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Review Article

3D Bioprinting for Skin Regeneration: Emerging Biomaterials, Techniques, and Clinical Potential

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ABSTRACT

Skin tissue engineering seeks to fabricate neo-tissue and enhance the integration of host tissue for usage in regenerative medicine. In most cases, biocompatible polymers and growth factors induce tissues in the surroundings of the defect site and also act as scaffolds for the attachment and growth of the transplanted cells. Recently, 3D bioprinting has shown good scope in tissue fabrication using a layer-by-layer technique with structural control through scaffold-based or scaffold-free approaches from micro-scale to macro-scale. This review focuses on the advances in biomaterials of bioprinting techniques and various categories of 3D printing technologies to address important issues in skin tissue engineering by highlighting improvements in scaffold structural fidelity, cellular viability, and integration of vascular and appendage characteristics. Key findings show that bioinks significantly improve cell adhesion and proliferation, while 3D printing techniques enhance structural and functional mimicry of native skin. By combining diverse findings, this review aims to close the identified knowledge gaps and provide a thorough understanding that guides future research and clinical applications.

Keywords: 3D bioprinting, Biomaterials, Skin tissue engineering, Tissue regeneration, Wound healing.

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INTRODUCTION

Skin tissue engineering involves the application of tissue engineering techniques, including the fabrication of scaffolds and the arrangement of cells, to produce skin substitutes designed to restore damaged skin or achieve other therapeutic objectives. *Ex vivo* tissue engineering, as well as *in situ* tissue engineering, are the two categories of tissue engineering. Stem cell isolation from a donor and scattering them on the scaffold external surface in bioreactors to enhance the proliferation of the cells into the desired tissue is the principle of *ex vivo* tissue engineering (Fu *et al*., 2021). This scaffold must have a similar size and shape to the flawed area and be inserted into the intended tissue area, dissolve slowly, and be substituted with recently regenerated tissues. *Ex vivo* tissue engineering has good mechanical properties (Todros *et al*., 2021). *In situ* tissue engineering is a simpler and more convenient method than *ex vivo* tissue engineering, which involves prefabricating a scaffold from biologically compatible biomaterials with particular dimensions and shapes and implanting it directly into the target damaged tissue area without first introducing the seeding cells. It attracts surrounding cells and promotes tissue regeneration (Sun *et al*., 2021). This approach is an alternative to the *ex vivo* procedure, as rejection of implanted scaffolds is avoided and considered to be immunologically compatible (Poudel *et al*., 2022).

First, using a computer, 3D printing may obtain the length, depth, and other details of the trauma site. Based on the collected injury data, the executive end of the 3D printer can then create artificial skin tissues that are specific to the trauma site. This method is exceptionally convenient, timely, and repeatable. Furthermore, 3D printing can achieve *in situ* printing capabilities by directly depositing bioink in the wound layer by layer, depending on its specificity. This development increases the efficiency of deposition and has great potential, especially for military and first aid applications. Lastly, the physical characteristics of 3D-printed scaffolds can be fully autonomous, with traceable control over the size and quantity of pores that support nutrition transport and cell proliferation and differentiation. With the aforementioned benefits, 3D bioprinting is currently thought to be the best method for creating viable tissue-engineered skin *in vitro* (Huang *et al*., 2016). The use of scaffolds and the fabrication process are two important elements that influence the selection of biomaterials. A lot of work has been done on developing and altering biomaterials. Biomaterial refers to a material or structure that interacts with biological processes. It might be of natural origin or produced artificially. In addition to being aseptic, they should be safely absorbed into the tissues of the host and degrade after completing their action, and also be biologically compatible

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to prevent the emergence of an immune response (Li *et al.*, 2018). The role of various biomaterials in skin tissue engineering is listed in Table 1.

OBJECTIVE AND SCOPE OF THE REVIEW

The review main objectives are to classify the various 3D printing technologies used to create skin tissue scaffolds, assess bioink material and their effects on the mechanical and biological characteristics of scaffolds, benchmark bioprinting methods for skin regeneration based on printability, cell viability, and structural fidelity, pinpoint issues and constraints with existing bioprinting strategies for vascularization and skin appendage integration, and explore gaps in research and future directions.

LITERATURE SEARCH METHODOLOGY

A review of reputable databases, including PubMed, Scopus, Web of Science, Google Scholar, and Embase, was conducted to find scientific literature. To find pertinent information, many keyword combinations were used, such as "Biomaterials in 3D printing," "3D printing techniques," "3D printing in skin tissue engineering," "Advancements in 3D printing " These search terms were chosen to encompass relevant research related to advances in 3d printing.

EXTRACTION OF DATA

The data extraction process was designed to directly support the review's objectives by gathering essential study details, including printing modality, technical parameters, bioink composition, and scaffold performance outcomes. By collecting crucial study information, such as printing mode, technical parameters, bioink composition, and scaffold performance outcomes, the data extraction procedure was created to directly support the review's goals. Bioprinting performance was benchmarked across research using printability evaluations, structural fidelity criteria, and viability percentages. Persistent difficulties were identified with the use of information on vascularization techniques and appendage integration initiatives, such as angiogenic factor incorporation. While highlighting gaps in long-term functionality, standardization, and integration, extracted limits and suggested future directions also pointed to new potential. When taken as a whole, these data allow for the methodical comparison and knowledgeable assessment of 3D bioprinting techniques for skin tissue engineering.

APPROACHES USED FOR SKIN REGENERATION

Fundamentally, an ideal engineered skin construct should replicate the intricacies of the original three-dimensional structure and perform the normal physiological skin tissue functions. Additionally, it ought to encourage neovascularization and give the cells there encouraging stimuli in the neighborhood. Lastly, it must be able to integrate into the host with little scarring and provide a regulated inflammatory reaction if implanted *in vivo*. Many solutions have been created in recent years in an attempt to accomplish these objectives. A lot of them entail delivering cells or signals that can promote or take part in tissue restoration. There are various approaches used for tissue engineering. These methods are designed based on the severity of damage caused to the patients. Despite the regeneration capacity of skin when the damage is more intense, different types of skin grafts are used.

The Conventional Skin Grafts

The grafting is done by the surgical method (Ferreira *et al.*, 2006). As the graft covers the damaged area, it protects from infections and regenerates the matrix of the tissues more quickly. These methods include autograft (individual tissues are used), allograft (donor tissue is required for grafting), and xenograft (tissues are taken from animals). Autografts are preferable among all techniques as the chances of rejection are limited, but for patients with severe injuries, as there is a limitation of autologous skin availability, this method can't be adopted. There are chances of rejection in allografts as well as xenografts because the donor is from the same/different species.

The conventional approaches available for skin tissue engineering are listed in Table 2.

3D BIOPRINTING AS AN ADVANCED APPROACH FOR SKIN TISSUE ENGINEERING

3D bioprinting is considered as a breakthrough technique for the fabrication of skin. It is used for direct skin application to the patient for customized wound care. Biomaterials are printed using bioink, which contains living human cells, in a 3D bioprinter. Scaffolds are also used to target tissues in the necessary structure (Yu *et al.*,2016). By using 3D bioprinting, researchers have produced a blood vessel-like layer that mimics skin, preventing the body from rejecting grafted tissue. Using various cell types, 3D bioprinting may be a useful method for reconstructing burns. A distinct product combination is used in skin 3D bioprinting. This method can help to repair skin and enhance a burn patient's functional prognosis by providing physiologically similar tissues. It makes it possible to restore damaged skin with precisely positioned skin cells. 3D-printed skin cuts down the count of surgical treatments and the length of hospital stays needed by patients (Emily *et al.*, 2019). Designing, choosing bio-ink, printing process, and post-processing are the most important steps in this procedure. 3D bioprinting provides an excellent achievement by recreating functional skin. The uniqueness of this technique is to produce patient-specific biomimetic structures.

A 3D computer-aided design model with the intended internal architecture and spatial location of the bioink components is made as a first step toward printed skin construction. The required geometry can be produced using a range of design software programs tailored to 3D printing. Non-invasive imaging methods

are utilized to map the architecture to be printed and scan patient features, including optical coherence tomography (Hani *et al.*, 2009), computed tomography, magnetic resonance imaging, and ultrasound. Magnetic resonance imaging and ultrasound imaging can differentiate between different layers of the skin; they are more frequently used for soft tissue components. The 3D printable stereolithography format is then used to translate the medical images, allowing for the assignment of distinct bioinks to each printed layer or area. For these methods to be consistently useful for patients, their effectiveness depends on variables like image resolution, depth penetration, and cost. Nonetheless, the precision with which multilayered structures may be scanned and converted into printable grafts will surely increase over time and be crucial to the future development of customized tissue-engineered therapies.

Bioink is a fluid or hydrogel made of biomaterials, live cells, and physiologically active ingredients. In the field of skin tissue engineering, biomaterials must be designed with printability, adequate mechanical qualities, and superior biocompatibility in mind. When it comes to bioprinting techniques, printability means the ability of bio-inks to create precise and superior structures. The gelling time, viscosity, and rheological characteristics of biomaterials can all be used to gauge their printability (Cui *et al.*, 2019). Natural biomaterials can promote cell migration and proliferation because they share biological functions with healthy skin tissue. Synthetic materials are produced through industrial processes on a large scale, and also, they also have better mechanical qualities when compared to natural materials. However, synthetic materials are inferior in terms of biological characteristics.

Bioinks are also developed using two types of cells: Skin-derived cells and non-skin-derived cells. Fibroblasts, keratinocytes, melanocytes, adipocytes, Langerhans cells, and epidermal stem cells are examples of cells generated from skin-induced pluripotent stem cells (Li *et al.*,2018), embryonic cells, mesenchymal stem cells, amniotic fluid-derived stem cells (Skardal *et al.*,2012), and others are examples of non-skin-derived cells. The cells are used because cell growth is an essential phase in the biomaterial design process. But it's important to make sure the cells are readily available, non-immunogenic, and suitable for *in vitro* growth. Among all, fibroblasts and keratinocytes are abundant in the dermis and epidermis, so more studies are going on these two cell types. The effective use of such multicellular, multimaterial bioink is necessary to realize the potential for enhanced skin tissue engineering to aid in wound healing or the creation of *in vitro* models. Components of bio-inks are shown in Figure 1.

3D Printing Techniques

Inkjet bioprinting of skin

Continuous inkjet and drop-on-demand are two subtypes of inkjet bioprinting. In this bioprinting method, a combination of cells and bio-ink is deposited into a chamber that adheres to the printhead (Binder *et al.*, 2010). The printhead is deformed during the manufacturing process by the piezoelectric transducer. Tissue constructions are established by spatially specified droplets. Less cost and more cell viability are the advantages of this technique (Xu *et al.*, 2005). However, this approach has many drawbacks, including the difficulty of printing viscous materials, uneven cell distribution, and printhead clogging. Researchers have given inkjet bioprinting less thought in recent years as a result

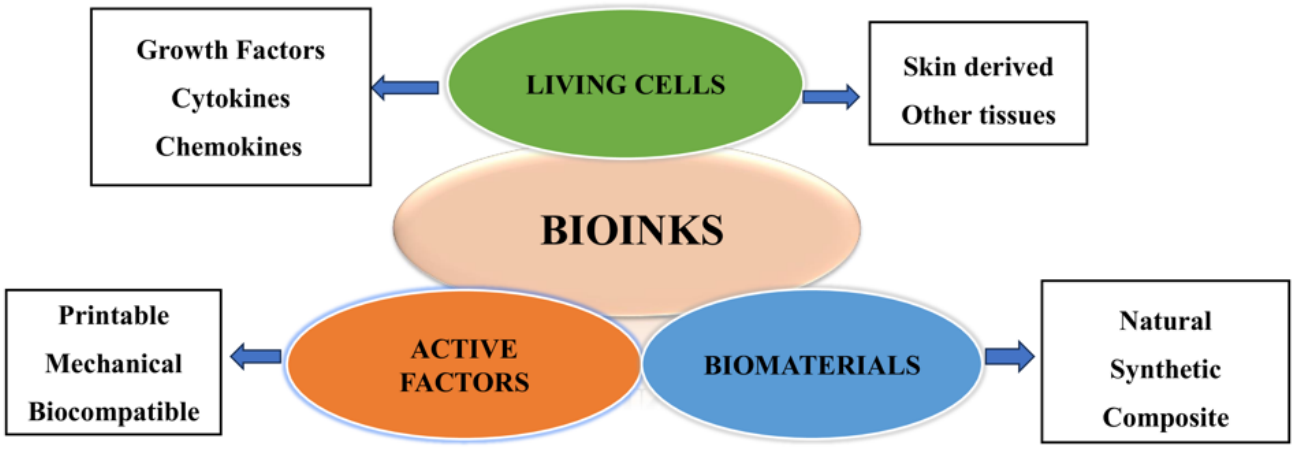

Figure 1: Compositions of the bio-ink components utilized in 3D bioprinting.

of these issues. Compared to drop-on-demand continuous inkjet technique-based bioprinters, which produce drops substantially more quickly. Because of its great precision and little bioink waste, drop-on-demand is more appropriate for deposition and patterning than continuous inkjet (Pepper *et al.*, 2012). To create a spatially diverse tissue structure, drop-on-demand material primarily uses piezoelectric, thermal, or electrostatic forces to create droplets that can precisely and flexibly deposit various biological ingredients (Derby *et al.*, 2010). For *in situ* biological printing, its non-contact printing approach is more suited. In certain investigations, bio-ink containing human keratinocytes and fibrocytes has been directly printed over the full-thickness wound on the back of athymic mice by using drop-on-demand technology. The experimental group's skin grafts accelerated the healing of the wound and decreased the occurrence of skin contracture when compared to the control group, which didn't receive any biological dressing (Saunders *et al.*, 2014).

Laser-Assisted Bioprinting

In the laser-assisted bioprinting bioink layer, a laser energy absorbing layer, a donor, and a collecting layer are four layers typically used to create a structure (Guillemot *et al.*, 2010). A laser beam is projected on the laser energy-absorbing layer throughout the process. A bubble of air is then formed between the donor and bio-ink layers as a result of this layer vaporizing. The bioink, on the collecting layer, is ejected in the required

Table 1: Role of biomaterials in skin tissue engineering.

Name of biomaterial	Role	References
Natural biomaterials		
Acellular matrices	Acellular matrices promote the adhesion of cells and wound healing because they restore collagen, glycoprotein, various growth factors, and other constituents	(Li *et al.*, 2018)
Acellular Dermal matrix	Stimulate the differentiation of endothelial, fibroblast, and epidermal cells	(Ma *et al.*, 2014)
Acellular amniotic membrane	The amniotic membrane has low immunogenicity, is rich in neurotrophic factors and enzymes that aid in wound healing	(Murri *et al.*, 2018)
Proteins		
Collagen	Collagen activates and aggregates fibroblasts and macrophages to assist in the healing process	(Indu *et al.*, 2015)
Gelatin	Gelatin preserves the arginine-glycine aspartic amino acid sequence that enhances cell adherence	(Kim *et al.*, 2011)
Keratin	Keratin support in nerve tissue regeneration, as well as a leucine-aspartic acid-valine cell adhesion sequence	(Vipin *et al.*, 2008)
Fibrin	Fibrin can stimulate angiogenesis, granulation tissue development, and fibroblast differentiation	(Caiado *et al.*, 2011)
Silk Fibroin	Silk fibroin is considered to be non-toxic, and it can nourish the tissues of the nerve and the skin	(Yang *et al.*, 2007)
Polysaccharides		
Chitin and Chitosan	Chitosan helps in cell adhesion and differentiation Cations in chitosan have antibacterial properties, so they may help in the prevention of wound infection	(Revi *et al.*, 2014)
Alginate	When alginate is exposed to the body, a large proportion of D-mannuronic acid residues reduce their tacky texture and triggers immunogenic reactions	(Davidovich *et al.*, 2011)
Synthetic materials		
Polylactic acid	Polylactic acid in scaffolds enhances the healing capacity of full-thickness wounds in rats	(Ghorbani *et al.*, 2018)
COMPOSITE MATERIALS		
Collagen, elastin, polycaprolactone	Enhanced fibroblast and keratinocyte proliferation They also helped in tissue integration and increased angiogenesis in the mouse model in the early stages	(Chong *et al.*, 2019)

quantity when a bubble forms. The process of creating a tissue structure is droplet by droplet (Hakobyan *et al.*, 2014) as shown in Figure 2(a). Moreover, studies conclude that this technology is distinguished by a high level of cell viability (95.1%) and eliminates the blockages. However, it is a highly economical procedure that results in extremely high costs for industry applications (Salah *et al.*, 2020).

Extrusion bioprinting of skin

This technology uses automated machine systems and fluid distribution systems to provide highly regulated printing (Mironov *et al.*, 2003). Pneumatic, piston, or screw drive methods are used to move the bio-ink comprising cells via a micro nozzle as continuous filaments while being controlled by a computer, as shown in Figure 2(b) Layer-by-layer printing creates a finished 3D structure (Shafiee *et al.*, 2016) because they can preserve the filament state after extrusion, shear thinning properties of hydrogels work well in pneumatic-based extrusion bioprinting. Viscosity of bioink printing is possible with the screw-driven structure (Fielding *et al.*, 2012). Newer extrusion bioprinters have many printer heads that allow for the simultaneous depot of various bio-inks with minimum cross-contamination (Ahmed *et al.*, 2016). Additionally, they provide enhanced control over the printed construct's form, porosity, and cell distribution. The extrusion methods have various advantages over laser-assisted bioprinting, like faster printing speeds, a greater variety of bio-printable bio-ink types (cell clumps and acellular matrix components), and enhanced printed product's mechanical strength (Matai *et al.*, 2020). Moreover, by using this method of bionic effects, printing a porous grid structure can be achieved. The most suitable use for this method is the fabrication of scaffolds. The extrusion method has a very low resolution. Typically, the minimal resolution is greater than 100 μm (Liu *et al.*, 2017).

Stereolithography

Rapid prototyping was first established with stereolithography, which was expanded in the late 1980s (Chan *et al.*, 2010) A 2D layer is managed by a laser beam in stereolithography by bioink polymerization, as shown in Figure 3(a). After every layer of a substance is deposited, curing takes place. A photosensitive hydrogel is exposed to visible or UV light while it cures. Until the entire scaffold is finished, the process is performed again and again, covering the former layer, after a particular layer has been polymerized. Polyethene glycol diacrylate and Gelatin methacryloyl (Magalhães *et al.*, 2020) are two hydrogel materials that can be used with this technique. Photo initiators can also be added (Sangermano *et al.*, 2008). To create a high-quality (including resolution) output, several polymerization process parameters, like intensity of light energy, printing speed, layer thickness, and exposure time, should be adjusted. Nevertheless, in contrast to the alternative techniques, the stereolithography procedure is tedious (Van *et al.*, 2012), and transparent liquid must be used, or else light won't pass through the material uniformly, leading to improper crosslinking. Recently, Stereolithography has frequently been used in the printing of scaffolds.

Fused Deposition Modeling

In this technique, the platform's nozzle is used to extrudate a heated coiled polymer filament. Solidification of the polymer takes place when it interacts with the platform (Wunner *et al.*,

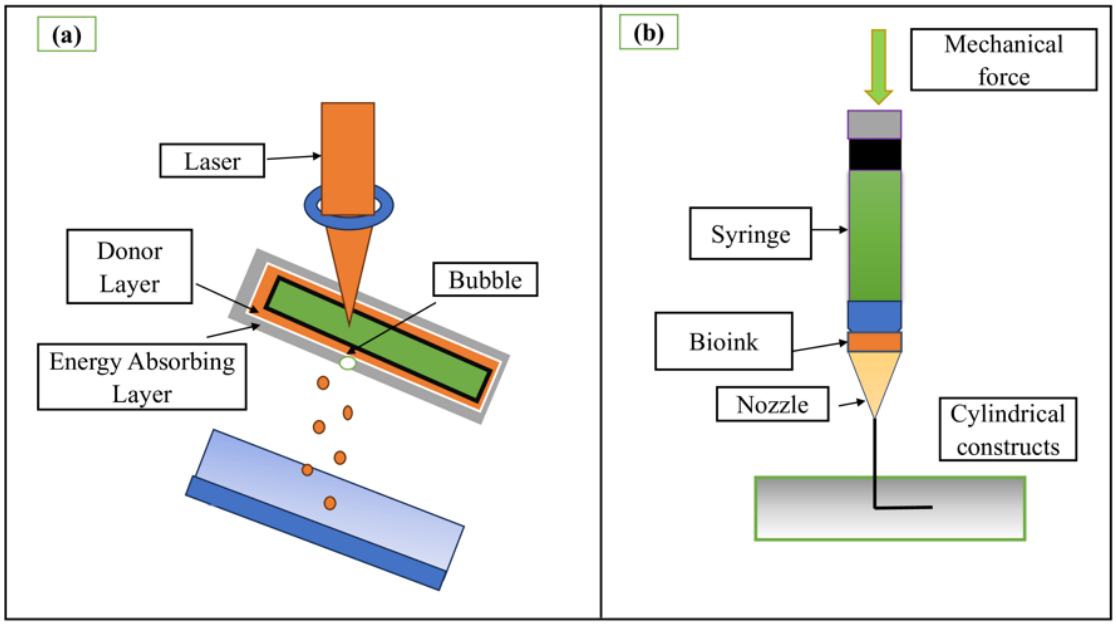

Figure 2: (a) Laser-Assisted Bioprinting;(b) Extrusion Method.

Table 2: Conventional approaches for skin tissue engineering.

Approaches	Description	References
Surgical methods	The surgical method involves growth factors as well as extracellular matrix injection for the healing of wounds and tissue regeneration Technique based on cells and tissues entails utilizing grafts that are comprised entirely of the epidermal cells and skin layers. The graft is prepared by creating interstices in it through meshing, which makes the skin more flexible and also allows the passage of fluids, which in turn enables more effective contact between the graft and the wound bed.	(Vig *et al.*, 2015)
Cell and tissue-based approaches	Epithelial autografts: They are prominent in patients with burn wounds. These autografts are fabricated using keratinocytes.	(Phinney *et al.*, 2015)
	Mesenchymal stem cells: These cells are considered as multipotent cells. They are self-renewable and undergo proliferation. They release substances that are responsible for immunomodulatory effects and extracellular vasculature that contribute to the trophic structure and cell signaling. Research also includes that mesenchymal stem cells can proliferate into endothelial cells, keratinocytes, and skin appendage cells.	(Dreifke, *et al.*, 2015)
	Autologous split-thickness skin graft: These Grafts are fabricated from the dermal or epidermal layers, covering large defect sites by providing skin flexibility and adequate tissue.	(Herskovitz *et al.*, 2016)
Electrospun nanofibers	Nanofibers and yarns obtained from the electrospinning method were utilized as surgical sutures. These suture manufacturing materials can be extracted from the spider's silk or from the silkworms They deliver drugs, cytokines, growth factors, and also bioactive molecules.	(Abbasipour *et al.*, 2013)

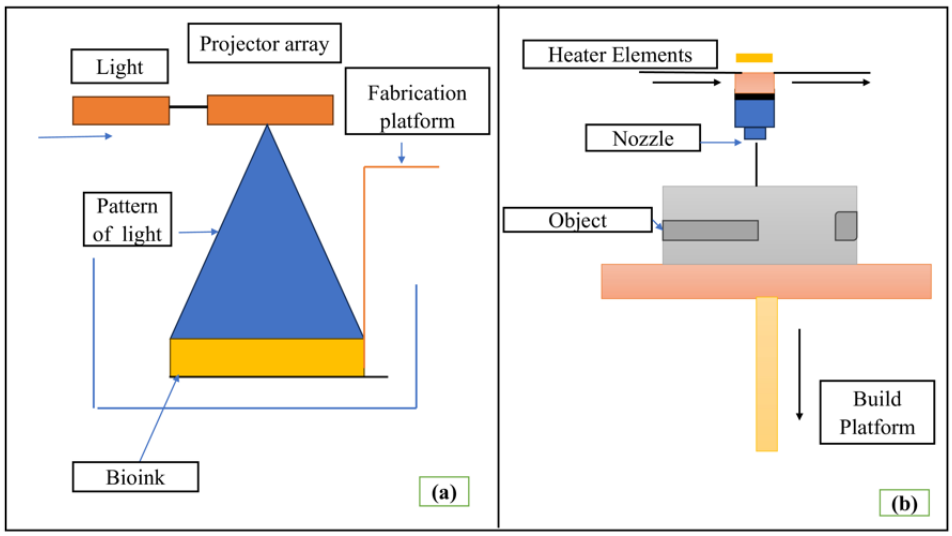

Figure 3: (a) Stereolithography; (b) Fused Deposition Modeling.

2017). The limitation of fused Deposition Modeling printers is spatial resolution and thermal degradation of the polymer. Fused Deposition Modeling permits the application of polycaprolactone and polylactide, which are considered to be thermoresponsive polymers. The selected materials for Fused Deposition Modeling should have high thermal stability. In devices that process polymer granules, thermal deterioration of polymers is a particularly evident issue. Long-term heating causes the polymer to reduce its viscosity, which is necessary for the correct manufacturing process. When using precision extrusion deposition, the material is provided as polymer granules that are extruded through a nozzle under pressure after being thermally plasticized, as shown in Figure 3(b).

Melt-Spinning Melt electrospinning

It is a novel 3D tissue engineering technology. This technique has limitations due to the toxicity of polymeric solutions (Dalton *et al.*, 2007). Solvent residues like chloroform, dimethyl

Table 3: List of outcomes of research in skin tissue engineering.

Printing Technology Type	Bioink Composition and Properties	Scaffold Structural Fidelity	Cellular Viability and Functionality	Vascularization and Appendage Integration	Authors
Extrusion, droplet and laser, light-based methods.	Specialized bioinks with focus on biocompatibility and mechanical properties.	High fidelity with multi-layered skin, including hair follicles and pigmentation.	Maintained cell viability and supported complex skin structures.	Enhanced appendage bioprinting.	(Derma*et al.*, 2024)
Extrusion-based with dual-photo crosslinking.	Optimized bioink had Human keratinocytes, fibroblasts, and endothelial cells.	Personalized organoid shape with dimensions of the wound site.	High cell viability and accelerated wound healing *in vivo.*	Enhanced vascularization and epithelialization were demonstrated.	(Zhang, *et al.*, 2024)
Extrusion and laser	Collagen, chitosan, agarose, and alginate bioinks analysed for properties.	Focus on biomimetic dermal structures; multi-layer complexity discussed.	Cell viability supported by bioink selection.	Vascularization and appendage integration remain challenges.	(Zhao *et al.*, 2024)
GelMA hydrogel extrusion bioprinting.	Gelatin methacrylate hydrogels with good mechanical properties.	High shape fidelity, supported cellular proliferation.	Promotes cell growth and angiogenesis.	Enhanced angiogenesis and microbial infection addressed.	(Hou *et al.*, 2023)
Decellularized ECM-based bioinks.	dECM materials providing native biochemical cues.	High-resolution, reproducible skin architecture was observed.	High cell viability and proliferation Was observed.	Supported wound healing and also vascularization potential.	(Bebian *et al.*, 2023)
Extrusion with ECM-based bioinks.	Placental ECM, sodium-alginate, gelatin biocomposites.	Native-like scaffold morphology and mechanical behaviour.	Non-cytotoxic and promotes cell adhesion and proliferation.	Enhanced angiogenesis and reepithelialization *in vivo*.	(Bashiri *et al.*, 2023)
Multiple bioprinting methods.	Novel biomaterials for stratified skin layers.	Fabrication of epidermis, dermis, and hypodermis, along with appendages, was achieved.	Supported cell viability and function.	Integration of blood vessels, nerves, and appendages was noticed.	(Zhang *et al.*, 2022)
Hydrogel-based extrusion bioprinting.	Synthetic, nanocomposite hydrogels.	Improved mechanical properties and printability.	Supported cell proliferation and survival.	Challenges in vascularization and scaffold stability.	(Yang *et al.*, 2023)
Extrusion bioprinting with recombinant collagen.	RhCol3 composite bioinks enhanced skin formation.	Supported full-thickness skin.	Promoted cell growth	Contributed to wound healing and vascularization.	(Yang *et al.*, 2022)
Extrusion bioprinting with GelMA/HAMA hydrogels.	Gelatin methacryloyl and hyaluronic acid.	Multi-layered skin with hair follicle structures.	Maintained hair inductive potency and cytocompatibility.	Hair follicle development and dermal layers were formed.	(Kang *et al.*, 2022)

Printing Technology Type	Bioink Composition and Properties	Scaffold Structural Fidelity	Cellular Viability and Functionality	Vascularization and Appendage Integration	Authors
Extrusion bioprinting with polysaccharide bioinks.	GelMA combined with ulvan polysaccharide.	Improved mechanical strength and also scaffold stability.	High cell viability and proliferation were observed.	Supported dermal ECM deposition; vascularization potential.	(Chen *et al.*, 2021)
Inkjet, extrusion, laser bioprinting.	For skin and appendages.	High-resolution skin substitutes with vascularization were observed.	Supported cell viability and tissue regeneration.	vascularization and appendage printing were observed.	(Weng *et al.*, 2021)
Inkjet, extrusion, light-based printing.	Diverse biomaterials and cells for skin constructs.	Precise spatial distribution was observed.	Cell viability was maintained, and clinical challenges were noted.	Vascularization was observed.	(Unal *et al.*, 2021)

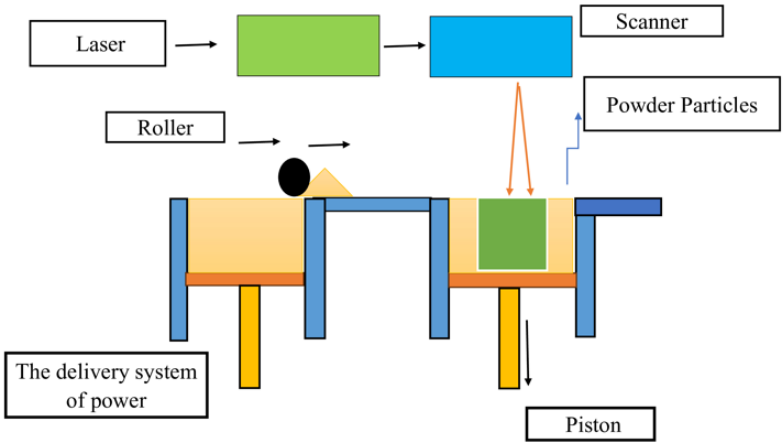

Figure 4: Selective Laser Sintering.

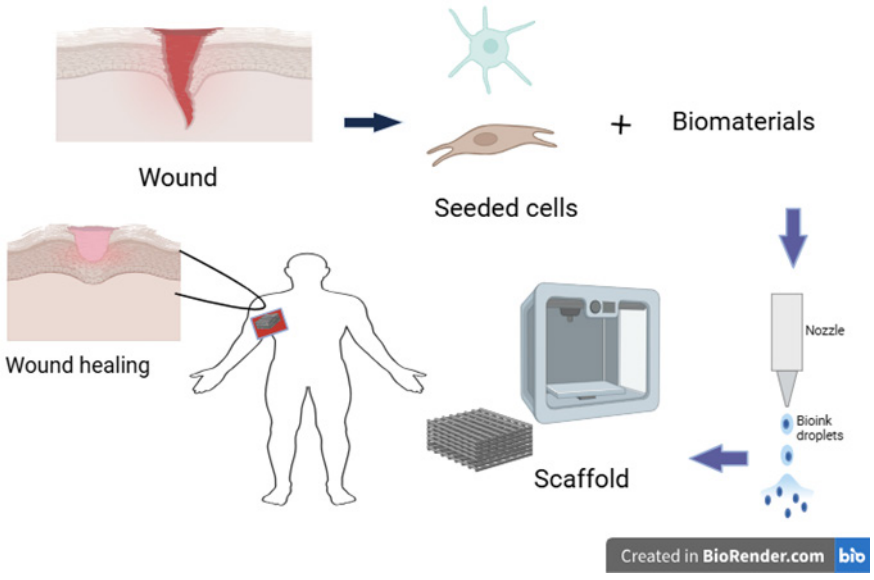

Figure 5: Outline of skin tissue engineering by 3D printing (created by Biorender).

Table 4: Gap areas and future directions of 3D printing research.

Gap Area	Description	Future Research Directions	Authors
Integration of Skin Appendages and Neural Components	The functional integration of appendages like hair follicles, sweat glands, and nerves—essential for skin functionality—is rarely achieved in bioprinted skin models.	Investigate how bioprinted appendages interact with host neuronal and circulatory systems; concentrate on using multi-cellular bioinks and spatial patterning to produce appendage progenitor cells; create techniques for *in vitro* maturation to improve appendage functionality.	(Derman *et al.*, 2024)
Vascularization of Bioprinted Skin Constructs	Graft survival and integration with the host tissue are limited by the ineffective and slow vascularization of current bioprinted skin scaffolds. Current approaches are unable to produce a vascular anastomosis within the crucial post-implantation periods.	Create bioinks and bioprinting techniques that incorporate angiogenic factors or pre-vascularized networks; enhance scaffold architecture to facilitate quick host vessel infiltration.	(Zhang *et al.*, 2022; Kang *et al.*, 2022)
Balancing Bioink Printability and Biocompatibility	The mechanical strength and cell compatibility of many bioinks are compromised; natural polymers are not very printable, and synthetic additions may make them less biocompatible.	Create composite bioinks with adjustable biological and rheological characteristics; investigate cutting-edge crosslinking techniques that maintain cell viability; standardize bioink formulations for repeatability.	(Zhang *et al.*, 2022)
Long-term Stability and Functionality of Bioprinted Constructs	Limited data exist on the long-term viability and mechanical integrity of bioprinted constructs.	Develop bioinks with controlled degradation rates that correspond with tissue regeneration; carry out long-term *in vivo* research evaluating scaffold degradation, immune response, and functional recovery.	(Zhang *et al.*, 2022)
Scalability and Manufacturing Standardization.	Most studies don't focus on scalable, reproducible manufacturing procedures suitable for clinical translation.	Create automated, high-throughput bioprinting systems; set up regulatory-compliant procedures and quality-control measures; improve preservation and transport techniques for bioprinted skin transplants.	(Derman *et al.*, 2024; Zhang *et al.*, 2022)
Regulatory and Ethical Challenges for Clinical Translation	Clinical application is hampered by ethical issues, regulatory approval, and uniformity of cell sources and bioinks.	Establish GMP-compliant biomanufacturing processes; create ethically sourced, well-characterized cell lines; collaborate with regulatory authorities to establish standards.	(Zhang *et al.*, 2022)

sulfoxide, and dimethylformamide, which are used in this method, are toxic to cells that are seeded upon the scaffold. These drawbacks were resolved by the use of the molten polymer in place of the polymer solution. The molten polymer must have an appropriate viscosity to be blasted in an electric field. A revolving drum would collect the molten polymer, but the accurate deposition of fiber in the X and Y axes is made possible by the use of numerical control (Hochleitner *et al.*, 2015). According to recent research on melt electrospinning, continuous fibres with a diameter of less than 1 mM can be deposited, which is similar to the traditional electrospinning solution (Pattanayak *et al.*, 2011).

Selective Laser Sintering

In this technique, powdered polymer particles are slightly heated using a laser beam above the polymer's glass transition temperature (Pattanayak *et al.*, 2011). This results in partial melting of the particles (Lohfeld *et al.*, 2010), so partial fusion is caused by molecular diffusion on the particle surface. Each item layer is fabricated, then the building platform is lowered, and a fresh layer of powdered polymer particles is applied on the upper and the two layers that are joined. Figure 4 illustrates the selective steps of the laser.

Before testing or implantation, construct maturation is the last stage in the fabrication process. Typical methods for creating the air-liquid interaction are to form the layer of keratin by prematuration of the cell-laden scaffold in the proper medium with subsequent deposition of the outermost layer of keratinocytes. After that, the scaffold is lifted out of this medium until the surface reaches the air (Ramadan *et al*., 2016). Bioreactors are considered primary tools for the maturation of vascularized tissue constructs because vascularization is enhanced by flow-induced shear stress (Melchiorri *et al*., 2016). As a result, dynamic flow plays a crucial role in creating full-thickness skin tissue with sufficient vascularization for rapid integration*in vivo*.

RESEARCH OUTCOMES OF SKIN TISSUE ENGINEERING

Most of the researchers on skin tissue engineering in recent days are focusing on wound healing by 3D printing, as outlined in Figure 5. Recent outcomes are enlisted in Table 3.

GAPS AND FUTURE DIRECTIONS IN BIOPRINTED SKIN

The main research gaps that still impede the development and clinical application of bioprinted skin structures are compiled in Table 4.

CONCLUSION

3D printing and bioprinting make it possible to precisely create intricate, biomimetic structures. The fields of tissue engineering and regenerative medicine have undergone a fundamental transformation and capacity to create high-resolution scaffolds customized for a range of biomedical applications. In order to support viable cell encapsulation and tissue formation, the development of adaptable bioinks—which include natural polymers, synthetic polymers, and microbial polysaccharides—has been crucial to maintain a balance between biocompatibility, mechanical properties, and printability. Despite these technological advancements, problems including sustaining cell viability while printing, improving bioink rheology, replicating complex tissue topologies, and scaffold stability still exist. These obstacles need to be removed before the technology can fully convert laboratory achievements into useful clinical solutions.

To fully fulfil the therapeutic and industrial potential of 3D bioprinting, a number of significant research gaps must be filled. The design of bioinks is one important area for improvement. Existing bioinks frequently have trouble achieving the best printability, mechanical durability, and biological functionality at the same time. In order to precisely tune mechanical qualities and degradation rates, future research should concentrate on developing standardized, adaptable bioinks that combine the benefits of natural and synthetic polymers. Furthermore, it is often acknowledged that a crucial bottleneck in bioprinted constructions is the integration of vascular networks. Rapid and stable vascularization appropriate for clinical translation has not yet been fully achieved, despite the potential of co-culture systems, angiogenic bioinks, and multi-material printing techniques in promoting angiogenesis and epithelialization. Even less explored is the integration of nerves, lymphatics, and fully functional appendages, which is a crucial area for the advancement of biomimetic skin regeneration. Ethical issues, manufacturing scalability, regulatory permission, and reproducibility are just a few of the many challenges that clinical translation must overcome. Although bioprinted skin structures have been shown *in vivo* to speed up wound healing and reduce inflammation, fully functional, vascularized, innervated, and appendage-integrated skin substitutes are still mostly in the preclinical stage. In order to enable patient-specific, responsive tissue constructs that may transcend present constraints, emerging developments emphasize the combination of bioartificial tissues, artificial intelligence, and smart bioinks.

By filling in these research gaps through interdisciplinary innovation, 3D bioprinting will move more quickly from experimental technology to commercial and clinical use, ultimately revolutionizing regenerative medicine and customized healthcare.

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ABBREVIATIONS

3D: Three-dimensional; **UV:** Ultraviolet (light); **dECM:** Decellularized extracellular matrix; **ECM:** Extracellular matrix; **GelMA:** Gelatin methacryloyl; **HAMA:** Hyaluronic acid methacryloyl; **RhCol3: R**ecombinant human collagen type III; **GMP:** Good manufacturing practice.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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