labeled data, that is, they learn to map specific inputs to outputs based on examples. Unsupervised models work with unlabeled data and aim to identify patterns or groupings within the data.

Supervised Learning Models	Unsupervised Learning Models
Linear Regression and Logistic Regression	Hierarchical Clustering
Support Vector Machines	Principal Component Analysis
Neural Networks	Independent Component Analysis
Naive Bayes	K-Means Clustering
Gradient Boosting Machines (e.g. XGB)	Gaussian Mixture Models
Gaussian Processes	Association Rule Learning (Apriori, Eclat)
Decision Trees	Self-Organizing Maps
Random Forests	
K-Nearest Neighbors	

allows for the analysis of images produced by cameras, thus facilitating data extraction and the use of complex data. One group developed an adaptive printing system that integrated real-time feedback control to a robotic printer through a computer vision model.[84] This setup allowed bioprinting on dynamic freeform surfaces, e.g. a moving hand, enabling more precise and flexible bioprinting. In another study, AI-based control loops, including a convolutional neural network (CNN), were developed to automatically adjust printing parameters and monitor the process in real time, leading to a reduction of material waste.[92] Other studies have similarly employed computer vision as a means of detecting deviation of the printing trajectory from the reference and correcting it.[93–95]

Additionally, ML-based anomaly detection systems informed by other sensors are being developed. The CNN in one study was particularly good at detecting nonuniformity.[96]

AI contributions to bioink fabrication

Besides improving the action of printing itself, AI techniques can assist in identifying and producing the best materials and bioinks for bioprinting. These methods can link bioink characteristics and fabrication parameters with outcomes such as cell viability and the mechanical properties of the final construct. Neural networks (NN), for instance, can integrate data from laboratory experiments and literature to correlate bioink parameters, for example, concentration, with the desired features of bioprinted constructs.[97] An ML algorithm called extreme gradient boost (XGB) was used to gain insights into how different hydrogel preparation parameters influence stiffness.[98]

One challenge is predicting and ensuring the correct viscosity of the bioink. Traditional predictive models often fall short in accurately doing this. One study employed Bayesian optimization (BO) to predict viscosity with a relatively small data input, achieving good agreement with empirical knowledge.[99] Techniques like this can reduce the waste of valuable materials that would otherwise be spent in repetitive trial-and-error experiments to obtain the correct flow properties.

One group established a relationship between rheological properties of bioinks and their printability using AI-driven methods.[100] They found that a high elastic modulus improves shape fidelity. This finding underscores the potential of AI methods to provide critical insights about the interaction of multiple variables throughout the bioprinting process.

Varied AI contributions

As a collective effort, one of the significant advances in integrating AI and bioprinting is the development of large, open-source datasets of experimentally tested parameters.[101] These datasets can be used to train various AI models which can then be fine-tuned for specific applications.

AI is sometimes paired with bioprinting in larger experimental designs, even when the former does not have a direct impact on the latter. One research group combined them for bacterial classification. They employed an acoustic bioprinting technique to produce droplets with a volume of less than 5 picoliters, with only a few cells in them, at a high rate. High-throughput surface-enhanced Raman spectroscopy (SERS) was then employed in each droplet and coupled with a machine-learning classification model.[102] They were able to achieve highly accurate cell classification.

Still in the realm of high-throughput detection and classification, a study used bioprinted organoids that were imaged using high-speed live cell interferometry (HSLCI); this data was then analyzed using ML algorithms.[103] This pipeline was used, for example, to quantify drug responses in cancer models.

In another innovative example of integrating AI and bioprinting, one group bioprinted artificial skin to study mosquito biting patterns.[104] Mosquito feeding platforms mimicking skin were printed using hydrogels perfused with blood. The developed CV model was able to identify the mosquitos' engorged or non-feeding abdomens with good precision.

Tumor treatment responses have been evaluated using bioprinted patient-derived glioma tissues.[105] Machine learning was able to predict drug efficacy on these constructs.

Uses of bioprinted skin

The main goal of research in skin bioprinting is to produce adequate substitutes for natural tissue that resemble real skin and promote regeneration. However, bioprinted skin constructs have also been used for other applications that are not, at least immediately, related to tissue regeneration; for instance, as an *in vitro* disease model.

Bioprinting was used to create skin models to study aging with a group.[106] Printed samples included microrelief, a natural surface topography of the skin, present from birth, that has an impact on wrinkling. Constructs with skin textures corresponding to different ages were fabricated. Bioprinted skin can serve to test a variety of chemicals, for example, cosmetic products, potential irritants and drugs.[19,32] It may serve as a substitute for animal models in certain tests, without the ethical concerns associated with it. It may also be a platform for training artificial intelligence algorithms, as discus-

sed above.

One group used bioprinted skin to test the permeation of nanocapsules containing quinizarin, a candidate for skin inflammation.[107] Another study combined bioprinting with microfluidic platforms for high-throughput testing of chemicals and drugs on complex skin models.[108] Bioprinted skin models have been employed for large scale toxicology testing in a high-throughput screening platform.[81]

In a study outside the area of disease or toxicology research, one group bioprinted skin constructs that mimicked different skin tones across the Fitzpatrick scale by using polydopamine as a "synthetic melanin".[109] These constructs could be used to study the effect of skin phototypes on biomedical optic devices. As mentioned earlier, bioprinted skin has also been used in mosquito research.[104] This could contribute to the development of repellents or other more effective methods to protect against mosquito-borne diseases.

Conclusion

Skin bioprinting is rapidly developing, driven by advances in areas that are having an impact on techniques, materials, the availability of cells and other factors, contributing to printed constructs that are complex and effective for a variety of uses. Among the innovations, artificial intelligence is playing an expanding role in several steps crucial to bioprinting.

Acknowledgments

Figure 2 and the visual abstract were created using icons made by Freepik from www.flaticon.com.

Dedication

In memory of Dr. Jorge Vicente Lopes da Silva, whose contributions to the field of 3D printing were invaluable. His dedication and passion for science continue to inspire us all.

Support

The Office of Naval Research Global (ONRG Award N62909-21-1-2026) and National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq/Brazil

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