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3D BIOPRINTING OF TISSUE AND ORGAN: AN OVERVIEW

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ABSTRACT

This review article delves into the exciting realm of 3D bioprinting, an innovative technology with the potential to revolutionize regenerative medicine. With a primary focus on addressing the critical issue of organ shortages, 3D bioprinting has emerged as a beacon of hope. This technique enables the precise fabrication of living tissues and organs by strategically assembling biological materials, cells, and support structures. The text explores the fundamental principles, methods, and materials used in 3D bioprinting, emphasizing the importance of biocompatible "bioinks." The applications of 3D bioprinting are diverse and promising, ranging from the generation of multilayered skin to the development of high-throughput tissue models for drug discovery and toxicology studies. However, this technology is not without its challenges. Complexities such as material selection, cell incorporation, and the recreation of intricate biological micro-architectures demand a multidisciplinary approach. The collaborative efforts of experts from various fields are propelling innovation and progress in 3D bioprinting. As we advance in research and technology, the dream of routinely printing functional human tissues and organs draws closer, promising a future where transplantation becomes more accessible and effective.

KEYWORDS: Biofabrication, 3D bioprinting, Biomaterial, Tissue generation.

INTRODUCTION

The process of creating complex living and non-living biological products using living cells, chemicals, extracellular matrices, and biomaterials as starting points is known as biofabrication.[1] A new frontier in regenerative medicine, 3D bioprinting offers a glimpse of

a time when tissue replacement will be a common medical procedure and organ shortages will be less of an issue. Fundamentally, 3D bioprinting is a cutting-edge technique that makes it possible to create three-dimensional live tissues and organs by precisely assembling biological materials, cells, and supporting elements. Due to its potential to solve the urgent demand for transplantable tissues and organs, which has grown more urgent over time, this developing field has attracted a lot of attention.

The severe lack of organs available for transplant Is the main factor propelling the development of 3D bioprinting. Despite an increase in willing donors, there is still a huge shortage of transplantable organs. Due to this gap, patients now have to wait longer between appointments, have worse medical results, and occasionally even pass away. Scientists turned to 3D bioprinting as a method to close the supply-demand gap in organs after realizing how urgent the problem was.[2]

Understanding 3D bioprinting's foundational concepts and methods is crucial for understanding it completely. Layer-by-layer biological materials, including living cells, are deposited utilizing computer-assisted transfer techniques in bioprinting. It is possible to build intricate 3D structures that resemble the shape and functionality of natural tissues and organs because to the precise positioning of biological components and spatial control over functioning aspects. There are three main approches of bioprinting- biomimicry, autonomous self assembly, mini tissue.[3]

One of the most critical aspects of 3D bioprinting is the choice of materials. Unlike traditional 3D printing that uses plastics or metals, bioprinting necessitates materials that are biocompatible and suitable for sustaining living cells. These materials, often referred to as bioinks, can be composed of natural or artificial polymers, growth factors, and living cells. The selection of bioinks is paramount, as it impacts the viability, functionality, and safety of the printed tissues.[4]

The applications of 3D bioprinting are as diverse as they are promising. Notably, this technology has been successfully employed in the generation and transplantation of various tissues, including multilayered skin, bone, vascular grafts, tracheal splints, heart tissue, and cartilaginous structures. These accomplishments represent significant strides towards addressing the organ shortage crisis. Moreover, 3D bioprinting has found utility in developing

high-throughput tissue models for research, drug discovery, and toxicology studies, thereby reducing the need for animal testing and accelerating pharmaceutical research.[5]

However, the path to widespread adoption of 3D bioprinting is not without its challenges. The complexities of this technology extend beyond the 3D printing process itself. Factors such as the choice of materials, the selection of appropriate cell types, the incorporation of growth and differentiation factors, and the technical hurdles related to the sensitivity of living cells all pose significant challenges. Addressing these complexities requires a multidisciplinary approach, involving experts from fields such as engineering, biomaterials science, cell biology, physics, and medicine. The need to adapt technologies originally designed for non-biological materials to the realm of sensitive living biological components presents a central challenge. Moreover, the intricacies of reproducing the complex micro-architecture of extracellular matrix (ECM) components and multiple cell types with sufficient resolution to recapitulate biological function remain a daunting task.[5]

In short 3D bioprinting stands as a become of hope in the quest to overcome the organ shortage crisis and revolutionize regenerative medicine. By enabling the fabrication of living tissues and organs with precision, this technology offers a path towards a future where transplantation is more accessible and effective. Although challenges exist, the collaborative efforts of experts from various fields continue to drive innovation and progress in 3D bioprinting. As research advances and technology matures, we move closer to a reality where the dream of printing functional human tissues and organs becomes a routine and life-saving medical practice.

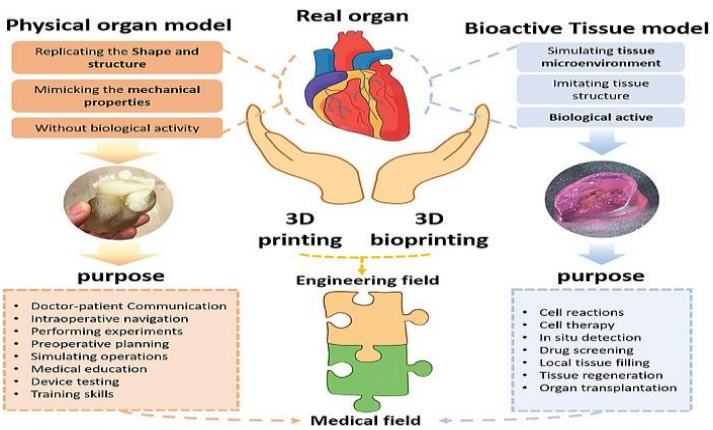

Fig. 1: The difference between organ model and tissue model (e.g., tissue/organ construct. Reproduced with permission.[15] Copyright 2019, IOP Publishing), where 3D printing and 3D bioprinting play their respective roles.[6]

ADVANTAGES AND DISADVANTAGES

Advantages

- Removing the need for organ donors: Instead of relying on organ donors, 3D organ printing would allow patients to acquire an organ in a matter of days.
- Building a microenvironment: Membranes, biological latex, and biotapes can all be made via bioprinting in addition to artificial organ construction. These can be utilized to develop a microenvironment and simulate the human body in real time.
- Minimal organ rejection—Because bioprinting uses cultivated cells or tissue, the rejection rate will be low. By doing this, it would be made sure that the organ would not be rejected after surgery. Why 3D bioprinting for tissue repair is not just for making organs. Additionally useful for tissue grafting and repair, this method.
- Teaching – Another fantastic application of this technology is the provision of organs and tissues to physicians or medical students so they can perform surgery and gain a deeper knowledge.
- Research – Bioprinting is useful in research as well. There would be less need to use animal sacrifice or do human or animal experiments if scientists or pharmacists studied numerous diseases and their pathogenicity or the effects of medications on the organs.

Disadvantages

- Lack of accessibility – 3D printing requires appropriate technology and tools, which are difficult to obtain, just as other technologies. Why Affordability: For the majority of individuals, organ printing may not be the most cost-effective technology.
- The hard process of creating full organs and the ongoing evolution of complicated organs like the heart, liver, and pancreas pose the biggest obstacles to the development of organ printing.
- Authorized and skilled individuals, as well as highly qualified establishments, are essential but have not yet been established.
- Many beneficiaries lack the time necessary for the process.
- Moral and ethical issues.[7]

APPROCHES OF 3D BIOPRINTING

1) Biomimetic
2) Self autonomous assembly
3) Mini tissue

Biomimetic

Otto Schmitt created the term "biomimetics" in the 1950s to describe the transfer of concepts and analogs from biology to technology.[8] The Greek words "bios" and "mimesis," which mean "life" and "to imitate," are the source of the term "biomimetics."[9] Biomimetic is the technique that entails the creation of composite materials that imitate the properties and/or structures of various natural materials.[9] Making human tissues and organs with biological properties for therapeutic use via 3D bioprinting is still difficult. To meet this challenge, several important concerns, including the creation of biomimetic microenvironments, need to be properly addressed. The capacity of 3D printing to construct structures that resemble native tissues as well as biomimetic microenvironments has been widely demonstrated.[10] Understanding the microenvironment, cell types, substances, ECM composition, and biological forces is crucial for micro-scale tissue replication. Research in engineering, imaging, biomaterials, cell biology, biophysics, and medicine can contribute to this knowledge foundation.[11] creation of microstructures For the purpose of developing artificial tissues with biological functions, mimicking native tissues is essential. For example, 3D bioprinting has been used to organize various cell types to produce lamellar and complex tissues and to produce vessel-like networks to provide cells with initial nutrients and oxygen.[12] For application in the medical business (such as biosensing and tissue engineering), biomimetic medicinal materials are biocompatible and/or biodegradable substances that have been developed by carefully observing nature's models and then mimicking their natural designs and processes.[9]

Self autonomous assembly

Autonomous self-assembly is a process that duplicates a particular organ or tissue in vitro, much like embryonic organ development. Early cellular components in a tissue's development create extracellular matrix elements and suitable cell signals, which result in the tissue's autonomous structure and patterning. It is possible to employ cells for histogenesis and to modify the composition, location, functional, and structural features of the tissue using autonomous self-assembly, as previously mentioned. To successfully accomplish this, it is crucial to have a thorough understanding of the mechanisms underlying embryonic organogenesis and the aptitude to control the environmental factors that govern these systems.[5] [3] [13] In early embryonic development, morphogenesis refers to the carefully regulated spatial organization of cells that gives rise to tissues and organs. While physical mechanisms play a crucial role in the development of specialized biological structures with a

particular shape, morphogenesis is strictly governed by genetic factors. To create replacement organs for regenerative medicine, tissue engineering (TE) seeks to replicate morphogenesis in the lab or in vitro. By seeding and growing cells in suitably formed biocompatible scaffolds, tissues and organs are traditionally generated, with the intention that the maturation process will provide the required structure. We set up and deployed an innovative TE approach based on developmental biology principles that uses bioprinting, the automated delivery of cellular composites into a living organism, to achieve this goal more organically and effectively.[14] Early cells in a tissue's development create their own ECM components, the proper cell signaling, and autonomous organization and patterning to achieve the correct biological micro-architecture and function.[15]

Mini tissue

The mini tissue building block methodology makes use of both of the earlier methods.Mini-tissues, or tiny functional organ and tissue units, are created using this bioprinting technique.The smallest structural and operational unit of the organs, such as the kidney neuron, is represented by the tiny tissues.Then, either autonomous self-assembly or biomimicry can be used to create these mini-tissues.Using physiologically inspired organization, mini-tissues are first assembled into macro-tissues in the bioprinting process. Next, tissue units that can self-assemble into useful structures are reproduced.[5][16][11]

PROCESS

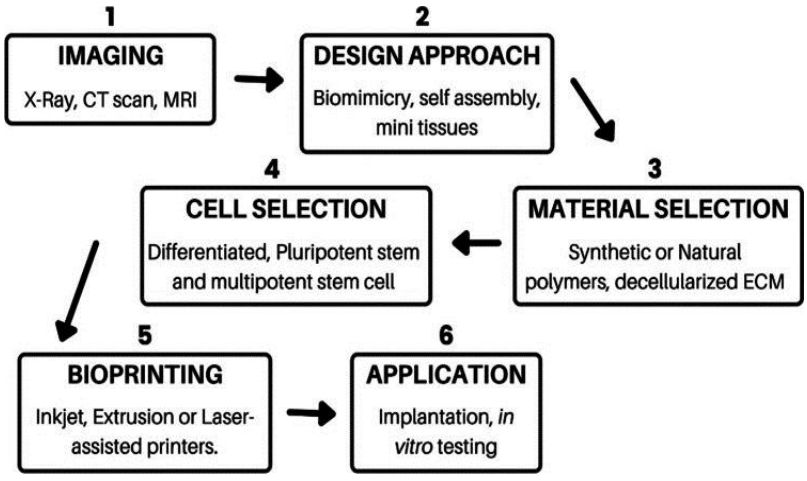

Fig. 1: Steps in 3d Bioprinting.[17]

Organ printing incorporates three consecutive steps are as follows:

Pre-Processing, which involves creating "blueprints" for organs

Processing, which involves printing the organs

Post-Processing, which involves conditioning the printed organs and accelerating their maturity.[1]

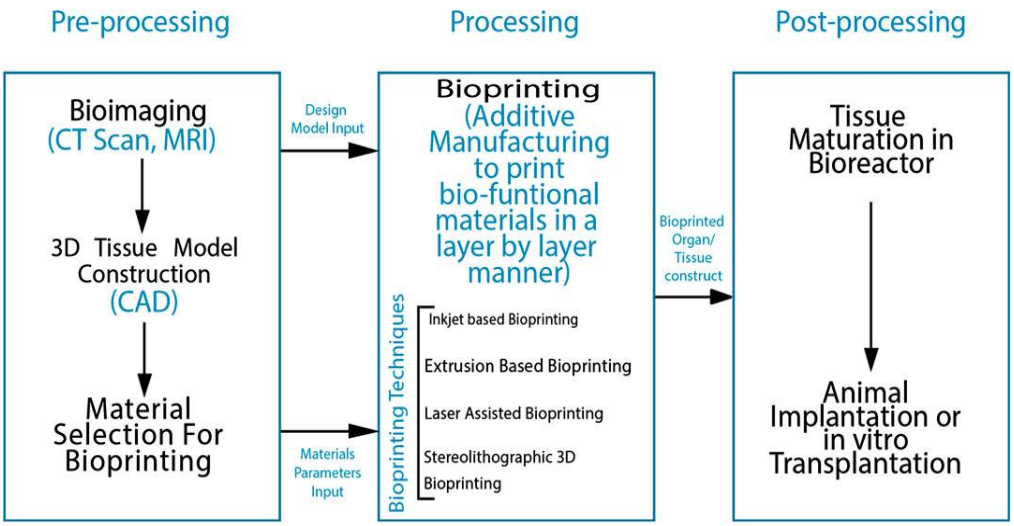

Fig. 2: General overview of process.[18]

Pre-bioprinting

Pre-bioprinting is the process of selecting the materials to be utilized and designing a model that will be used by the printer to make the final product. Getting an organ biopsy is one of the initial stages.[19] The target tissue or organ's 3D anatomical structure and function are first recorded during the pre-processing phase utilizing medical imaging technologies, often noninvasive magnetic resonance imaging (MRI) and computed tomography (CT). Using computer-aided design/manufacturing (CAD/CAM) tools (such as SOLIDWORKS, Autodesk 123D, and CATIA), imaging data obtained from these noninvasive modalities is then converted to 3D computational models.[20] Tomographic reconstruction is performed on the images before printing using a layer-by-layer method. After that, the printer is used to create the now-2D images. Certain cells are separated and multiplied following the image's creation.[19] Then, these cells are combined with a unique liquid substance that supplies them with oxygen and other nutrients to maintain their viability. In some procedures, 500 m in diameter cellular spheroids surround the cells. The placement of these cells in the tubular-like tissue fusion necessary for processes like extrusion requires them to aggregate together without the aid of a scaffold.[12]

Processing

The actual printing of the tissues using additive manufacturing methods is the processing step.[18] When creating biological constructs with 3D bioprinting, cells are often dispensed

onto a biocompatible substrate in order to produce tissue-like three-dimensional structures. It has been demonstrated that 3D bioprinted artificial organs, such as livers and kidneys, lack essential components that have a significant impact on the body, such as functional blood arteries, tubules for collecting urine, and the proliferation of billions of cells necessary for these organs. Without these elements, the body is unable to obtain the vital nutrition and oxygen needed for its deep interior.[2] Given that every tissue in the body is made up of several cell types naturally, the ability of various printing methods to maintain the stability and vitality of the cells during the production process varies. Photolithography, magnetic 3D bioprinting, stereolithography, and direct cell extrusion are a few of the techniques utilized for 3D bioprinting of cells.[21]

Post-processing

According to Papaioannou et al. (2019), post-processing is the maturation of the manufactured construct in a bioreaction and its structural and functional characterisation.[18] In order to produce a stable structure from the biological material, post-bioprinting is required. The mechanical integrity and functionality of the 3D-printed product are at risk if this process is not kept up with.Mechanical and chemical stimulations are required to maintain the object. These stimuli cause the cells to release chemicals that regulate tissue growth and remodeling. Additionally, recent advancements in bioreactor technologies have made it possible for tissues to mature quickly, to become vascularized, and to be transplant-survivable.

Convective nutrition transfer, the creation of microgravity settings, pressure changes that cause solution to flow through the cells, or the addition of compression for dynamic or static loading are all ways that bioreactors function. Different types of tissue respond well to different types of bioreactors, such as compression bioreactor are ideal for cartilage tissue.[21]

TECHNOLOGIES IN 3D BIOPRINTING

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

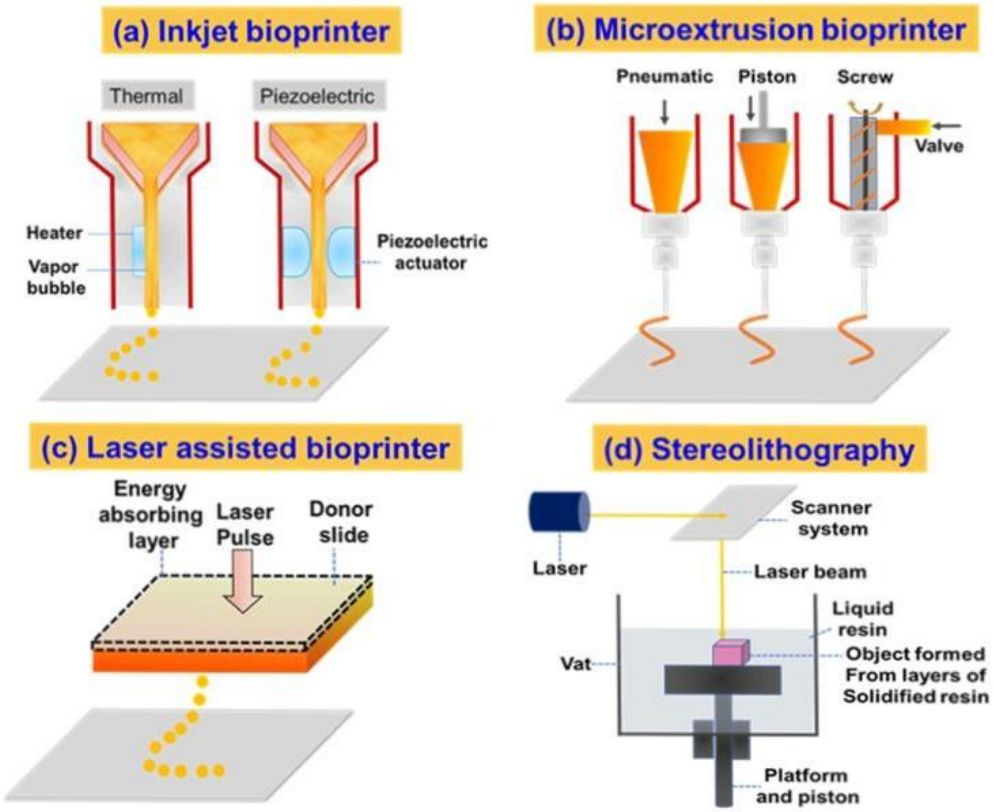

Fig. 3: Schematic representation of a) inkjet bioprinting, b) microextrusion bioprinter, c) laser assisted bioprinter &d) sterolithography.[22]

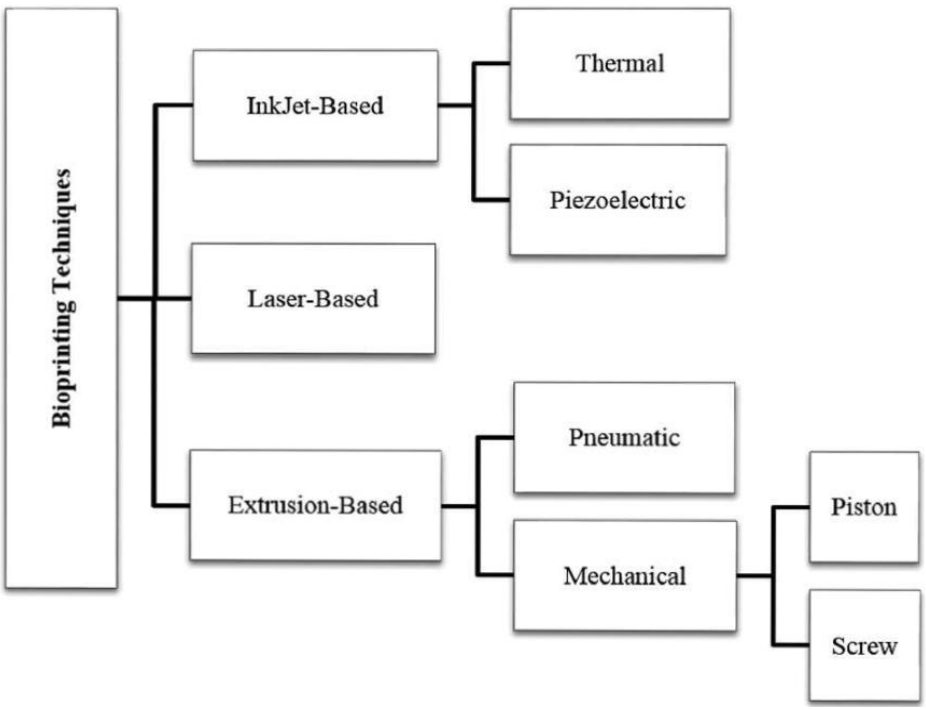

Fig. 4: Classification of bioprinting technology.[23]

Extrusion based bioprinting

The first study in the context of EBB involved printing living cells using the bioplotting method, which produced great flexural and mechanical strength and a high rate of cell multiplication by extruding hydrogel bioink into a liquid solution. Later, the developing technology attracted a lot of attention and developed quickly.[24] Extrusion-based printing methods use a constant stream of material.[25] Theoretically, extrusion-based bioprinting uses mechanical or pneumatic drive to extrude bioink (often from a syringe) via a nozzle to create continuous micro filaments that are then deposited on a receptive substrate and finally stacked into the required structures. The substrate might be either solid (like a culture dish), liquid (like a growth media), or something made of gel. After configuration, software often generates the nozzle's path based on digital models. The final bioprinted structures would be influenced by factors like temperature, nozzle diameter, extrusion pressure, movement speed, extrusion speed, route interval, etc.[26] Extrusion bioprinting may be printed on a variety of materials because to three key factors:

a) Viscosity adjustability

b) the bioink phase before extrusion

c) biofabrication window for a particular material.[28]

Common extrusion-based bioprinting can be categorized into a) pneumatic, b) piston, and c) screw-driven actuation modes, which correspond to the various liquid dispensing systems.[26]

a) Pneumatic:- A pneumatically driven extrusion device uses compressed air to dispense liquid bioink, typically a syringe filled with bioink attached to an air pump. Hydrogels with shear-thinning properties work well with pneumatic systems. To reduce contamination, use a filter on the airway and ensure smooth extrusion with additional liquid or gel-based medium.[26]

b) Piston driven extrusion:- Mechanically powered liquid dispensing systems are ideal for extrusion of highly viscous biomaterials like high-molecular polymers. Piston-driven extrusion, with related products like micro-infusion pumps, uses a guiding screw to force bioink out of the nozzle.[26]

c) Screw driven extrusion:- Screw-driven devices provide volumetric control and aid in extrusion of biomaterials with higher viscosities. They work similarly to piston-driven systems but may harm cells loaded with bioink and increase pressure, requiring careful design.[26]

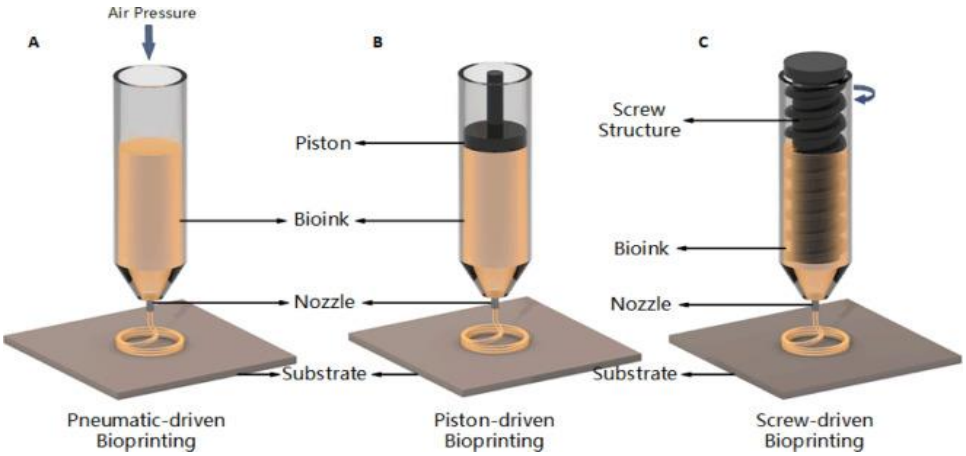

Fig. 5: Types of EBB printing.[26]

Phases of EBB bioprinting

Phase I) involves applying force to start and maintain extrusion,

Phase II) involves extrusion and filament formation

Phase III) involves 3D deposition under the control of the robotic arm

Phase IV) involves crosslinking bioprinted constructs to ensure mechanical integrity.[28]

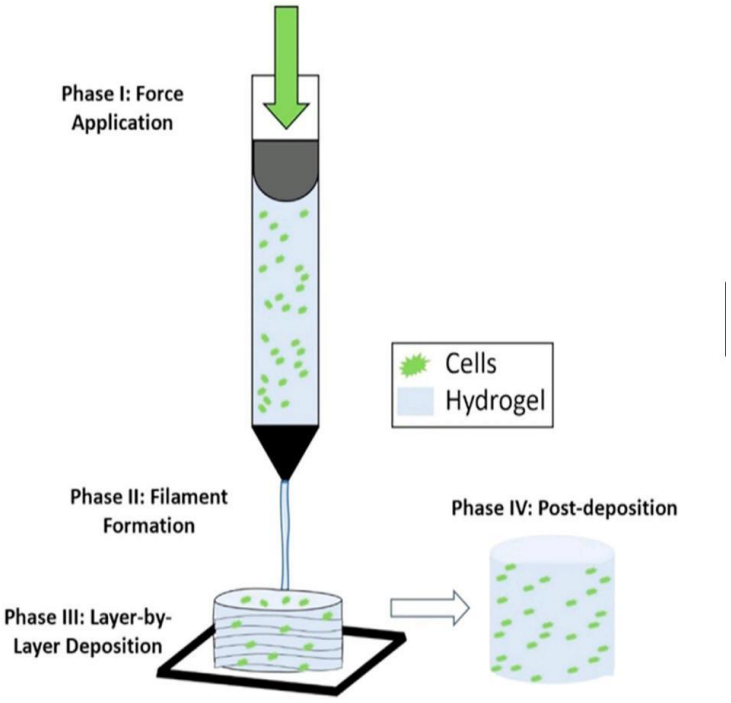

Fig. 6: Phases of EBB.[28]

Generally, extrusion-printed scaffolds have higher structural stability. A high viscosity cell suspension could be printed using the microextrusion process. Nevertheless, as compared to

inkjet and laser bioprinting, its resolution is quite low. Stress-induced cell deformation and a small range of materials are two further restrictions.[29]

Inkjet bioprinting

In this technique, a "bio paper" made of a hydrogel substrate, a culture dish, a polymer construct, etc. is covered with "bioink," which is basically a low-viscosity suspension biomaterial containing live cells, etc. The printing is done in a pattern that is digitally controlled using the AM process, which is a non-contact printing method.[18]

Types of inkjet bioprinting

a) **Thermal inkjet bioprinting:-** A heating element and an ink chamber with a few nozzles make up the thermal inkjet technology. A brief current pulse is supplied to the heat element to create an ink droplet. Ink is forced out of the nozzle orifice by a bubble that forms when the temperature of the ink near the heating element rises.
b) **Piezoelectric inkjet printing:-** Piezocrystals are used in piezoelectric inkjet printing and are found at the back of the ink chamber. The crystals are given an electric charge, which makes them vibrate. A small amount of ink is forced out of the nozzle by inward vibration.[23]

Ink-jet printing can be done in two different ways: continuously (continuous ink-jet printing) and drop on demand.

I) Continuously inkjet bioprinting

By exerting pressure on the bioink and forcing it out of a nozzle, continuous ink-jet printing creates a continuous jet of droplets. The jet of bio-ink is then redirected onto the substrate by an electric field that is applied next. The extra droplets that don't follow the necessary pattern are diverted in the direction of a gutter, where they are collected and recycled.[18]

II) Drop on demand inkjet bioprinting

The technology used to create droplets in drop-on-demand inkjet printing is identical to CIJ, with the exception that the droplets are only produced when needed. Therefore, a pressure pulse rather than a constant pressure is employed to drive the droplets out.[18]

High throughput, high resolution, cheap cost, reproducibility, and ease of use are all benefits of inkjet printing. Additionally, it is simple to adapt inkjet printers to print cells and biomolecules. However, to handle and print biological materials with increasing resolution,

accuracy, and speed, specially developed inkjet printers were employed.[30][31] Thermo-mechanical stress exposure of cell materials, non-uniform droplet size, low droplet directionality, frequent nozzle clogging, and unstable cell encapsulation are some of the drawbacks of thermal inkjet printing.[31]

Pressure assisted bioprinting

As more and more affordable systems become commercially available, pressure-assisted systems are increasingly being used by various research groups studying bioprinting. These systems typically come with one or more cartridges that can be used to dispense various cell and biomaterial combinations. Selected biomaterial inks or bioinks are placed within plastic or glass cartridges. The material is ejected in the form of a filament through a needle or nozzle by applying gas pressure. The resolution in this situation is typically determined by the type of material, gas pressure, nozzle diameter, and deposition speed. As the shear stress increases with decreasing nozzle diameter and typically impacts cell survival, high resolution is challenging to accomplish. With these systems, the typical strand diameter ranges from 200 micrometre to millimetre.[32] Extrusion is the foundation of pressure-assisted bioprinting (PAB), which uses it to produce desired 3D patterns and constructions. The biomaterials used for printing are typically solutions, pastes, or dispersions that are extruded onto a stationary substrate by synchronizing the motion of pneumatic pressure, plunger- or screw-based pressure, in the form of a continuous filament through a microscale nozzle orifice, or a microneedle. Complete 3D patterns and constructions are eventually produced after layer by layer application.[4] Direct integration of cells, room temperature processing, and homogeneous cell distribution are all benefits of PAB technology. The use of PAB for the printing of cells and organs has been demonstrated to retain activity.[4]

Laser assisted bioprinting

Light-assisted bioprinting platforms are being utilized more frequently for cell printing and tissue engineering applications in addition to inkjet printing and extrusion-based printing technology. These technologies can print several cell types with high cell viability and primarily use photo-polymerization of biomaterials. Laser-based printers and digital light processing (DLP)-based printers are the two subcategories of light-assisted bioprinting devices.[33]

Four part make up this system

a) Receiving substrate

b) Metallic ribbon film that absorbs laser energy
c) Pulsed laser source
d) Laser focusing tool

The construction ribbon consists of two layers, with the upper layer, which absorbs energy, being primarily glass covered in a thin titanium and gold coating.

Continuation of the production process:- With the aid of film evaporation on the upper layer, the laser pulse focuses on the intended location. At the interaction with the suspended bioink at the bottom layer, a high-pressure bubble is created. The bioink is subsequently released in droplet form onto the receptive substrate. An elevator system is used to sustain the z-axis movement. Notably, despite the lack of nozzles, this printing process can use a wide range of materials, including cell-encapsulated materials, ceramic materials, hydrogels, and the epoxide-based photoresist SU-8.[17]

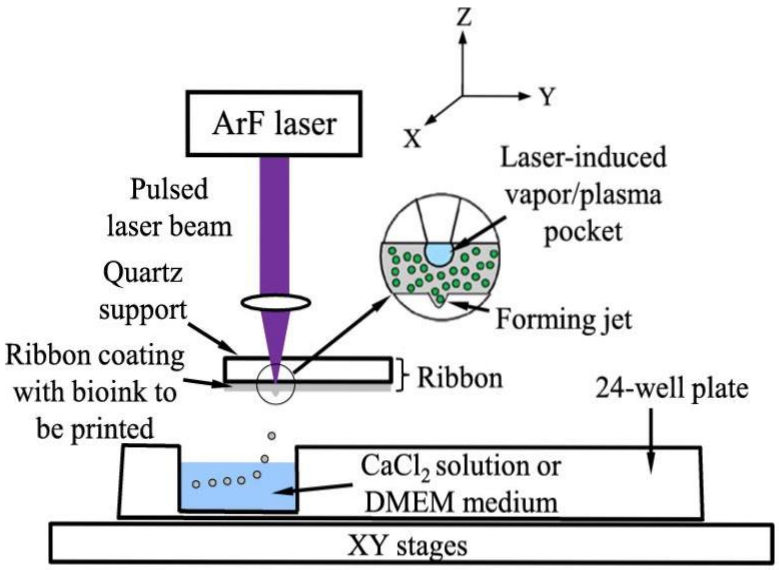

Fig. 7: Experimental setup of laser assisted bioprinting.[34]

Stereolithography

Both Kodama (1981) and Marutani et al. (Nakai et al. 1986) introduced and advanced stereolithography at the same time (in the 1980s), despite working independently.[35] This approach and laser-assisted bioprinting are closely related: Light stereolithography printing uses ultraviolet (UV) to selectively harden the photosensitive polymer layer by layer, resulting in the creation of a multiplex structure. A computerized design that resembles a building is first printed. Computed tomography (CT) scan and magnetic resonance imaging

(MRI) pictures are translated into the structured organ structure in the stereolithography file.[17][35] STL is a top-down process used for polymerizing light-sensitive polymer materials, regulating light intensity using digital micromirror arrays. It is used to create cured structures from epoxies and acrylics, with high fabrication precision and the ability to layer-by-layer print hydrogels.[4] The use of UV-sensitive photoinitiators has been discontinued because UV damage to cellular DNA and skin cancer. A commercial beam projector, bioinks made of a combination of PEGDA, methacrylated gelatin (GelMA), and an eosin Y-based photoinitiator were all used in Wang's 2015 investigation into the use of tailored visible light in an STL system. They began by outlining the thorough protocol of the visible light-based STL system and highlighting the requirement for an IR-filtering water filter for the system. The researchers' experiments using NIH 3T3 cells showed that this system with tailored visible light was capable of supporting the bioprinting of visible light-curable hydrogels with a 50-m resolution and high cell survival (85%) for at least 5 days.[4] There are many limitations, including the absence of suitable biocompatible and biodegradable polymers, the detrimental effects of lingering toxic photocuring reagents, the impossibility of totally removing the supporting structure, and the impossibility of forming horizontal gradients in the structures.[4]

BIOMATERIAL AND BIOINK

Hydrogel, metallic, ceramic, polymeric, and composite materials can all be classed as biomaterials. The best printing method is determined by the physical properties of biomaterials. For instance, low-viscosity materials are more desirable for bioprinting because cells can thrive in the environment of low pressure. The encapsulated cells are influenced by a variety of material characteristics, including pore size and interconnectivity.[4]

Parameters

a) Mechanical properties
b) Porosity and interconnectivity -Pore shape, volume, size and geometry
c) Biocompatibility

In order for the final tissue structures to be precisely represented, the bioprinting materials (1) need to be strong and long-lasting to assure high-quality forms of the created pieces, and (2) need to have qualities similar to those of living tissue.[22]

Table 1: Common material used in bioink and Gel Formation Mechanism.[36]

Compound	Mechanism of gel formation	Chemical structure
Agar	Thermal	Polysaccharide
Collagen	Spontaneous gelation Photoinitiation	Protein
Alginate	Ionic	Polysaccharide
PLGA-g-PEG	Thermal	Poly(lactic-co-glycolic acid)
PEGDMA	Thermal/chemical	Poly(ethylene glycol) dimethacrylate
Pluronic	Thermal	Poly(ethylene glycol)
Agrose	Thermal	Polysaccharide
Carrageenan	Thermal	Polysaccharide
Fibrin	Spontaneous gelatine	Protein
Elastin	Photoinitiation	Protein
Silk	Photoinitiation	Protein
Chitosan	Chemical	Polysaccharide
Hyaluronic acid	Chemical	Glycosoaminoglycan
NIPAAM	Thermal	N-isopropyl Acrylamide

Bioink

In 2003, along with the phrase "biopaper," the term "bioink" was first used in relation to organ printing. The original idea was to create or even print a biopaper (hydrogel) and then use bioprinting to insert living cells or tissue spheroids as the "bioink." Therefore, the cellular component that was positioned in three dimensions (3D) on or within hydrogels was what the word "bioink" initially alluded to. Cells and cell aggregates were the bioink in numerous groundbreaking experiments in the field.[37] The cell viability during 3D bioprinting is directly impacted by the print speed, print pressure, and moving distance. To keep the cells viable, researchers combined them with bioink. Bioink's primary function is to load cells and give them an environment of external support akin to the extracellular matrix during printing. Selecting bioink that is appropriate for bioprinting that has strong mechanical, biodegradable, and biocompatible qualities is crucial.[38] The viscosity, surface tension, and cross-linking characteristics are the three factors that make up bioink. Print accuracy and cell loading capacity are significantly affected by these variables. Consider viscosity as an example; the polymer solution has a greater viscosity with poor fluidity, allowing for the long-term preservation of form structure and excellent cell-binding properties. High-viscosity solutions, however, also call for more pressure while impairing cellular activity.[38]

Material for bioink

Natural:- Sodium alginate, silk fibroin, chitosan, and collagen.

Synthetic:- Polycaprolactone (PCL), polyethylene (poly(ethylene glycol) [PEG]), and hydroxyapatite (HA).[38]

The right mechanical, rheological, chemical, and biological properties are what a bioink should have in order to be considered optimal.These characteristics should enable the creation of tissue constructs with the following benefits: (i) sufficient mechanical strength and robustness while maintaining tissue-matching mechanics, preferably in a tunable manner

(ii) adjustable gelation and stabilization to support the bioprinting of structures with high shape fidelity

(iii) Biocompatibility and, if necessary, biodegradability mimicking the natural microenvironment of the tissues

iv) suitability for chemical modifications to meet tunable requirements

Table 2: Summary of the most recent studies on bioinks.[43]

Bioink type	Application	Outcomes
Gelatine-based bioinks	A resource for bioink designing	Influence of parameters on printability
Hyaluronic acid	Tissue engineering	Dual-crosslinking hyaluronic acid hydrogel
Hyaluronic acid	Cartilage bioprinting	Development of 3D bioprinted tissues
PU-gelatine	Muscle unit	Complex tissue for muscle-tendon
Alginate-chitosan	Neural tissue	3D neural tissue construction
Collagen-gelatine	Tissue engineering	Print of cell-laden hydrogel
Hyaluronic acid-gelatine	High viable liver construct	Development of stable bioinks
Collagen	Tissue engineering	Novel bioinks of various tissues
Collagen-chitosan	Tissue engineering	Influence of printing parameters on quality
Gelatin-methacrylamide	Tissue engineering	Improvement of mechanical and rheological properties
Alginate-hyaluronic acid	Tissue engineering	Bioprinting and cartilage matrix development
Alginate-PCL	Tissue engineering	Investigation of layer effect on cell proliferation in-vitro and in-vivo
PEG-gelatine	Tissue engineering	Characterization of gel-phase bioinks
PLA-gelatine	Living tissue constructs	Development of gelatin-based bioinks
Gelatine-PE	Tissue engineering	Development of bioinks using FFF process
Alginate-PCL fibbers	Bone organ engineering	Mechanical in-vitro and in-vivo analysis
Alginate	Tissue bioprinting	Development of tissue strands as bioinks

APPLICATION

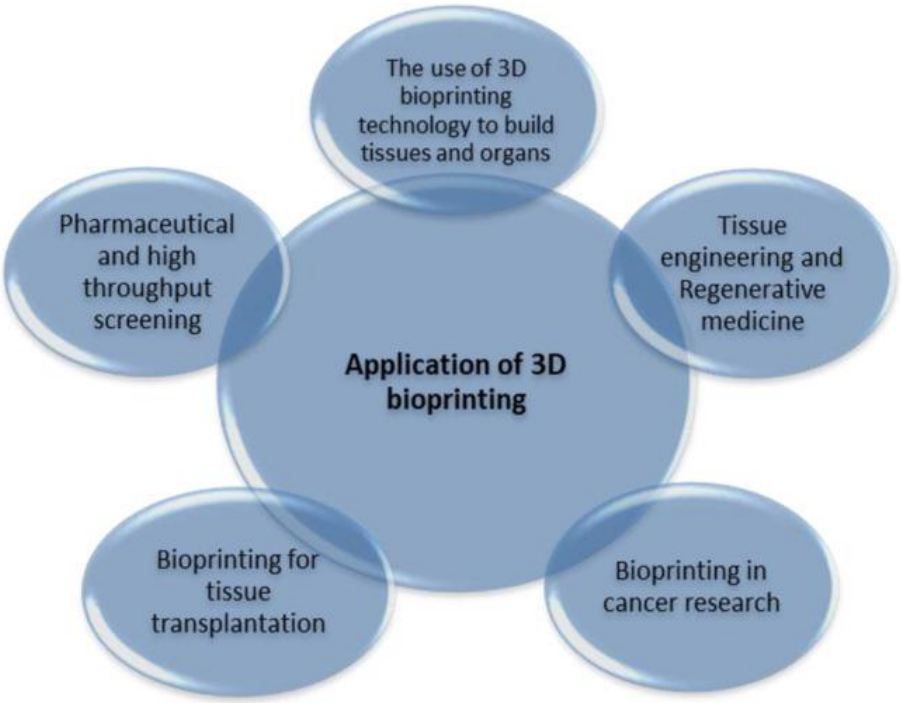

Fig. 8: Application Of 3d Bioprinting.[17]

USE OF 3D BIOPRINTING IN RESEARCH RELATED TO THE SKIN

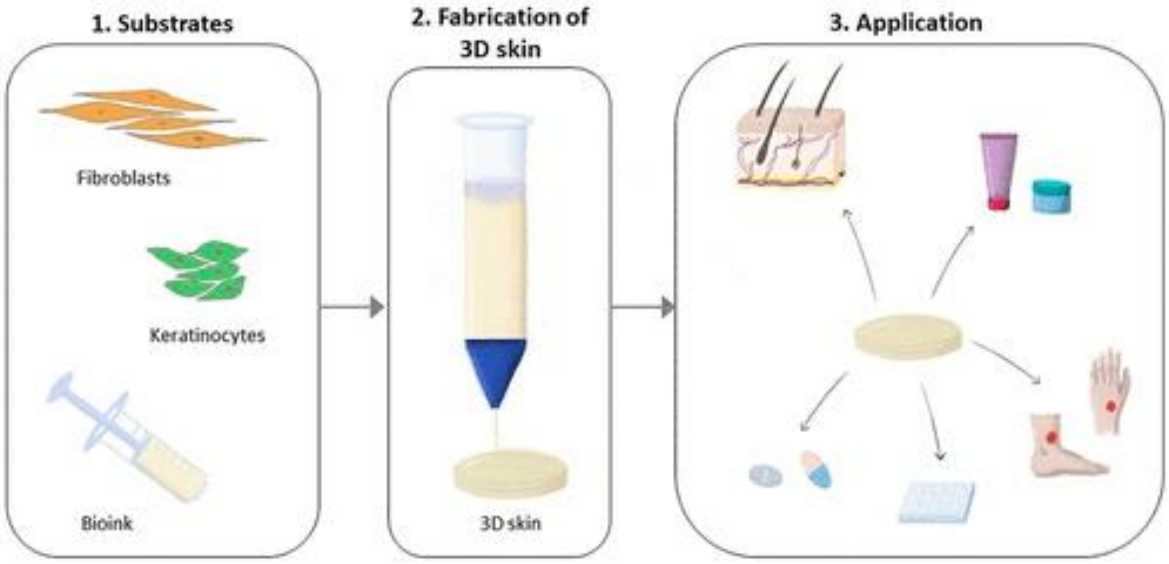

Fig. 9: Overview of 3d skin bioprinting technique.[39]

Artificial skin made using human bioengineering may be employed in a variety of therapeutic and academic settings. The need for skin biofabrication is still increasing due to the growing popularity of cosmetic/aesthetic surgeries, as well as the increased prevalence of obesity, diabetes, and aging populations.

Skin bioprints are proposed as an alternative strategy for the following:

- Clinical applications of regenerative medicine (chronic wounds, burn injuries, ulcerations, reconstructive surgery following significant oncological resections).
- Modeling physiological and pathological circumstances, including wound healing, UV response, aging, skin barrier permeability, drug reactivity, photoirradiation, skin cancer, genodermatoses, and inflammatory diseases.
- The cosmetic/pharmaceutical business (drug metabolization, drug absorption, and safety of active ingredients).[39]

RECONSTRUCTION OF BODY TISSUE

It served as a foundation for additional uses. Reconstructing essential biological tissues is made easier with 3D bioprinting.[40]

CONVERTIBLE ORGANS

Due to the fact that 3D bioprinting may reconstruct various kinds of body tissues. The right environment is given to these newly created tissues so they can continue to divide and develop into organs. Hence offering artificial organs for transplants and procedures with natural-looking characteristics.[40]

Case study: A tracheal splint was administered to a baby patient who had tracheobronchomalacia a few years ago. It is claimed that the tracheal splint is a result of 3D bioprinting. The procedure was successful, saving the baby. Because of this, 3D bioprinting is viewed as a windfall for the medical industry.[40]

ORGANIZATIONS FOR RESEARCH

The success of 3D bioprinting in the production of biomaterials and organs. A new era of clinical research is thus inaugurated. The organs are used to explore the interactions and responses between drugs and organs.

For instance, in 2019, Israeli scientists created a heart the size of a rabbit using human cells.[40]

BIOFABRICATED IN VITRO MODELS FOR STUDYING INFECTIOUS DISEASES

The lack of experimental models hinders the rapid understanding of infection pathophysiology and the development of effective medications, as they cannot accurately

simulate human-pathogen interactions, resulting in low dependability.Although conventional in vitro human cell culture platforms are affordable, they have difficulty faithfully recreating complicated human organ responses. To bridge the gap between traditional models and human infectious diseases, advanced models, such as microfluidic organs-on-chips, are emerging. However, techniques based on 3D bioprinting have not yet produced reliable results. This section discusses technology for creating in vitro human tissue models, such as organoids, microfluidic organs-on-chips made with soft lithography, and 3D bioprinting options.[41]

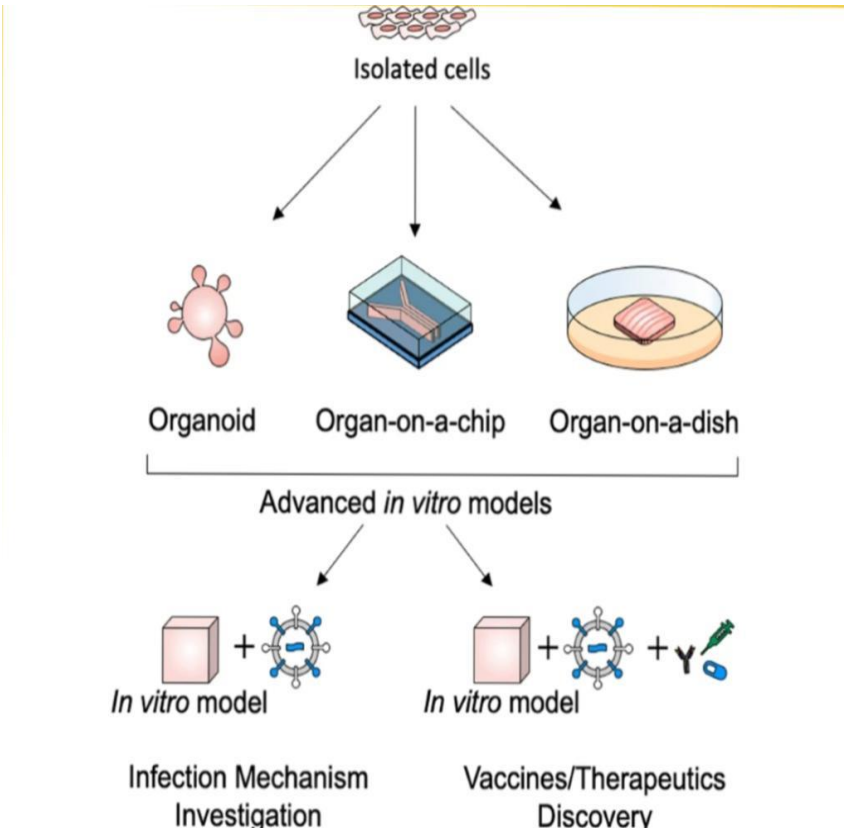

Fig. 10: Modern in vitro models are utilized in research on infectious diseases to determine the causes of infection and effective vaccines and treatments.[7]

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Tissue engineering and regenerative medicine (TERM) are the integration of medicine and bioengineering, focusing on restoring tissue functionality to the body. Tissue regeneration involves implanting cells and biomaterials into the body, promoting tissue growth and supporting self-healing abilities. Stem cell technology is often used in biomaterials. Tissue engineering has potential to increase successful outcomes in organ systems where

regeneration is biologically unable to occur. It can also be used to regenerate tissue using our own cells, addressing genetic or acquired tissue defects.[41]

CANCER INVESTIGATION

The development of 3D bioprinting for use in several medical fields has made it a viable approach in cancer research. The creation of 3D bioprinted models is one of the potential strategies that researchers have been exploring recently to get over the restrictions that currently exist in this sector. For instance, scaffold-free cellular spheroids can mimic the in vivo milieu of cancer cells due to their biocompatibility, which makes them a startlingly important component of cancer investigations. A synopsis of the most significant and recent studies has been offered in order to give readers a better understanding of the research that has been done in the use of 3D bioprinting in cancer investigations.[43]

CHALLENGES

However, 3D printed organ models are still only being used on a small scale, and the following are some major obstacles to their wider application:

1) Expensive, both in terms of time and money. Commercial 3D printers, materials, and required software can be quite expensive, and labor-intensive techniques for segmenting images and building models can take several days to complete and take a lot of practice.[44]
2) A constrained simulation feature. Current materials still struggle to accurately replicate soft tissue.a few soft materials that resemble well but are unsuitable for 3D printing directly.[44]
3) Poor manufacturing precision. The resolution of images and 3D printing has a significant impact on accuracy. A 3D printer has a finite amount of printing space accessible.[44]

Bioprinting technology, despite significant advancements in the last decade, faces challenges such as resolution, repeatability, cell viability, practicality, and biocompatibility. It is promising for advancing tissue and organ fabrication for transplantation, mitigating organ shortages, and saving lives. However, there are still challenges in developing a standardized scalable fabrication method for robotic cell delivery, improving nozzle and cartridge design, increasing the diversity of bioprocessable and functional bioink with high cell density, printing intraorgan branched vascular trees, developing enabling technologies for multibioink multiscale hybrid bioprinting processes, and developing accelerated tissue maturation technologies.[23]

4D bioprinting is a promising direction for fabricating living tissues in a shorter period of in vitro culture time. It allows for rapid fusion, folding, and remodeling of cell-aggregate-based bioinks, enabling bioprinting in the fourth dimension "time." However, there is still much research needed to accelerate tissue maturation further. Integrating well-connected capillaries inside the bioink will be another milestone towards functional tissue and organ printing. This will advance the tissue folding and remodeling process, improve cell viability, and enable larger-scale models.[23]

Regenerative medicine and tissue engineering have been greatly impacted by 3D bioprinting. The development of 3D tissue constructions with heterogeneous structures that mimic the architecture of biological tissue and organs is made possible by advancements in computer science, tissue engineering, and nanotechnology. Engineering design, bioink development, and the creation of printable and biocompatible biomaterials are among the difficulties. dECM-derived hydrogels are intriguing biomaterials for printing, however creating capillary networks is still difficult. Integrated organ printing is still in its infancy. Scriptable tissue scaffolds can be made using smart polymers.[29]

CONCLUSION

In conclusion, 3D bioprinting of tissue and organs represents a groundbreaking technology with the potential to revolutionize medicine and healthcare. The bioprinting process, which involves layer-by-layer deposition of living cells and bioink, offers a promising avenue for creating complex, patient-specific structures. The bioink, often composed of cells, biomaterials, and growth factors, plays a critical role in achieving successful bioprinting outcomes.

The applications of 3D bioprinting are vast and include tissue engineering, regenerative medicine, drug testing, disease modeling, and personalized medicine. This technology has the potential to address the organ transplant shortage by producing functional replacement organs. Furthermore, 3D bioprinting allows for the creation of intricate tissue models for drug testing, reducing the need for animal testing and enabling more precise drug development.

Advantages of 3D bioprinting include its ability to create complex, patient-specific tissues and organs, potentially reducing rejection rates in transplant recipients. It offers a non-invasive alternative to traditional surgical procedures, thereby reducing patient risk and

recovery time. Additionally, it can be used to study disease processes in a controlled environment.

However, there are certain disadvantages and challenges associated with 3D bioprinting, such as the need for optimized bioink formulations and biocompatible materials. The technology is still in its infancy and faces regulatory hurdles. Furthermore, the cost of bioprinting and the time required for printing complex structures can be substantial.

In conclusion, while 3D bioprinting holds immense promise for the future of medicine, it also presents several challenges. With continued research and development, overcoming these challenges can lead to more widespread adoption and the realization of its full potential in improving healthcare and addressing critical medical needs.

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