



# 3D Bioprinting is Emerging technology in biomedical industry: Methods, Applications, and Challenges.

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## **ABSTRACT:**

Three-dimensional (3D) printing has come a long way, and it now has a lot of medical applications. One of the most major developments leading to 3D bioprinting was the development of biomaterials, cells, and supporting components that enabled the fabrication of functioning living tissues. Several different methods and techniques of 3D bioprinting are briefly described in this review article, and applications of 3D printing in tissue engineering and regenerative medicine, tissue transplantation, cancer research, artificial development of skin, bone and cartilage, blood vessels, liver tissue and also include the future of bioprinting in pharmaceutical applications that is disease modelling and drug optimization, Drug toxicity screening, Precision medicine this article also include limitations and future challenges in 3D bioprinting. Further breakthroughs in the manufacture of patient-specific vascular tissue constructions will be aided by advances in additive manufacturing techniques, medical imaging modalities, biomaterials, and cellular engineering. Tissue engineering and regenerative medicine are likely to undergo significant transformations as a result of future transdisciplinary research and breakthroughs.

Key words: Three-dimensional (3D) printing, Tissue engineering, Regenerative medicine, Tissue transplantation, Precision medicine, Additive manufacturing.

## INTRODUCTION:

Bioprinting advances have opened up new and intriguing prospects for producing patient-specific medical treatments. Drug testing, tissue engineering, biomimetic sensors, and 3D tissue models all need the construction or printing of biomimetic tissue structures. Because of the rejection issues that come with ex vivo approaches for tissue/organ transplantation are being investigated due to allo-geneic organ transplantation and donor shortage. These approaches entail the multiplication of autologous cells generated from patients and their use as the principal cell source for the development of tissues and organs for transplantation. These 3D tissue analogues can be made by fusing native cells with biocompatible materials in a precise and regulated manner. (1) 3D bioprinting is a type of additive manufacturing that prints live structures layer by layer using cells and other biocompatible materials as inks, often known as bioinks, to replicate the behavior of natural living systems. The increasing demand for bioprinting has sparked new biomedical sector innovation. Researchers are attempting to envision a future in which damaged organs could be replaced using printer. Bioprinting is slowly becoming more and more feasible. The word of 3D printing is one of the most exciting sectors as well as one of the most practical and useful. Bioprinting is actually very similar to the additive manufacturing processes. As with other types of additive manufacturing bioprinting uses a digital file as a blueprint to print an object but unlike 3D printing bioprinters print with cells and biomaterials creating organ like structures that let living cells within them multiply to form a new organ just like Fused Filament Fabrication (FDM) or stereolithography (SLA) printer on your desktop these organ are printed layer by layer however rather than using a CAD file researcher create a digital model using computed tomography and MRI scans. So far, researchers are 3D printed an antibacterial tooth, an ovary, a bionic ear, elastic bones, lungs and even heart. (2) Bioprinted 3D constructions are designed to imitate the native tissue's cell density, organisation, niche, and anatomical geometry, and hence could be a potential option for various regenerative medicine applications. (3) “Biofabrication is the process of making complex living and non-living biological products out of raw materials such living cells, chemicals, extracellular material, and biomaterials.”

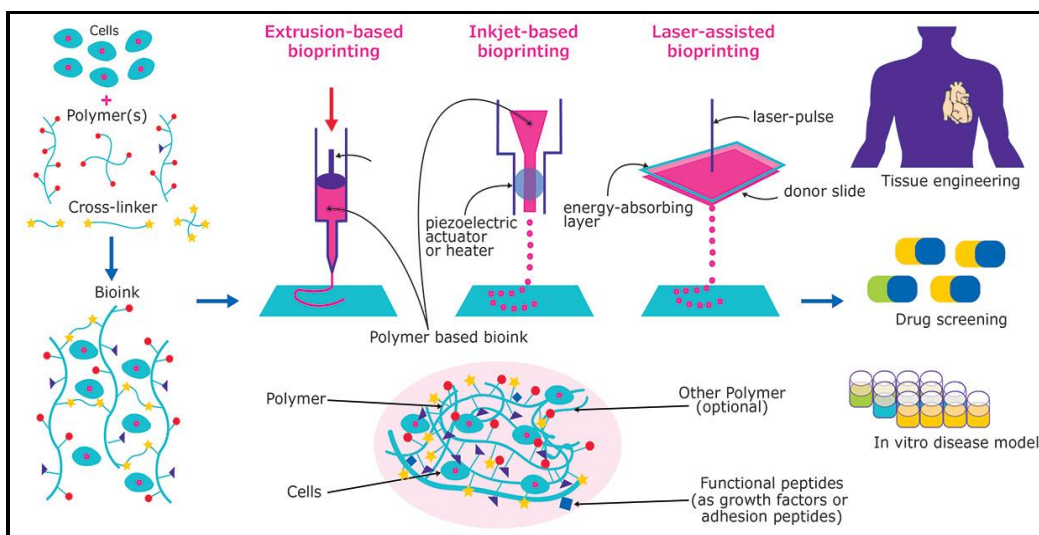


Figure: 1

**History of 3D printing:**

The rise of the printing (press) business in the 15th century allowed for the rapid reproduction of text and images, as well as the mass distribution of knowledge, which had enormous social, political, and economic consequences. After five centuries, 3D printing technology is a new revolution with the potential to revolutionise industry and other disciplines such as medicine. After the first developments in 3D printing, which was described as "stereolithography" by Charles W. Hull (5) in the early 1980s, new methods and techniques for the construction of 3D objects have been developed and used for educational, research and even clinical purposes. Originally, stereolithography, also known as photo-solidification, optical fabrication, or resin printing, was used to create 3D structures by printing thin layers of a material that had been exposed to UV light in a sequential manner. Since then, many additive manufacturing techniques have been developed to automate the fabrication of individualised, computer-modeled tissue replicas and even organs.

**Basic principle of 3D bioprinting:**

3D bioprinting, in general, is based on the layer-by-layer exact positioning of biological constituents, biochemicals, and living cells, as well as spatial control of the placement of functional constituents of the manufactured 3D structure. 3D bioprinting is based on three approaches: (a) biomimicry or biomimetics, (b) autonomous self-assembly, and (c) mini tissue building blocks. (6)

In a nutshell, biomimicry, also known as biomimetics (from the Greek words bios, which means life, and mimesis, which means to imitate), is the study of nature, its systems, processes, and elements in order to be inspired and stimulated to find the best answers to human problems. Biomimetic components used into a bioprinted product have a dynamic effect on adhesion, migration, proliferation and function of both endogenous and exogenous cells. (6) Cell attachment, as well as cell size and form, are all influenced by materials, allowing for the control of cell proliferation and differentiation in a scaffold. Cell adhesion, proliferation, and cytoskeletal assembly can all be influenced by nanoscale features like ridges, steps, and grooves. (6)(7)

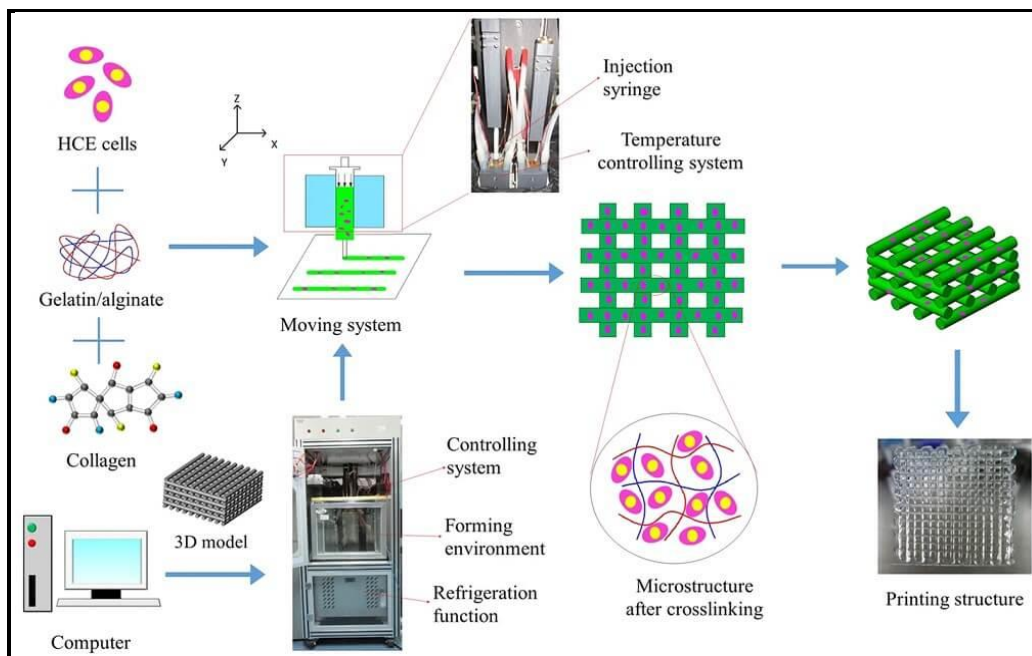


Figure: 2 Schematic illustration of the 3D bioprinting process and optical images of the printing set up and printing constructs.

### Basic steps in 3D bioprinting process:

Preparation, printing, maturation, and application are the main steps in the process. This may be summed up in three simple steps:

**Pre-bioprinting** involves creating a digital model that the printer will produce. The technologies used are computed tomography (CT) and magnetic resonance imaging (MRI) scans.

**Bioprinting** is the actual printing process, where bioink is placed in printer cartridge and deposition takes place based on the digital model.

**Post bioprinting** is the mechanical and chemical stimulation of printed part so as to create a stable structure for biological material. (4)

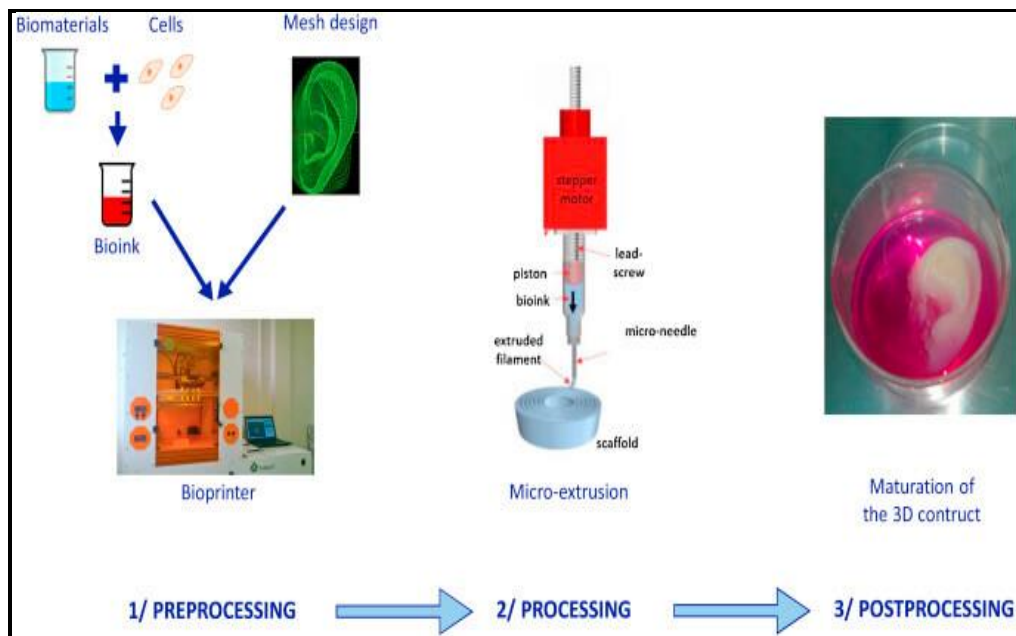


Figure: 3 Basic steps in 3 D bioprinting

**3D Printing technology types:**

1. Extrusion based bioprinting
2. Inkjet-based bioprinting
3. Pressure-assisted bioprinting (PAB)
4. Laser-assisted bioprinting (LAB)
5. Stereolithography (STL)

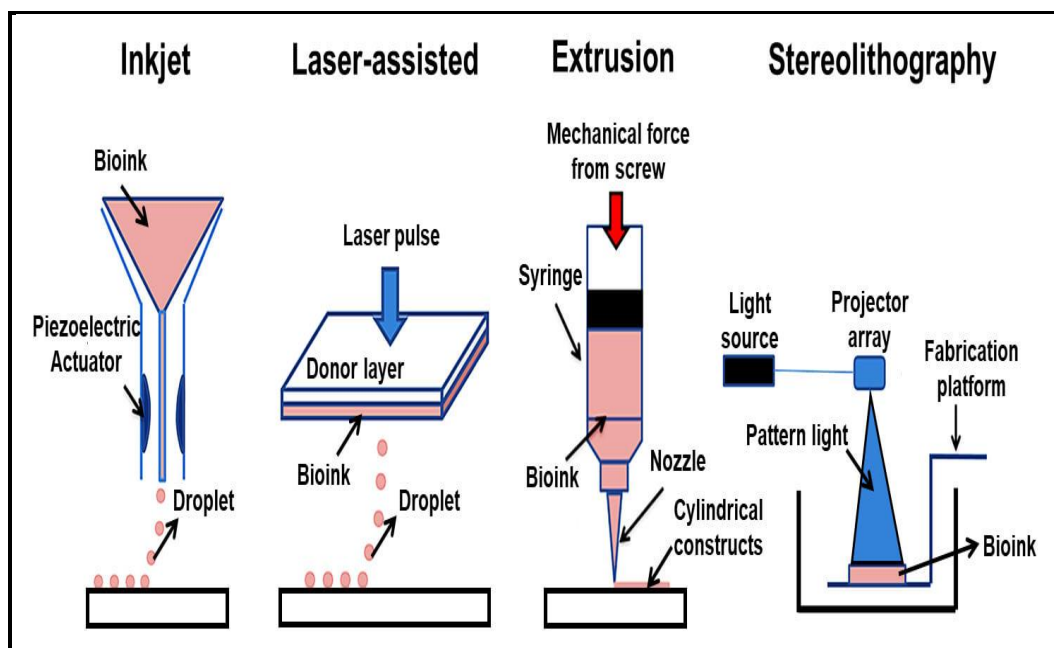


Figure: 4 Types of 3D printing technologies

### Bioinks:

Bioinks are biological materials used in 3D bioprinting to construct synthetic live tissues. The name "bioink" refers not only to the cells utilised in the manufacturing process, but also to the carrier molecules that help the cells proliferate. Biopolymer gels, which operate as a 3D molecular scaffold for cells to connect, develop, and multiply, are common carrier materials utilised with cells during bioprinting. The biopolymers employed in bioink are critical because they retain water, which gives synthetic tissues mechanical stability. The choice of bioink for a given process is critical, as the bioink must have the appropriate physicochemical qualities, which include mechanical, chemical, biological, and rheological qualities. (8) Cells, whether as single cells, coated cells, or cell aggregates (of one or more cell types), or in combination with other materials, are a crucial component of a bioink's printing formulation (for example seeded onto microcarriers, embedded in microgels, formulated in a physical hydrogel, or formulated with hydrogel precursors. In the case of biomaterial ink, any biomaterial can theoretically be utilised for printing, with cell seeding occurring after production. (4)

### The qualities of the bioinks used in the bioprinting process should be as follows:

The bioinks utilised should have enough mechanical strength and robustness to maintain tissue-matching mechanics in the resulting tissue constructions. To achieve excellent shape fidelity during bioprinting, the bioink molecules should have tunable gelation and stability. Bioinks must be biocompatible and degradable in the tissue's natural microenvironment. Chemical alterations to generate specific tissue should be possible with bioinks. (4)

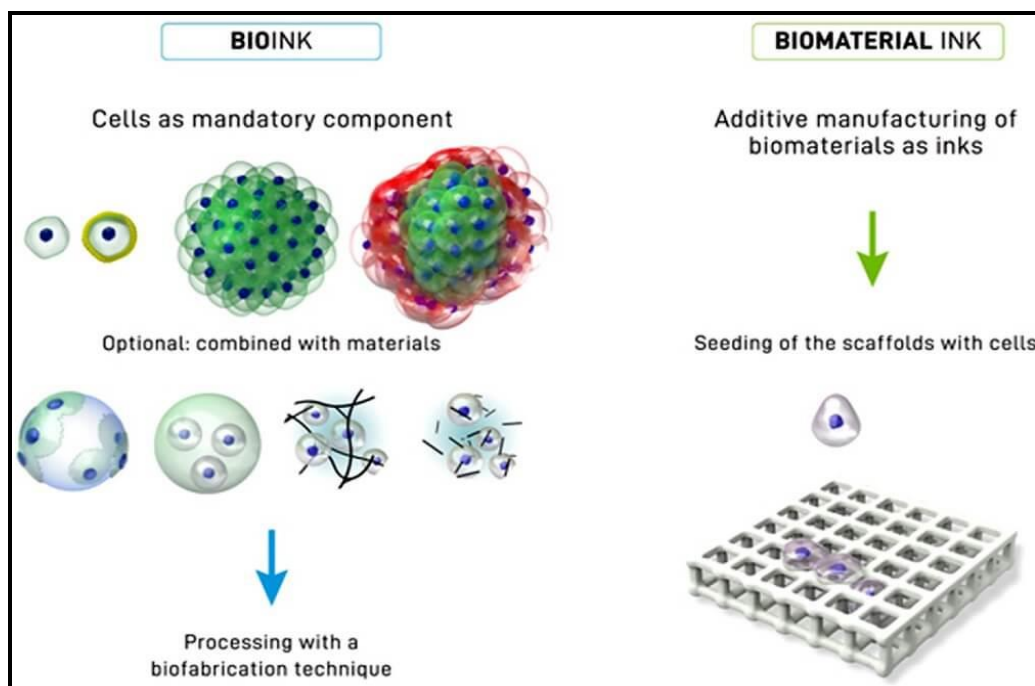


Figure: 5 Distinction between a bioink (left side) and a biomaterial ink (right side).



## Bioprinter:

Bioprinters, also known as 3D bioprinters, are automated devices that fabricate 3D functional tissues and organs from digital models obtained through different scans using biomaterials.

Bioprinters are robotic devices that work in a variety of ways. Bioprinters are 3D printers that can only produce cell-free scaffolds and can't dispense living cells. Prof. Ralf Mulhaupt's group at Freiburg University developed the first commercial 3D bioprinter. The advancement of 3D bioprinters has been a continual process including the hybridization of new technological techniques to create new advanced bioprinter forms. There are three types of bioprinters: inkjet bioprinters, extrusion-based bioprinters, and laser-based bioprinters. These bioprinters use various methods and are used for various applications depending on the biomaterials employed. (9)(10)

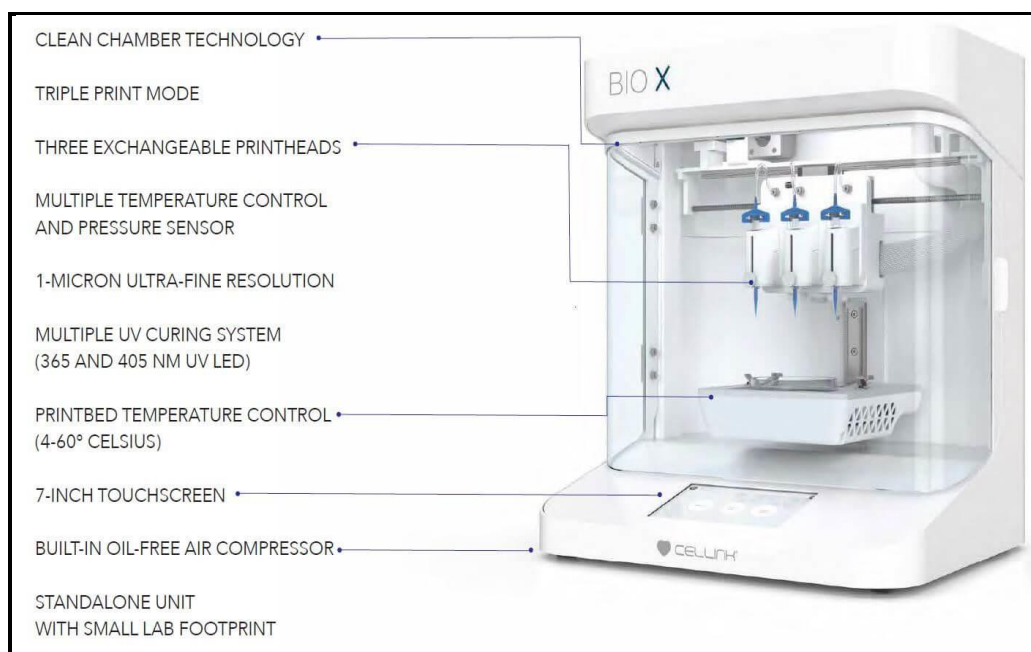


Figure: 6 3D bioprinter

## APPLICATIONS OF 3D BIOPRINTING:

### (A) Tissue engineering and regenerative medicine:

The integration of the circulatory network from arteries and veins down to capillaries, as well as the incorporation of numerous cell types to replicate complex organ biology, remain obstacles to bioprinting of functional organs at clinically relevant dimension structural and mechanical integrity are compromised, as is long-term functionality. (11) Despite these challenges, a wide range of tissues, including thin or hollow tissues like blood vessels, have been successfully bioprinted. (12) Tissues that don't need to be vascularized, like cartilage. (13)

### (B) Bioprinting for tissue transplantation:

Several bioprinted tissue types have been transplanted into animals to explore their functionality within the host, including neural, cardiac, blood artery, bone, and skin. Due to a lack of FDA permissions, such researches have not been conducted in humans yet. Bone tissue replacement with 3D-printed plastic,

ceramic, or metallic implants, on the other hand, has been done successfully. After the surgery, there were no negative side effects. The difficulties in transplanting bioprinted tissue and organs include duplicating the organ's vasculature and metabolic state. In situ bioprinting of tissue and organ constructions directly into defect locations, rather than bioprinting whole tissue outside, maturing, and testing them in vitro before transplanting, could be one of the solutions to this problem. The recruitment of endothelial cells and integration into the host vasculature can be achieved by bioprinting the tissue in situ. (14)

### **(C) Bioprinting cancer research:**

The fundamental disadvantage of two-dimensional tumour models is that they lack three-dimensional interactions with nearby cells and substrates, making them unfit to simulate physiologically relevant environments. As a result, bioprinting provides a method for understanding three-dimensional cellular interactions and making clinically meaningful findings on cancer development and spread. An inkjet-based bioprinting technology was used to bioprint human ovarian cancer cells (OVCAR-5) and MRC-5 fibroblasts, for example. Furthermore, scaffold-free bioprinting of a breast cancer model has been demonstrated, with cancer cells surrounded by a physiologically appropriate stromal milieu of MSC-differentiated adipose cells, mammary fibroblasts, and endothelial cells. These tissues were alive in vitro for two weeks, with obvious tissue compartmentalization. The effect of other chemotherapeutic medications, including tamoxifen, was then investigated using this model. (14)

### **(D) Skin:**

A variety of tissue engineering techniques are used to fabricate skin tissue.

Tissue engineering can be used to create autologous split-thickness skin grafts, allografts, acellular dermal substitutes, and cellularized graft-like commercial items, among other things. Skin tissue may be bioprinted utilising an eight-channel valve-based bioprinter that creates a 13-layer tissue out of collagen hydrogel. To make constructs with tightly packed cells in epidermal layers, keratinocytes are bioprinted on top of alternating layers of human foreskin fibroblasts and acellular collagen layers. After around 10 days in the stratified epidermis, the tissue constructs are engrafted with the host. The stratum corneum, as well as certain blood vessels, show early signs of differentiation and development as a result. The biomaterial employed in the procedure varies, however keratinocytes and fibroblasts are the most common cells used. (4)

### **(E) Bone and cartilage:**

Because the nature of such hard tissues is simple and primarily made up of inorganic materials, bone and cartilage creation is the most advanced application of bioprinting. Despite the fact that various procedures like as gas foaming, salt leaching, and freeze-drying have been used to create such hard tissues, 3D bioprinting creates the most precise architectures. Polymethacrylate scaffolds are made from human mesenchymal stem cells generated from bone marrow using a thermal inkjet bioprinter. To manage the spatial location of cells, the cells are coprinted with nanoparticles of bioactive glass. A printed bioink is



created by combining nano fibrillated cellulose and alginate with human chondrocytes as living soft tissue in cartilage tissue engineering. (4)

#### **(F) Bleed vessels:**

The fabrication of tissues and organs is dependent on vascularization to give oxygen and media to the bioprinted constructions hence bioprinting of vascular networks is critical. Extrusion- and laser-assisted bioprinting techniques were employed to create bioprinted vascular networks. Hydrogen gels containing sodium alginates and chitosan, as well as encapsulated cells, are bioprinted directly in tubular form during bioprinting. The tubular structures created as a result of this process have increased metabolic transport and cellular viability. (4)

#### **(G) Liver tissue:**

Because liver cells have a high potential to regenerate, bioprinting of liver tissue is rather uncommon. However, the number of healthy donors is limited, and the regeneration time for such a liver is lengthy. Cells such as primary hepatocytes and stem-cell-derived hepatocytes are used as bioink in this process. The liver can be printed in the exact size and shape required by the patient using 3D printing technology. Canaliculi are created via bioprinting and are joined together by the collagen matrix to build bigger structures. (4)

### **The future of bioprinting in pharmaceutical applications:**

#### **Disease modelling and drug optimization:**

Better disease models are necessary for a better knowledge of disease behaviour, progression, therapy, prevention, and cure. When combined with micro fluidic systems, bioprinting of 3D cell-hydrogel constructs has allowed the creation of complex, repeatable organ-on-a-chip models that can replicate body-wide diseases by incorporating many external features such as fluid or air flow and a combination of tissue types into a single 3d model. Researchers can use this technology to study disease progression in order to better understand and treat the condition. (15)

#### **Drug toxicity screening:**

Toxic effects must be thoroughly investigated and monitored in vivo in animal models when new medication treatments are being developed. The ability to more properly replicate such effects prior to animal research using 3D cell culture models created with bioprinting technology may allow the medicine to be tweaked or removed before costly in-vivo testing. Overexposure to toxins can cause toxic hepatitis, liver inflammation, and eventually cirrhosis if exposed for a long time. This is why liver toxicity is one of the most heavily researched side effects in drug development. The liver's intricacy, paired with its important role in drug metabolism, has produced a demand for physiologically enhanced liver models, which can be answered with new bioprinting techniques and enhanced liver models. (15)

**Precision medicine:**

Precision medicine is the tailoring of treatment based on a patient's genetic information. Researchers can collect a vast amount of data for long-term studies of illness development and drug response using a patient's own cells. In precision medicine, the use of 3D cell culture models allows cells from a patient to be cultivated and expanded in order to research the condition ex vivo while keeping the patient's in vivo genotype. 3D bioprinting can be used to create a large number of patient-specific disease models using the patient's own cells, which can be used in parallel with clinical trials or independently through precision medicine initiatives to target patient or disease-specific genetic, proteomic, and phenotypic characteristics. (15)

**Limitations and future challenges in 3D bioprinting:**

Bioinks with high biocompatibility and mechanical strength are the key challenges to bioprinting. The present bioprinter technology has a lesser resolution and speed than previous generations, which makes further improvement difficult. Bioprinters should be able to because the existing bioprinting technology is slow, the pace should be enhanced to mass-produce biomaterials at a commercially acceptable level. Tissue structures require continuous oxygen and nutrients; therefore vascularization is a significant barrier the expense of 3D bioprinting may make it unavailable to the poor, which raises ethical concerns. Because bioprinting is a new technology, it needs to be thoroughly researched to verify that it is human-safe. Personalized 3D printing technology could result in a slew of regulatory issues in terms of overseeing printed goods. Personalized 3D printing technology might lead to a serried of regulatory problems to ensure printed product supervision.

**CONCLUSION:**

3D bioprinting is accelerating at an ever-increasing rate due to its multidisciplinary character. It is an exciting period, but we must be cautious not to overestimate the capabilities of this technology. The human body is extremely complicated, and replicating all of its functions is difficult and time-consuming. Every day, those working in the sector make improvements in both technology and their understanding of how it might be used and enhanced. While we aren't quite there yet, bioprinting will undoubtedly change the future of medicine.

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