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Review Article

3D BIOPRINTING: TECHNIQUES, APPLICATIONS AND FUTURE DIRECTIONS

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ABSTRACT

3D bioprinting represents a transformative approach in tissue engineering and regenerative medicine, enabling the precise fabrication of complex biological structures. This review examines the current state of 3D bioprinting technologies, focusing on their techniques, applications, and future directions. Key bioprinting techniques—including inkjet-based, extrusion-based, and laser-assisted methods—are analysed for their strengths and limitations in terms of resolution, material compatibility, and scalability. The review highlights a range of applications, from the creation of tissue models for drug testing and disease research to the development of bespoke implants and prosthetics. Future directions emphasise the need for advancements in biomaterials, scalability of processes, and regulatory considerations to enhance the clinical translation of 3D bioprinting technologies. This comprehensive review aims to provide insights into the current capabilities and future potential of 3D bioprinting, serving as a guide for researchers and practitioners in the field.

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History

A number of years ago, The rational combination of technologies, such as cell patterning and commercial inkjet printing, was suggested by Thomas, Gabor Forgacs, and Vladimir Mironov, to construct an organ that might be utilized in an organ transplant. Since then, the field of bioprinting has evolved significantly [1].

In the early 1980s, American engineer Charles Hull created the first 3D printer that could produce solid items by depositing a successive layer of acrylic-based photopolymer and simultaneously crosslinking by UV light followed by computer-aided design; this technique is called stereolithography (SLA) [2].

Evolution in the 1980s:

In 1984: SLA was invented.

In 1988: The first use of bioprinting involved the 2D micro-positioning of cells.

Evolution in 1990s:

In 1996: Observations of cells adhering to one another during embryonic development.

In 1999: According to reports, the first use of laser technology revealed two-dimensional patterning in living cells.

Evolution in the 2000s:

In 2001: Using 3D printing, a synthetic structure for a human ladder was made.

In 2002: A bioprinter based on extrusion was first used, and it was later commercialized as a "3D- Bioplotter."

In 2003: An HP ordinary inkjet printer was modified to become the first inkjet bioprinter.

In 2004: 3D tissue made entirely of cells—no scaffold involved.

In 2009: Vascular constructions without scaffolds were made.

Evolution at the 2010s:

In 2012: On animals, in situ bioprinting was accomplished.

In 2015: By coaxial technology Tubular structure was printed.

In 2016: Using an ITOP system, a cartilage model was created by the application of rapid continuous optical 3D printing based on DLP.

In 2019: The first bioprinted cardioid structure was created at Tel Aviv University; FRESH technology was used to construct the human heart's collagen at different scales.

1. Introduction

Tissue engineering is a broad field.[3]. Different conventional methods of tissue engineering include solvent casting and particle leaching, freeze-drying, TIPS, gas foaming and electrospinning, and modern methods include different types of 3D bioprinting methods [4].

3D bioprinting is a process of precise placement of living cells and biomaterials known as bio-ink arranged layer by layer to create complex composite tissue-like structures. It is a microarchitecture of cells,

biomaterials, and polymers that develops artificial tissue substitutes to mimic complex structures [3,5].

It is leading the following fields: [3,6]

a. Despite tremendous progress in the field of tissue engineering there is restricted accessibility to biological structures needed for restoration or transplantation of lost or damaged organs and tissues.
b. provide a suitable substitute for tissue implants and animal testing practices while investigating the causes of diseases and creating new therapeutics.
c. 3. Provide in vitro models to screen medications and gain a better understanding of tissue development. The inherent incapacity of these conventional methods to replicate the intricate microstructures of biological tissues limits their ability to specify the spatial placement and distribution [7]. The 3D bioprinters give an advantage over conventional methods by depositing biomaterials with micrometre precision in suitable conditions and showing efficient management of scaffold construction and cell distribution [8].

1.2. Bioprinting Approaches:

1.2.1 Biomimicry

The first approach of bioprinting is biomimicry. The aim of this approach is the production of exact identical shape and framework of specific tissues and organs' extracellular and cellular components. These biomimicing products are influenced by the materials used in the process and environment of culture [5]. Thus, knowledge of the microenvironment, the biological forces operating within it, the specific arrangement of supporting and functional cell types, solubility variables, and the extracellular matrix's composition are all required[9].

1.2.2 Autonomous assembly

The second method of bioprinting is self-assembling autonomously. It is a method of reproducing biological tissue by emulating the structure and development of embryonic organs. The cellular component of a developing tissue generates its extracellular matrix building blocks and cell signals throughout the early stages of embryo development. These processes enable autonomous organization and patterning to provide the necessary biological function and desired microarchitecture. Self-assembling cellular spheroids are used to create a scaffold-free version that satisfies the self-assembly technique by allowing cell configurations and differentiation to resemble emerging tissue. This self-assembly approach demands detailed knowledge of the cell as the primary catalyst for histogenesis, structural and functional properties of the tissues, mechanism of embryonic tissue

development and the microenvironment [5,6,9].

1.2.3 Mini-tissue

The third strategy Mini tissue building blocks are the result of combining earlier tactics. This technique forms and assembles mini-tissues—small, functional components of tissues and organs—to create a bigger framework [5,6,9].

1.3 Basic steps of 3D bioprinting

The bioprinting of tissue is accomplished in three steps: pre-processing, processing, and post-processing [3].

1.3.1 Pre-processing

The first step of pre-bioprinting begins with selecting materials for the biopsy extraction procedure and creating a scaffold model for 3D printing. To obtain information on the structure and morphology of the targeted tissue, imaging technologies including magnetic resonance imaging (MRI) and computed tomography (CT) are used. To create 3D bioprinting models, the captured images are reconstructed and then transferred to model files, like gcode, that the bioprinter can read. Professional commercial software, such as TradeSync Integration Manager, BioAssemblyBot, and BioCAD, is also offered by certain bioprinting companies. Next, the cells required for the procedure are chosen and multiplied. To maintain their viability, the resulting mass of cells is mixed with oxygen and other nutrients. [10,11].

1.3.2 Processing

The second step starts with forming bioink by mixing cells, nutrients, and matrix together. Once a computer model has been produced to build a three-dimensional structure, this bioink is then deposited onto the printer cartridge.To fabricate 3D cell-laden different 3D bioprinting strategies: Inkjet, Extrusion and Laser based bioprinting. The right bioink selection is essential for successful bioprinting. It would provide the mechanical attributes required to ensure printability and continued functionality during deposition, as well as the necessary attributes for appropriate printing fidelity [9,12].

1.3.3 Post-processing

The final stage of the bioprinting process, known as post bioprinting, is crucial for maintaining stability and allowing cell-laden structures to mature, which supports the formation of desirable tissue constructs.. carefully selected growth and differentiation factors as chemical stimulants to promote particular cell responses including cell division, matrix formation, and tissue differentiation [13]. Tissue engineering requires in vitro mechanical stimulation to rearrange and sustain tissue growth under certain biomechanical settings in vivo. Tissue remodeling and regeneration are significantly aided by the mechanical conditions [14].

The whole process is illustrated in fig.1

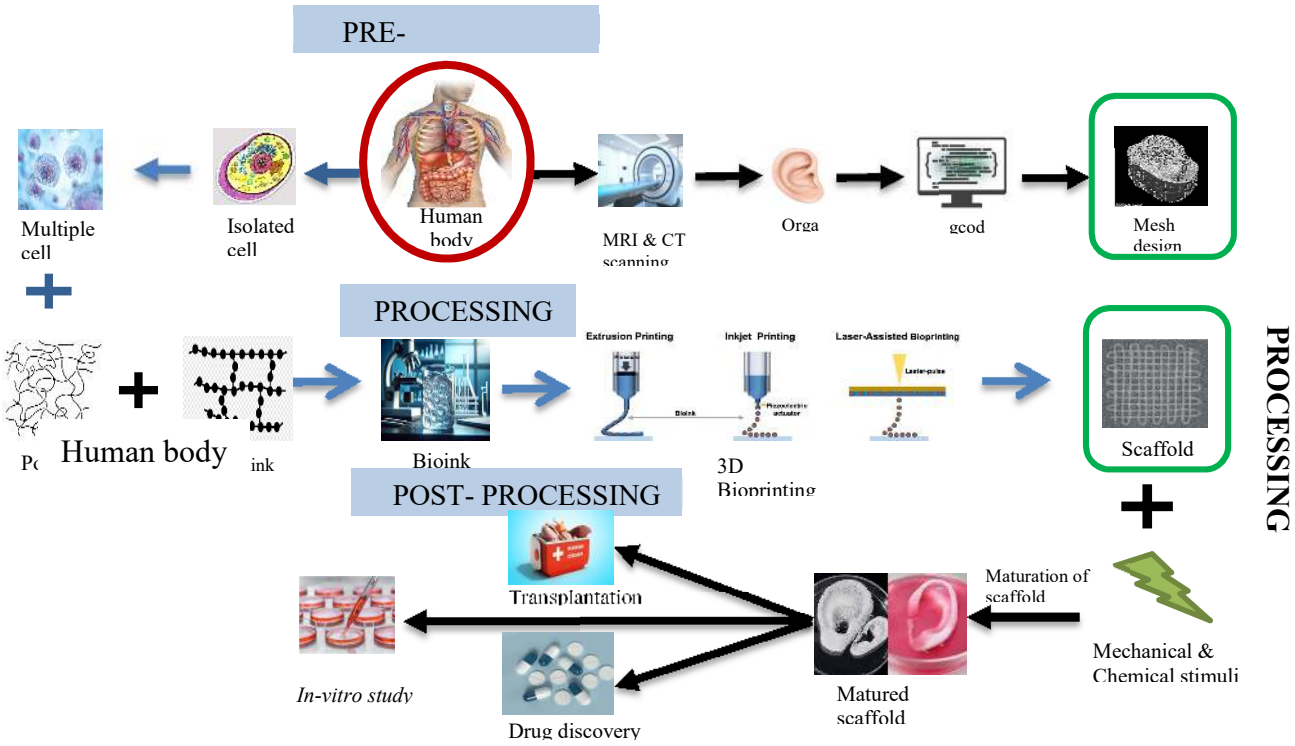

Fig. 1. Processes involved in 3D bioprinting of human tissues. (**1**) Pre-processing: Isolation of human body cells and cultured in vitro. Computed tomography (CT) or magnetic resonance imaging (MRI) were done to get the target tissue's structural details and generate the printing model, such as kidney, bone, and ear; (**2**) Processing: preparation of bioink, 3D bioprinting of scaffolds loaded with cells under the guidance of tissue models from CT or MRI scans; (**3**) Post-processing: Bioreactor culture system for in vitro scaffold maturation to be 3D functional human tissues and potential applications of the 3D bioprinted human tissues[3].

2. Bioink formulation and its properties:

2.1. Biomaterial(ink)

Bioink is a scaffolding substance used in bioprinting that carries living cells and enables their accurate layer-by-layer deposition to produce complex tissue designs. Bioink consists of a biomaterial solution (ink), a fundamental component that provides structure, support, and a conducive environment for the development of cells and tissues and as per targeted tissue in the presence or absence of growth factors. One of the primary obstacles to the 3D bioprinting of cell-filled scaffolds for human tissues is its formulation. The physical and chemical cues of the cell containing the biomaterials are one of the causes of this, which necessitates knowledge of cell physiology and cell-ECM interaction. There are

different natural, artificial and decellularised materials are used as polymers for bioink formulation [15,16].

2 Natural polymers have the same properties as ECM from humans and their innate bioactivity

Ex- Hyaluronic acid, gelatin, collagen, fibronectin, alginate, chitosan, and silk fibroin

- According to physical properties synthetic polymers are tailored for suitable effect.

  Ex-poly (lactic-co-glycolic) (PLGA), polylactide (PLA), polyethylene glycol (PEG), and PCL
- Hybrid biomaterials i.e.; a combination of natural and synthetic mass are used as bioink to get both advantages in combination.

3 Regarding Bioink Decellularized extracellular matrices, or dECM, are a material that is becoming more and more promising since it comprises a variety of ECM components that are particular to different tissues.. It is processed by decellularization process to remove cellular components from tissues and organs eg; urinary elements that are typical of various tissues and, hence, more closely resemble the original tissue. While the mechanical characteristics and shape integrity of the bioprinted 3D construct are compromised by the low viscosity of dECM bioinks, they nevertheless exhibit potential as a bioink. [16,17].

According to the wide variety of hydrogels, the gelation process of bioink has three different crosslinking mechanisms: chemical (ion compound [18], pH [19] physical (temperature [20], light [21], and enzymatic [22] crosslinking to print stable and complex scaffolds [23,24].

2.2. Cell Selection

Researchers have effectively integrated the selection of cells based on the intended function and the targeted tissue or organ or stem cells which have the capacity to self-renew and differentiate into a range of specialized, functional cell types [16]. Autologous cells, or cells obtained from the patient, are suitable for a bioink because they help to prevent immunological rejections [12]. Different types of stem cells can be used to generate human tissues for use in 3D bioprinting procedures, such as human mesenchymal stem cells (hMSCs) [25], adipose-derived stem cells (ASC) [26], and human amniotic fluid-derived stem cells (hAFSC) [17], induced pluripotent cell(iPSC) [27]. Among these principal stem cells, the multipotent iPSCs cause tumorigenesis. While MSCs are not as multipotent, they are still readily obtainable and can be further divided into numerous important cell types, such as cardiac, neural, endothelial, osteoblast, and smooth muscle cells. [28,29].

Besides stem cells, endothelial progenitor cells (EPC) have been seeded in bioink for angiogenesis. Peripheral blood-derived EPCs aid in the MSCs differentiation in vitro, while MSCs promote EPC growth and sustain the cellular networks that are created [30]. To encourage the in vitro differentiation of the stem cells into a desired cell phenotype, certain external supplements are given to the culture medium. As for example, stem cell in vitro differentiation seeded in three-dimensional bioprinted MSC-loaded GelMA/gellan gum scaffolds necessitate adding particular substances known as osteogenic media, such as β-glycerophosphate, ascorbic acid, and dexamethasone, as cells' growth medium. [31]. There has been a lot of interest in the potential of 3D bioprinting techniques to produce many cell types simultaneously and accurately in space. For better chondrogenesis and osteogenesis, chondrocytes and MSCs or MG63 cells have been co-cultured in hydrogels [32,33]. In order to create osteochondral tissue, A multi-head tissue assembly device has been created by Shim et al. to dispense MG63 cells and human chondrocytes separately [33].

2.3. Growth factor selection

Besides stem cells, endothelial cells' bioactive molecule, such as growth factors, which are soluble signalling molecules are added to control the bioink-induced cell growth, proliferation, and differentiation by selective transmembrane receptor binding to target cells [34]. To encourage stem cell differentiation, some of the most widely used growth factors and hormones include transforming growth factor-β (TGF-β) [35], insulin-like growth factors (IGF) [36], bone morphogenetic proteins (BMP) [37], vascular endothelial growth factor (VEGF) [38], and parathyroid hormone (PTH) [39]. It makes sense to employ growth factors to encourage tissue regeneration since they have a strong correlation with the restoration of injured human tissue [40].

Guo et al. described the embryonic development, tissue morphogenesis, cell proliferation and cell differentiation in osteocyte, chondrocyte and osteochondral tissue in the presence or absence of growth factor TGF-β. At last, they confirmed the increasing gene expression of collagen II and aggrecan and decreasing gene expression of collagen I (produced by undifferentiated MSCs) in chondrogenic, osteogenic medium [41]. But in mature non-human primates, TGF-β has only had sporadic success in endochondral bone production. [40]. Osteogenic molecules BMPs, particularly BMP-2, BMP-4, and BMP-7, are extensively used for inducing de novo bone formation in ectopic and orthotropic sites, including critical size defects, where that periodic exposure of PTH can stimulate bone formation in rats

and humans[42,43]. Du et al. created a collagen-binding domain (CBD), which induced the differentiation of MSCs into osteocytes within 14 days more efficiently than the osteogenic media [44]

3. Bioprinting Methods :

The first biomaterials were printed in 1988 when Kleibe et al. used a graphics plotter and an HP inkjet printer to execute the micropositioning of collagen, fibronectin, and cells by using cytoscribing technology [45]. However, the bioprinting revolution began in 1999 when technology especially meant for organ printing was developed at that time. As the new millennium approached, researchers kept modifying conventional printing techniques. Bowland et al. conducted the first inkjet-based bioprinting in 2003 using a modified HP inkjet printer. In 2009, Organon developed the NovoGen MMX printer, which paved the way for bioprinting to become widely available for purchase. Since then, numerous bioprinting tools and techniques have been created and improved [46]. In order to create bio-engineered structures used in regenerative medicine, pharmacokinetics, and fundamental cell biology research, computer-aided transfer processes for patterning and assembly of living and nonliving materials with a specified 2D and 3D organization are currently used to define bioprinting [30].

Commercial 3D bioprinter manufacturers have assessed the characteristics needed for the perfect bioprinting process as the field has grown. Those characteristics: high-speed movement, the ability to disperse several bioinks at once, simplicity of use, a manageable size, ease of sterilization, the capacity to operate entirely on its own, affordability, and adaptability [47]. Despite the fact that all bioprinting techniques yield comparable results, they can still be categorized according to their printing dispensing modalities i.e, the ways in which they print and distribute. Three primary categories comprise the majority of bioprinting modalities: extrusion-based, droplet/inkjet-based [48], and laser-based [49].

3.1. Inkjet based bioprinting-

The first bioprinting method was published in 2003 and was called inkjet bioprinting which is extremely comparable to traditional 2D inkjet printing [50,51]. Out of three primary bioprinting methods, this technique is thought to be the most widely understood. According to the type of flow it is two types: flow continuously (continuous inkjet printing) or drop out from the nozzle (on-demand inkjet printing). Generally, Placing a bioink—a mixture of cells enclosed in a hydrogel pre-polymer solution—into a typical ink cartridge and connecting it to a printer head allows it to build scaffolds using electronic

designs created with CAD software [12]. Both thermal and piezoelectric actuators are used in printer heads to differentiate between setups. Modern methods rely on micro-electromechanical system (MEMS) architectures, which cause a small droplet to deform when the nozzle is opened. The ink cartridge for thermal inkjet systems has a nozzle that holds a thin-film resistor for a heating element. Then, a quick electric pulse is administered, creates heat and causes a bubble to develop. When the pulse ends, the thermal energy is released, the bubble either breaks or expands, and a drop comes out of the nozzle [52]. The largest resolution of these drops is typically about 30 μm, although they can range in size from 10 to 150 μm. in the case of piezoelectric printing an electric charge is applied to piezoelectric crystals, a pulse is produced that creates pressure and results in droplets which are forced out by the vibrations. Therefore, the driving voltage and vibration frequency of the electric signal that operates the actuator influence the droplet deposition velocity [53]. The preferred and more commonly used configuration [12] is thermal inkjet bioprinting [54], as it is a simpler, more cost-effective, and more efficient technology than piezo-electric [55].

Even though we have already covered a lot of the benefits of inkjet-based bioprinting, we will list them all here. First, if they are adapted from commercially available printers, inkjet-based bioprinters may prove to be the most economical bioprinting technique [56]. Second, printing different cell types at once is made possible by parallel print heads, which streamlines the printing process. For instance, A multi-head inkjet printer invented by Weiss et al. enables them to produce a diversified scaffold with a gradient of materials concentration that increases over time [57]. In a similar vein, Wilson and Bowland et al. showed in a different study that up to nine biomaterials could be printed simultaneously [58]. Third, Cell viability with this technique is high (80–90% in experiments). Fourth, Piezoelectric dispensers are very versatile and have good control over droplet formation and positioning and also gives high-throughput and high- resolution results [54]. Another advantage of inkjet printing is that it is generally a noncontact method, which minimizes the chance of contamination and furthermore inexpensiveness, and reproducibility.

Despite its widespread use, inkjet bioprinting is not without its limitations. Thermal setups produce extra heat and shear pressures, which could negatively impact the survival of cells; however, further research is needed to thoroughly investigate this issue. Moreover, viscous bioinks with a maximum viscosity of 0.1

Pa/s cannot be used with MEMs-based printer heads [59]. Therefore, anything thicker may increase the likelihood of clogged printer nozzles. Furthermore, a formerly homogenous bioink mixture gradually starts to split, with the cells within it tending to settle toward the cartridge's bottom [60].

3.1.1. Acoustic bioprinting

A subtype of bioprinting called acoustic bioprinting makes use of an acoustic field instead of a nozzle to drive droplets out of the printer head [61]. One drawback of acoustic printing is the decreasing accuracy of droplet positioning as a result of sporadic acoustic field-induced substrate disruptions. The main advantage of acoustic bioprinting is its soft inclination, which could not work well when printing viscous bioinks with a high cell density. Thus, while helpful in certain situations, acoustic bioprinters are only appropriate for a limited range of applications [62].

3.1.2. Drop on-demand bioprinting

Another subset of inkjet bioprinting techniques is called drop-on-demand (DOD) printing, in which droplets are distributed on the substrate only when needed and not continuously so that it minimizes bioink waste [63]. DOD printers can be made by altering commercial Canon and HP printers, just as conventional inkjet bioprinters. But when printing with viscous bioinks, printheads for DOD are likewise prone to clogging, just like acoustic printers. Furthermore, even with the benefits of employing DOD printers that increase precision, occasionally unwanted secondary satellite droplets are inadvertently expelled after the target droplet. If the printer is made to employ a micro-valve, this issue can be resolved. [62,64].

Three primary issues were resolved by Takagi et al.'s 3D printing design employing DOD technology: the cells' size being significantly larger than pigments, which led to trapped air bubbles, cell sedimentation, and clogging inside the suspension. Although the piezoelectric portion is not novel the authors combined an open-head chamber without a limited flow channel with a bending-type piezoelectric actuator in DOD technology [65].

3.1.3. Extrusion-Based Bioprinting

Extrusion-based bioprinting, which integrates an automated robotic system for printing with a fluid-dispensing system for extrusion, has emerged as the most popular bioprinting approach in research [66,67]. It also has a lot of potential for creating living tissue products and is thought to be the most practical way to print 3D porous structures [12].

Inkjet and extrusion-based bio-printing techniques differ primarily in the way that bioink is expelled. It use micro-nozzle tips

to continuously extrude bioink as a continuous cylindrical filament that is layered into the desired shape, as opposed to inkjet printers that create droplets. [53,68]. The cylindrical filaments can be precisely produced to the necessary 3D custom-shaped structures under the control of the automated robotic system [69]. According to the source of power for the fluid-dispensing system, Extrusion-based bioprinting can be classified into mechanical and pneumatic systems. Moreover, there are two sorts of mechanical systems: screw and piston. [53,68,70]. A greater range of viscosities of bioinks can be printed using air (pneumatic) nozzles powered by compressed gas, which creates consistent air pressure. Pneumatic systems, however, have difficulty controlling the accuracy of their deposition... The piston-driven system produces a variety of viscosities by adjusting gating time and pressure and also it enables the random control of the bioink flow. Conversely, although screw-based nozzles are less expensive and do not require inlet airflow, they are not as effective with viscous printing materials. However, screw-based systems work better for printing high-viscosity or high-cell-density bioink than piston-driven systems do[71,72]. They do, however, produce greater pressure pressures on the cells, which may be detrimental to the survivability of living cells even though they do give greater spatial control. Therefore, a perfect printer can combine mechanical or pneumatic dispensing technologies with an automated robotic system to solve many of these problems [71].

There are a lot of extrusion-based bioprinters on the market, and they fall into five main types. These categories comprise conventional, substantial motion freedom, compatible with various bioprinting modalities, supporting bioplotting techniques, and supporting the printing of cell aggregates [73]. The Dong-Woo Cho group has a conventional extrusion printer with six dispensing heads and a three-axis motion control that can enable printing up to six different bioinks [69]. Additionally, they come with substrate plates that have controls for heating and cooling, which are useful for bioprinting materials that are sensitive to heat, such hydrogels. The newest extrusion bioprinters can print tissue and vasculature in parallel or numerous bioinks at once.. In recent years, research on developing extrusion technology has concentrated on the creation of branching vascular networks. If vascularization is successful, it may be possible to create tissues that are thicker [73].

Like any other technique, extrusion bioprinting has advantages and

disadvantages. A primary benefit of extrusion bioprinters is their capacity to print a greater variety of biomaterials with different viscosities, such as cell spheroids from 30 to 6 × 107 mPa/s, biocompatible copolymers, and crosslinked hydrogels. [74]. So a variety of biomaterials can be chosen through extrusion-based bioprinting [75]. Normally, Higher-viscosity materials typically support the printed construct structurally, whereas lower-viscosity materials offer an environment that is conducive to preserving cell viability and function [9]. The printers are reasonably priced and commercially available for use in research and development (R&D). It has the advantage of faster bioink printing due to its larger nozzle diameter than other bioprinting technologies. Furthermore, extrusion-based bioprinting works better when creating vast quantities of organ or tissue substitutes. Additionally, extrusion-based bioprinting works better when creating vast quantities of organ or tissue substitutes [68]. Furthermore the printers are reasonably priced and accessible commercially for use in research and development (R&D) and also capable of fabricating 3D constructs on a larger scale with ease as com- pared to all the other methods. One of the drawbacks is that during the extrusion-based bioprinting printing process, cells laden with bioink are exposed to shear stress as they go through the nozzle, which significantly affects cell viability. Therefore, researchers can modify the viscosity of the bioink and nozzle dimensions to lessen the impact of shear stress on cell activity. They can also adjust the printing temperature and duration to control the cell activity encapsulated in a bioink [53,52].

3.1.4. Laser-aided bioprinting

Using lasers as the energy source to transfer or deposit biological material, such as peptides, DNA, and cells onto substrates, is the basis of laser-assisted bioprinting, which developed from laser direct-write technology [76] and is a modified version of the laser-induced forward transform technique (LIFT) [77,78,79,80,49]. Originally LIFT was designed for direct writing of metals, but this nozzle-free bioprinting technique also allows for the high precision and resolution printing of living cells and other biologics down to the pico-micro scale. The components of a standard laser-assisted bioprinter are five: (1)A pulsed laser beam, (2) a focusing mechanism, (3) a layer of liquid bioink solution, (4) a layer of a "ribbon" structure donor layer with an energy-absorbing layer that reacts to laser stimulation, (5) a receiving substrate for bioink patterning and crosslinking are the first five components. [12]. The printer's energy comes from ultraviolet (UV) or wavelengths near UV, which are used in the

nanosecond laser pulses. Additionally, ribbons are put together in two sections to form a donor layer. These two components consist of a thin coating of a laser-absorbing substance, like titanium or gold, and a laser-transparent substrate, such as glass or quartz [81]. The cells are suspended in a liquid or gel to generate a bioink layer or film that is attached to the ribbon's metallic absorbent substance. The most specialized part is the substrate, which merely collects the bioink as it drops off the ribbon. Receiving substrates usually include biopolymers or other media that promote cell growth and adherence [82,83]. When the LAB is pulsed, the lasers heat the ribbon in a limited area, vaporizing the donor layer there. A high-pressure bubble is then created at the bioink interface and is ultimately deposited on the receiving plate [82,84]. The bioink droplet is propelled by this bubble and descends off the ribbon onto the receptive substrate. Then cross-linking takes place with the substrate [82]. Numerous variables, like the accepted substrate's surface tension, the viscosity of the bioink, and the laser's intensity, influence the LAB printing effect [85].

Given that the most technologically sophisticated bioprinting method is laser-based bioprinting, it offers numerous benefits. The main advantages are the lack of a nozzle and the non-contact printing technique, which lowers the possibility of contamination. Furthermore, by fine-tuning the biologic film's thickness, the bioink's rheological characteristics, printing speed, substrate wettability, and laser pulse energy, and organization, this technique can also yield extraordinarily high-resolution prints that more closely resemble native structures. Another benefit of laser-based techniques is that, because there is no physical contact causing mechanical stresses on the cells, their cell viability is usually as high as 95%, which is the best of all three basic approaches [86]. Additionally, a broad variety of viscosities (1–300 mPa/s) and many bioink types are suitable with laser-assisted bioprinting which could resolve the problems with the viscosity of bioinks seen in extrusion- and inkjet-based systems. [9]. Lastly, laser-based technologies provide control and precision during the fabrication of heterogeneous constructions with high cell densities, as well as automation, repeatable outcomes, and high throughput [86]. For example, According to Catros et al., 3D bioprinting with laser assistance facilitated the production of human osteoprogenitor cells (HOP) and nano-hydroxyapatite (nHA) without modifying the physicochemical characteristics of nHA, while preserving the proliferation, viability and phenotypic of HOPs [87].

However, as is the case with most cutting-edge technologies, laser-based printers are

more expensive and sophisticated than alternative techniques, and their intricate control systems necessitate a high level of knowledge and skill, which restricts their use and uptake in academia and business [12]. There are now fewer laser-based bioprinters and less research being done on the technology due to the high cost of production. Many basic knowledge gaps remain as a result of the lack of research, covering how exposure to a laser affects living cells, how characteristics such as droplet size, quality, wavelength, intensity, and pulse time affect pattern quality, and how results are affected by the gravitational settlement of cells in solution [86,49,9,88].**4. 3D Bioprinting Applications:**

In the field of biomedicine, 3D bioprinting technologies have found extensive applications. These include skin (such as wound dressings or full-thickness skin substitutes), orthopaedics, dentistry, osteochondral therapy, cardiovascular disease, and other engineering applications related to soft tissues (such as liver, pancreas etc.). Some of its applications are detailed below:

4.1. Skin

Although the skin's structure varies depending on the area of the body, it is primarily made up of an extremely vascular dermis coated with layers of cells called the epidermis. In cases where the skin is damaged or its integrity is compromised, it is necessary to eliminate any organic remnants and reinforce the affected area by incorporating fresh materials [89]. Extracellular matrix and soluble components for reconstructed cellular connections should be among them. Autologous skin grafts are the best materials for closing acute and chronic wounds with various causes [90]. Some other approaches, including bioengineering and synthetic substitutes, are required because skin transplants can cause problems like extra health risks and morbidity of deformed donor sites. Other wound dressings and tissue-engineered skin substitutes are intended to replace or support the form and function of the skin either for a permanent or temporary basis till the integrity and function are restored. A perfect analogous for skin shouldn't result in skin discolouration, scarring, or impaired sensitivity. It should resemble the flexibility of normal skin tissue, be resistant to infections, and keep you from becoming dehydrated.

Because skin bioprinting may produce complex and regulated results that are not achievable with conventional skin graft production procedures, it has garnered increased attention. It is one of the biomedical uses of 3DP technology that is developing the fastest.

For instance, Albanna et al. [91] created a transportable skin bioprinting device that allows for quick on-site wound care (Fig. 11). The primary elements of the system are a handheld 3D scanner and a printer featuring eight nozzles with a diameter of 260 μm, each powered by a separate motor for dispensing that facilitates particularly defined (XYZ) movement coordinates. With the use of an 8-channelled valve-based bioprinter, Lee et al. [92] demonstrated the bioprinting of skin tissue, utilizing collagen hydrogel to create a 13-layer tissue construct. Human foreskin fibroblast layers and acellular collagen layers were alternately bioprinted with keratinocytes, and the structures that formed showed the epidermis layers with densely packed cells as contrasted to the dermis, which had less ECM deposition and a lower cell density. Because amniotic-fluid-derived stem (AFS) cells do not produce antibodies, stratified skin substitutes can be bioprinted in situ employing alternating layers of fibrinogen–collagen and thrombin loaded with AFS cells, as demonstrated by Skardal et al. [91,92]. Skin substitutes were 3D bioprinted directly into the full-thickness wounds of pigs using in situ bioprinting; these skin substitutes resembled native skin more than control groups utilizing bio-ink injected with acellular hydrogels and mesenchymal stem cells (MSCs).

The integration of sweat glands and hair follicles has proven to be difficult to integrate, which makes bio-fabrication of skin substitutes that nearly resemble natural skin difficult even with advances in skin tissue bioprinting [93].

4.2. Bone tissue

The most often studied hard tissue in the field of 3D bioprinting is bone tissue. The most difficult task in this field is fabricating bone tissue constructs equal in strength to the real bone. Over four million procedures are carried out each year to repair bone problems, making bone the second most transplanted organ in the world [94,95]. The most effective methods for repairing broken bones are still autografting and allografting. Using an autograft is a costly and intrusive procedure that usually affects the donor and surgical sites due to the possibility of hematomas and infections. In addition, allografts come with drawbacks, namely the potential for an immunological response and subsequent host tissue rejection; if the graft is contaminated, the patient could also become sick. [96,97]

Vascularization and cell proliferation require an interconnectively porous bone tissue architecture. Several techniques were created to 3D bioprint these structures. To achieve such structural integrity and strength, hybrid constructs with strength-enhancing biopolymers employing cell-laden solutions were created, whereas

others bioprinted bone tissue using a single cell-containing solution. [98,99,100]

The use of biomaterials and artificial structures in the replacement of bone tissue has grown. Among all one of the most important elements influencing the success of the structure construct in the body is vascularity, which has not been effectively provided by a number of methods that have been employed in the past to construct TE structures. When bulk materials are used, prolonged biodegradation processes may take place, which may result in inflammatory reactions [89].

The main obstacle in this field is to fabricate bone tissue constructs with a mechanical strength equivalent to real bone [101]. Vascularisation and cell proliferation require an interconnectivity porous bone tissue architecture [102]. Several techniques were created to 3D bioprint these structures. To give such structural integrity and strength, hybrid constructs with strength-enhancing biopolymersq1 employing cell-laden solutions were created, whereas others bioprinted bone tissue using a single cell-containing solution [103,104,105].

Using amniotic fluid-derived stem (AFS) cells and alginate/collagen bioink, De Coppi et al. created a construct [Fig.2] for bone tissue regeneration [103]. An HP Deskjet 550C printer was modified to print the construct [107,108]. In another study, Fedorovich et al. bio-printed heterocellular tissue constructs made of Matrigel™ and alginate hydrogels [12]

Fabrication and Product

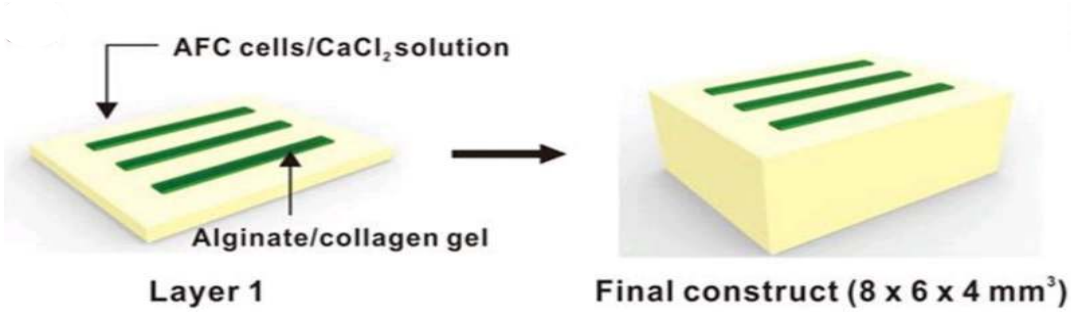

Figure. 2 Layer-by-layer printed AFS cells in CaCl2 solution by using an alginate/collagen composite gel that forms a rectangular sheet.

4.3. Cartilage Tissue

It is currently not possible to create cartilage tissue that is identical to native tissue by tissue engineering methods [108]. Because bioprinting offers such great potential for precise spatial and temporal deposition of cells and biomaterials with sophisticated patterns, it has recently

garnered increasing attention for its ability to create cartilage tissues that can mimic native tissues with zonally differentiated cells and extracellular matrix (ECM) composition. A hybrid printing system with electrospinning and an inkjet printing device was created by Xu et al. [109]. The scaffold made of polymeric fibres produced by the electrospinning device give the construction more mechanical strength. Step motors and a DC solenoid inkjet valve power a bespoke XYZ plotter on the inkjet platform. Their construct consisted of two inkjet-printed chondrocyte gel layers and three electrospun PCL/pluronic F-127 layers alternating in five layers [FIG.3]. The repair of osteochondral abnormalities, including those in the knee joints, is typically the focus of research on cartilage tissue bioprinting. To create heterogeneous tissue, Shim et al. used a special multi-head tissue/organ building system (MtoBS) [Figure 4(c)]. On day 1, 37 chondrocytes and osteoblasts having high cell viability (~90–94%) were printed onto a framework of PCL. Cells could multiply at a centimetre-scale level without diffusion thanks to pores in the building that carried nutrients and oxygen.F.

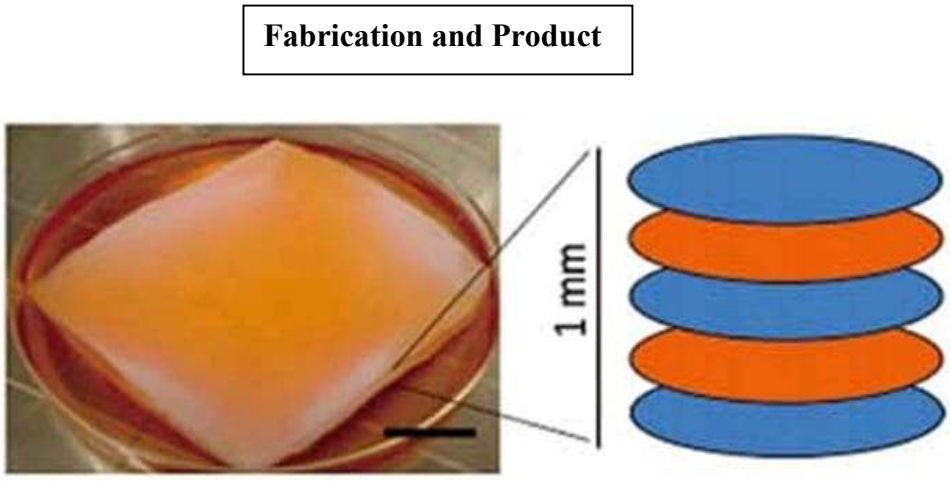

Fig. 3 Layer-by-layer 3d bioprinted cartilage tissue construct in culture media consisting of five alternating layers of PCL (blue) and chondrocytes/fibrinogen/collagen (orange).

4.4. Heart Valves

Heart valve engineering is crucial in cardiac tissue engineering. It is because malfunctioning heart valves must be replaced with mechanical or biological prosthetic counterparts if the damage or illness is severe [110]. Heart valves are not capable of regeneration. But calcification

and thrombogenicity limit such replacement valves [111]. Heart valve bioprinting has not received much attention, despite the heart valve's vital significance in the circulatory system. Butcher's team used a bioprinter with a dual-head that was altered from a home printer for the first time demonstration of the bioprinting of a heart valve. [112,113].

In a separate investigation by the team [114], thin hydrogel discs were created through dual-nozzle bioprinting of composite alginate–gelatin hydrogel. Spatial bioprinting was utilized to create smooth muscle cells (SMCs) and interstitial cells (VICs) of the aortic valve.

After a week of incubation, the samples showed a cell viability of 81.4 ±3.4% and 83.2 ±4.0% for SMCs and VICs, respectively.

4.5. Liver Tissue

Despite the liver's exceptional capacity for regeneration and healing, liver failure has laid an increasing interest in liver tissue engineering , when combined with the failure of other organs, is a major source of morbidity and mortality [115]. The great potential of bioprinted liver tissue models in drug testing and high-throughput screening, along with the extraordinary susceptibility of liver tissue to drug toxicity, suggest that engineering liver tissues may be a viable solution to the organ transplantation crisis. Human-induced pluripotent stem cells (hiPSCs) were bioprinted for the first time by Faulkner-Jones et al. [116]. For liver micro-organ engineering, the bioprinted hiPSCs were then stimulated and differentiated into hepatocytes. Using a modified NovoGen MMX BioprinterTM, Bertassoni et al. bioprinted HepG2 cells and fibroblasts within agarose strands and gelatin–methacrylamide (GelMA) hydrogel [117].

4.6. Neural Cells

To date, little research in the area of bioprinting for neural tissue has been done. Engineering nervous system tissues have great potential for replacing sick, elderly, or damaged nervous system components. The impact of VEGF (vascular endothelial growth factor) release on the migration and proliferation of C17.2 murine neural stem cells was investigated by Lee et al. [118]. In their investigation, C17.2 cells were bioprinted on a layer of collagen adjacent to a fibrin disk that contained VEGF. In contrast to the cells that were unable to proliferate within the collagen matrix, the study showed that brain cells proliferated and went near fibrin gel that released VEGF. Hsieh et al. recently provided bioprinting evidence for a thermoresponsive polyurethane hydrogel that can be bioprinted at 37°C and has adjustable stiffness without a crosslinker [119]. By adding neural stem cells to the bioink and injecting it into a model of

neural damage in zebrafish embryos, they demonstrated the bioink's efficacy. The findings showed that the injected gel restored the compromised nervous system's functionality after six days.

4.7. Vascular Tissue

Vascularization is critical to the bioprinting of scaled-up tissues and organs since the integration of a vascular network will essentially give cells with media and oxygen necessary for their survival and function [120]. Several bioprinting techniques have been used to create vascular tissue, including droplet bioprinting [121,122], extrusion [123–128], and laser-based bioprinting [127]. Many different extrusion methods have been used in bioprinting based on extrusion. Ozbolat et al. employed coaxial-nozzle extrusion to bioprint hydrogels, such as chitosan and sodium alginate, directly into a tubular form containing encapsulated cells [123,125]. This approach enabled practical direct bioprinting of vascular constructs. There are further direct methods for bioprinting vascular tissue, such as the layer-by-layer bioprinting of cell-filled hydrogel droplets by Nakamura and his colleagues using inkjet-based bioprinting[128].

4.8. Composite Tissues

In an attempt to mimic the complex biology, architecture, and functionality of organ-level structures, efforts have been directed toward bioprinting specific tissue types as well as composite tissues.Recently, Merceron et al. used a multi-head nozzle assembly to bioprint hybrid constructions that allowed for the creation of muscle-tendon units [129]. To support cellular constructs, a frame was created utilizing 3D printing of PCL and PU [s7], with half of the unit printed using PCL and the other half using PU.Tissue engineering has shown interest in osteochondral model bioprinting in addition to muscle-tendon units. Using alginate in a mesh pattern Fedovorich et al. [130] showed the method and process to bioprint MSCs and chondrocytes. Two distinct cell types were bioprinted within the scaffold's two opposing ends.

4.9. Lung tissue

Recently, only one attempt has been made and investigated for the creation of a lung tissue model through the use of bioprinting, as lung tissue engineering is a relatively young field. Using BioFactory® by regenHU, Hovath et al. showed how to bioprint an in vitro model of the air-blood barrier [131]. In this regard, they bioprinted a few layers of a zonally stratified tissue construct. To help cells adhere to the MatrigelTM layer, a single layer of EA.hy926 endothelial cells was bioprinted after a thin layer of MatrigelTM was bioprinted as a basement membrane. A fresh layer of MatrigelTM was bioprinted

on top of the previously constructed construct on day 2, and then a single layer of A549 epithelial cells was bioprinted. Manually, control samples were also built using the deposited layers. Samples characterisation was done on Day 5for endothelial and epithelial cells, respectively, cell viability of −86% and >95% was attained. Epithelial cells at the top and endothelial cells at the bottom were uniformly spaced from each other.Epithelial and endothelial cells were evenly distributed, with epithelial cells at the top and endothelial cells at the bottom.

4.10. In cancer research

Tumour models in two dimensions have been extensively employed in cancer research; however, due to their absence of 3D cell-matrix and cell-cell interactions, their representation to the physiologically relevant environment is poor. Bioprinting has therefore given considerable benefits to reproduce the cancer microenvironment, including the exact location of various cell types and microcapillaries and the investigation of cancer etiology and metastasis [132]. Nevertheless, bioprinting for cancer research is still relatively new, with few studies conducted in this burgeoning field of application. The process of bioprinting tumour tissue models for in vitro experiments was originally shown by Demirci's group [133]. Their study used inkjet-based bioprinting technology with dual ejectors to bioprint human ovarian cancer (OVCAR-5) cells and MRC-5 fibroblasts. In a high-throughput and repeatable process, different cell types spontaneously bioprinted on MatrigelTM to create multicellular acini in a spatially managed microenvironment with regulated cell density and cell-to-cell distance. In addition to demonstrating a useful tool for cancer research, the approach that was described offered an excellent platform for high-throughput screening. Recently, Sun's group demonstrated HeLa cell bioprinting which was used to create cervical carcinoma models [134]. In this context, >90% of the HeLa cells that were extruded and bioprinted were in a patterned hydrogel composed of gelatin, alginate, and fibrinogen. In contrast to the control groups, where cells in 2D culture developed cell sheets with lesser chemoresistance and lower level expression of metalloproteinase, HeLa cells moved toward one other and formed cell aggregates within hydrogel filaments in 5–8 days.

4.11. 3D bioprinted vaccines and drugs

Although 3D bioprinting is already being utilized in tissue engineering, it is also rapidly becoming a powerful method for producing pharmaceuticals and delivering drugs. Tailored medicine may now be produced thanks to 3D printing technology.

Because each patient has a unique dosage and set of prescribed drugs, producing tailored medicine typically involves sophisticated manufacturing techniques. This section outlines the 3D bioprinting technologies that can be used to produce medicinal medicines and RNA vaccines directly [135].

4.12. Application in pharmaceutics and high-throughput screening

The process of finding new drugs is time-consuming, expensive, and involves significant financial and human resource investment. Despite continuous attempts to boost the productivity of the drug development process, only one of the estimated 10,000 unique chemical entities and one out of every ten medication candidates completing clinical trials reaches the final approval stage and the market [136]. It will be quicker to get new treatments into clinics if it is possible to anticipate the toxicity and efficacy of drug candidates sooner in the drug discovery process. In order to overcome this bottleneck, recent efforts in 3D in vitro assay methods are suitable. This is because 3D tissue models can be produced on microarrays, which allows them to closely resemble natural tissue and be employed in high-throughput tests. Bioprinting is a more advanced technique for creating 3D in vitro systems than other approaches [137], with advantages including the low danger of cross-contamination, high throughput, controllability oversize and microarchitecture, and coculture ability. The prospective applications of bioprinted tissue and organ models in pharmaceutics, including drug toxicity and high-throughput screening, are being explored more and more [138].

5. Conclusion and future remarks:

Many 3D bioprinting technologies that can be used to create biomedical 3D organs or tissues in situ for in vivo applications are reviewed in this article. Bioinks can be printed in three dimensions, creating 3D microenvironments that resemble in vivo tissue. Within these settings, cells can initiate several biological processes, including migration, differentiation, proliferation, and viability. However, additional study is still needed to address the problems of today, like vascularization. Moreover, for bioprinting technologies to create multicellular tissues and organs, more sophisticated and intelligent bioinks are needed.

This paper introduces bioprinting technology and gives a comprehensive overview of its application areas, which include basic research in tissue engineering, regenerative medicine, and cancer pathogenesis, tissue bioprinting for transplantation and clinics, and some recent efforts in these areas. Early translational technologies in pharmaceutics for drug

testing and high-throughput screening are also included. Several other human tissues have not yet been explored in the field of bioprinting, but at the moment, about fifteen distinct tissue types are being experimented with. Soon, it will be important to shift the focus onto additional difficult-to-model tissues and organs that have the potential to transform medicine. The need to retain the cell-biomaterial suspensions in the material reservoir for an extended period of time is a key disadvantage of encasing living cells in biomaterials. This prolongs the storage duration of the cells and reduces their bioactivity. This is particularly troublesome in light of the longer manufacturing times needed to print larger-scale organs and tissues. Consequently, a more automated process for loading and ejecting the cell-biomaterial mixture is required for the synthesis of tissues and organs on a bigger scale.

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