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3D BIOPRINTING: A COMPREHENSIVE REVIEW

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Abstract : The Three Dimensional (3D) bioprinting, an innovative and interdisciplinary technology, holds immense potential for revolutionizing various fields, including tissue engineering, regenerative medicine, drug discovery, and personalized healthcare. This review article provides a comprehensive and critical overview of recent advancements in 3D bioprinting, covering key aspects such as bioink development, printing techniques, scaffold design, cellular behavior, and clinical applications. The discussion encompasses the state of the art in bioink materials, including natural and synthetic polymers, as well as the incorporation of cells and growth factors to replicate tissue-specific microenvironments. Various 3D bioprinting techniques, including extrusion-based, inkjet, and stereolithography, are analyzed for their advantages, limitations, and potential applications. Additionally, the role of scaffold design and its influence on cell behavior, tissue maturation, and vascularization is explored. Furthermore, this review assesses the integration of advanced imaging technologies and computational modeling in optimizing 3D bioprinted constructs. In-depth insights into cellular responses, tissue maturation, and integration within the host environment are discussed, highlighting the necessity for enhanced understanding and control of these processes for successful translation into clinical practice. In conclusion, this comprehensive review provides valuable insights into the current state of 3D bioprinting, highlighting its transformative potential in healthcare and research. It underscores the need for interdisciplinary collaboration, ongoing innovation, and ethical guidance to harness the full capabilities of 3D bioprinting for the benefit of humanity.

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IndexTerms **– 3D Bioprinting, tissue engineering, inkjet, regenerative medicine.**

INTRODUCTION

Tissue damage, degeneration, and regeneration are the basic nature of the human body. However, sometimes the regeneration capability will be insufficient. The classical method to treat that condition is organ or tissue transplantation, which is completely dependent on the availability and access of the donor. Even if a donor is available, the blood group must be compatible, or else there are high chances of graft rejection, which is fatal. As solutions, tissue engineering and regenerative medicine are making rapid progress. In the field of tissue engineering, additive manufacturing is among the most advanced techniques.**[1]** Tissue engineering has been developed since the 1980s, combining components like clinical medicine, biomaterials science, cytobiology, molecular biology, and bioengineering. Its main goal is to replace the organ or tissue that has been damaged by creating a new one. Its main usage is in the manufacture of tissue, organs, and stents used in tissue engineering. **[2]** In the 1980s, Chuck Hull brought 3D printing and tissue engineering into the limelight. Although it was introduced in the 1980s, the medical field saw the emergence of 3D printing as a viable technology only in the 21st century.**[3]** When printable materials are used for the manufacture of these strong 3D structures, the respective printable materials must satisfy all the required rheological properties, such as flow rate and response to stress and strain, to allow techniques like extrusion and solidification. Because the structure will be implanted in a human body, it must be manufactured with biocompatible and biodegradable materials that can support regeneration and appropriate functionality, as that is its sole purpose. Cell seeding onto a porous structure is technically challenging. Especially static cell seeding techniques are found to be inefficient, which leads to an uneven distribution of cells. To overcome it, organ printing is introduced.**[4]** 3D organ printing, which is a fractional part of 3D printing that has been developed in such a way that it subdues all the challenges caused by tissue engineering, Bioprinting uses additive manufacturing (AM) technologies that use organic and artificial materials in 3D structures, and it is achieved with the use of computer-aided design (CAD).**[5]** Bioprinting is a breakthrough technology used to create 3D structures using cells, tissues, and many other biomaterials. The major difference between 3D printing and 3D bioprinting is that the typical ink is replaced by the bioink, which comprises all the biomaterials used to manufacture a 3D structure with reliability, elasticity, and precision.**[6]** 3D bioprinting is a pioneering technology that can print various structures extending from muscle tissue, nervous tissue, cartilage tissue, and bone tissue to a whole organ. In the framework of the methodology, we get an X-ray, CT, or MRI of the patient and then print it progressively layer by layer, taking into account every possible minuscule intricacy, and then hold it together to function as a single entity.**[7]**

There are various steps involved in 3D bioprinting, such as pre-bioprinting, bioprinting, and post-bioprinting as shown in the Fig 1. 3D bioprinting is growing rapidly by creating structures that closely resemble the anatomical structures of the human body, such as the tissues of the heart, neck, bones, and cartilages.**[8]**

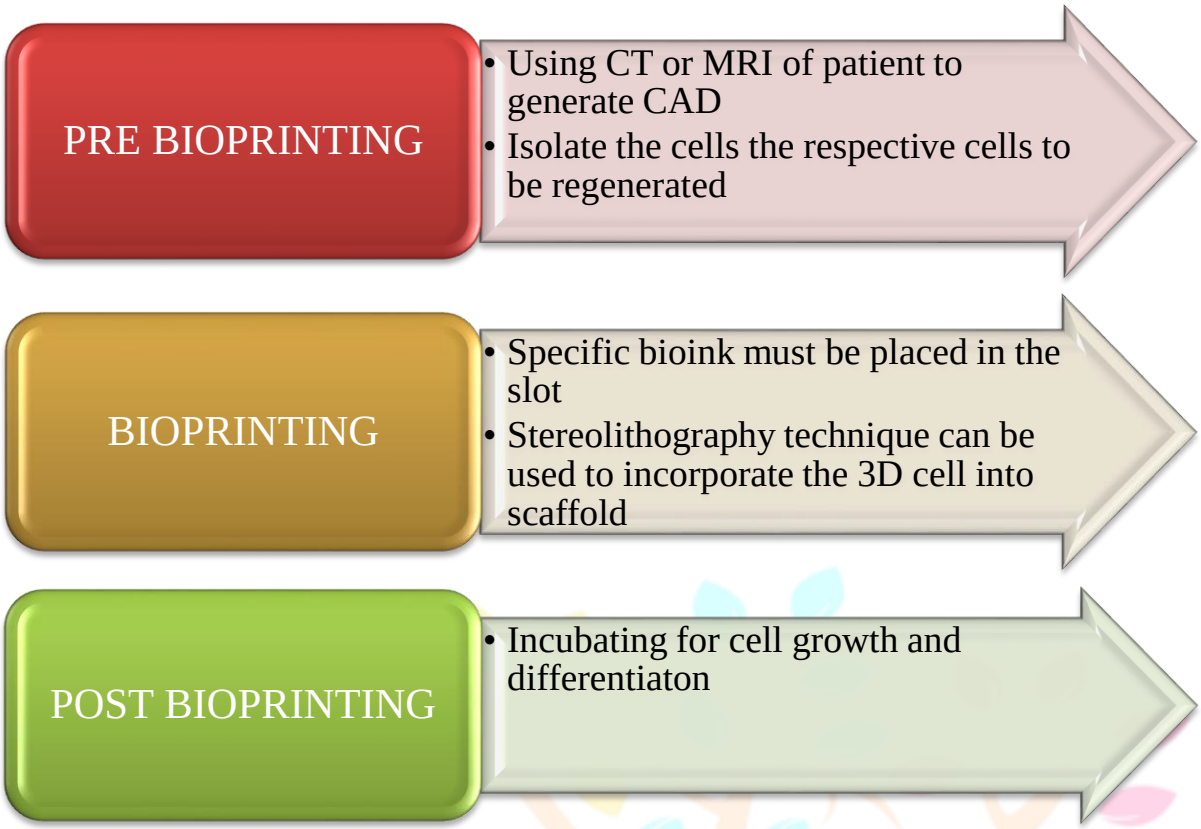

FIG 1: Steps of 3D bioprinting

Pre-bioprinting is all about scanning the patient and using those scans to generate CAD and STL files. Bioink plays a major role in the bioprinting step. The bioink consists of the cells and biomaterial that are used to incorporate the 3D cell onto the scaffold using STL or any other method, which is further incubated in the post-bioprinting step.

In 3D bioprinting, there will be a prominent usage of the bioinks, which comprise the necessary cells, biomaterials, and growth hormones for the construction of tissues or organs. Nowadays, hydrogel is a type of bioink that is mostly recommended in bioprinting due to its high resemblance to the human extracellular matrix (ECM), which facilitates the attachment, growth, and specialised development of enclosed living cells. The hydrogels for 3D printing are usually categorised into two types:

1. Natural
- Collagen
- Silk
- Alginate
- Fibrin

2. Synthetic
- Polyethylene Glycol (PEG)
- Polyvinyl Alcohol (PVA) **[9]**

Even though natural hydrogels are more compatible, synthetic hydrogels also offer adjustable mechanical and physical properties. The critical elements of bioink involve the following:

1. Robustness
2. Biocompatibility
3. Adaptability
4. Biodegradability
5. Ability to maintain stability **[3]**

The main objective of this article is to provide a clear image of 3D bioprinting, including the types of bioinks used, the techniques used, and the advantages and challenges involved. The techniques used in 3D bioprinting are:

1. Stereolithography
2. Extrusion printing
3. Laser-assisted bioprinting techniques **[1]**

3D bioprinting shows promising results in emerging structures very similar to the anatomical structure of humans. It is highly beneficial in treating patients in battlefields, disasters, or calamities. Muscle tears, bone breakage, or any other wound or injury can be treated by this method.**[9]** The drugs, genes, biomaterials, proteins, and cells must be accurately placed in order to bring out the best results in tissue manufacture. The detailing of every individual feature anatomically in tissue, including the cell junctions, connections, pore, and size of blood vessels, improves oxygenation, neovascularization, and cellular communication that help in tissue development and functionality.**[8]** Primarily, one must collect all the data regarding the structure (tissue or organ) and biomaterials being used in the construction. The next step is to convert the collected data into electrical signals for the 3D bioprinter to fabricate into the desired structure (tissue or organ). In the final step, the fabricated structure is incubated or developed. **[6]**

3D bioprinting is completely safe and harmless to use, even in veterinary medicine. During the research on humans, they also applied it to animals with similar anatomical features, and it showed efficient results. These investigations have laid the

foundations for human clinical trials and proved their application in veterinary science as well. **[10]**

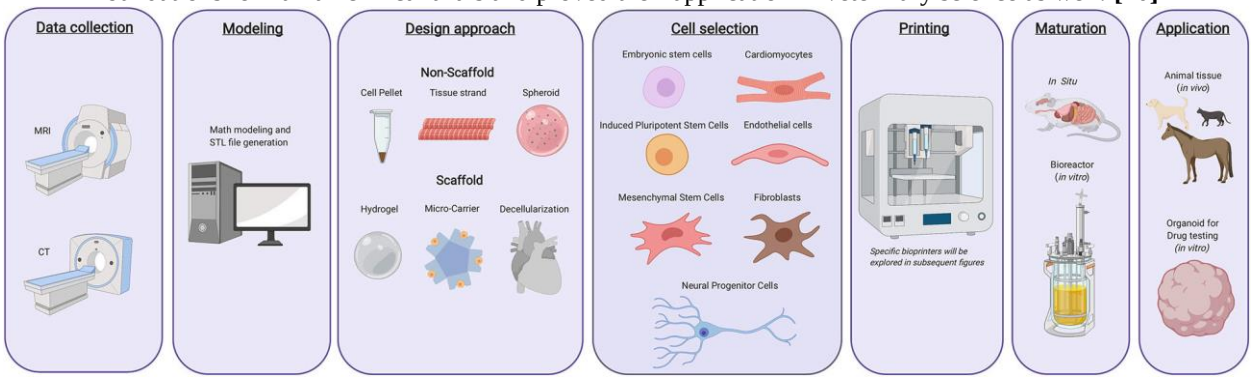

Fig 2: The patient's data is collected through an MRI or CT scan, and with the help of AM and CAD, a model is designed. In the design approach, biomimicry and self-assembly are used. According to the model, cells will be selected and then printed. Once the printing is done, that structure must be incubated and developed, and then there will be in vivo transplantation. [9]

In the FIG 2 there is a clear depiction of the procedure of bioprinting and its utilization in the medical world. There will be several applications of biopsies, like wound healing, treatment of bones and cartilage, artificial skin, bladder, and heart implants. Apart from the advantages, there will be many challenges on the road to bioprinting because it is a very expensive technique that leaves the poor behind to wait for the donor, and it is also not yet so advanced that there will be an ample number of risks that have to be considered before beginning the procedure. This article reviews bioprinting, its techniques, advantages, challenges, and applications.**[7]**

TYPES OF BIOINK

Bioinks used in 3D bioprinting is made up of several biomaterials like cells. These bioinks will act as a support system for the generated structure and its growth. It must be biocompatible and biodegradable in nature. The selection of bioink plays a very crucial role in 3D bioprinting. The appropriate selection of the bioink for the respective structure formation helps in precision, functionality growth and long term endurance. The structure that has been constructed using the bioink must be stable enough to retain its structure and not collapse or deteriorate. The stability and integrity is first tested in the artificial environment that is invitro before it is replaced by the deformed part in the human body. It will be transplanted into the patient only if it passes the tests that were done invitro to make sure it doesn't cause any trouble to the patient. The bioink formulation itself is a pivotal steps because all the phenotypic characteristics of the tissue depend on it.

There are several natural hydrogels such as collagen, alginic acid , agarose and chitosan which have osteo or chondro inductive or conductive properties and there are synthetic hydrogels such as PEGDA and hyaluronic acid.

Natural bioinks:

1. **Sodium alginate:** Sodium alginate is a kind of polysaccharide that is derived from brown algae and bacteria.**[2]** It is linear and also negatively charged polymer. Alginate is a combination of G (α-L-guluronic acid blocks) and M (β-D mannuronic acid monomers) domains. It is widely used for 3D bioprinting because of its biomimetic structure, suitable viscosity, high compatibility, good flow properties, low toxicity and it has a property of quick gelation when it comes in contact with calcium ion solution (such as calcium carbonate, calcium chloride and calcium sulfate). When mixed with biological materials, under favourable conditions it will be molded. A well known crosslinking polymer is calcium chloride but its hard to fabricate so alginate is added as an adjuvant to stabilize the construction.
2. **Chitosan:** Chitin is present in the exoskeleton of invertebrates. That chitin undergoes deacylation and forms chitosan. Unlike sodium alginate, chitosan has low gelation speed but it will be gelatinized rapidly with rise in its pH environment. It gelatinizes at 40°c under normal pH. It is biocompatible, non immunogenic, hydrophilic in nature and hemostat polymer. Bone, skin and cartilage tissue engineering extensively employs chitosan hydrogel. It can work as antimicrobial and anti inflammatory for wound healing. It also shows positive effect on proliferation and adhesion of keratinocytes and fibroblasts in the constructed structure.
3. **Agarose gel:** It is a linear polymer which reacts to heat and and is thermo reversible. When low melting point agarose is used in printing, upon contact with refrigeration platform it solidifies and retains shape and structure to the 3D bio printed structure. Campos *et al* has placed mesenchymal cells in agarose gel and it showed generation and growth of tubular structures in the presence of deposited cells and exhibited nearly 100% of cell survival in 21 days.
4. **Collagen:** It is the protein that is present lavishly in the human ECM and it consists of amino acids such as hydroxy proline, proline. It controls all cell fate processes. There are 28 variants of collagen present in the vertebrates. Collagen solution at neutral pH when heated at 20-37°c gets self-organized into cross linked polymer that provides support to the constructed structure. However, its gelation slow in normal environment thus is added with some biomaterials. Collagen type 1 is used for extrusion of skin bioprinting.
5. **Gelatin:** It is a permanent altered form of collagen thereby it possess all the characteristics of collagen like the cytocompatibility. The advantage of it is that it is cheaper and has better solubility than collagen. So is used as an alternative of collagen in 3D bioprinting. Gelatin exits as coiled structure at 40°c and retains back to its triple helical structure at normal temperature. The crosslinking strengthens the construct after it is finished. It shows epithelialization and granulation in the wound healing. Usually gelatin is used in combination of alginate as bioink due it reversible gelation property and can easily break the bonds in physical environment. There are certain GRD motif is a specific sequence that is present on gelatin which makes it more suitable for broad range of applications in the tissue engineering.
6. **Fibrin:** Fibrin is derived from fibrinogen by the action of thrombin. Fibrinogen is a protein present in the blood which helps in wound healing. Fibrin has great biocompatibility and cell binding nature. Lately it is being used as an additive in

the bioinks for skin construction. When the bioink that has fibrin is used in mice and pigs it showed rapid proliferation and accelerated growth.

7. **Hyaluronic acid:** Also called as sodium hyaluronate. It has huge significance in adjustment of cell behaviour and function, such as cell spread, proliferation and angiogenesis etc. The combination of hyaluronic acid with photo cross linkable materials like DexHEMA has shown increased cell viability in chondrocytes. Further, the physical blends of gelatin-alginate, fibrin-collagen, gelatin-hyaluronic acid have also been used as bioinks.
8. **Silk fibroin:** It is derived from silk worm and is a block copolymer which is amphiphilic in nature. The main heavy chain of silk fibroin has a frequent occurrence of G-X-G-X and it repeats for 12 times. Where X stands for Serine or Alanine and G stands for Glycine. The repeating units are connected by hydrophilic peptides. Silk fibroin is usually blended with gelatin to improve its flow and promote the redifferentiation of chondrocytes and multilineage differentiation of human nasal inferior turbinate tissue derived mesenchymal cells. If used alone it might cause needle clogging due to aggregation. It has high tensile property and good bioavailability.

Synthetic Bioinks:

1. **Poly(lactide-co-glycolide) (PLGA):** It is a copolymer of lactide and glycolide using ring opening mechanism. Its degradation rates can be altered by formulating it using different ratios of co polymers. This can be used when the Human umbilical vein endothelial cells are deposited on scaffold.
2. **Poly(ethylene glycol) (PEG):** It has various biomedical applications like nanoparticle coating and as bioink due to its high biocompatibility and hydrophilicity. It can be solubilized in water but to form as gel it must be modified chemically. It can easily form physical or chemical cross linkage. If its under UV light photo initiator must be applied to form cross linkages. Acrylated PEG is used for vascular grafts. PEG must be blended with dimethacrylate to print human cartilage.
3. **Poly(L-lactic acid) (PLA):** It is an aliphatic polymer which has biodegradable, biocompatible and semicrystalline properties. It being less viscous can be easily ejected by a needle. Acrylonitrile butadiene styrene-PLA blend was used as a bioink to produce a cartilage graft.
4. **Poly(ε-caprolactone) (PCL):** It is a synthetic polymer which is biodegradable, biocompatible and semicrystalline and has properties like low melting point, thermoplastic behavior, hydrolytic degradation and excellent mechanical properties. Due to its high viscosity there was difficulty in printing due to the requirement of wider needles but the problem has been overcome by electrohydrodynamic jet technique. This technique creates a temperature gradient in the ink and helps in construction of the structure.

TECHNIQUES USED

Inkjet bioprinting:

Ink-jet bio printing is the first organ printing technology and it is contact less. It is classified into two main types:

1. Continuous Ink-jet(CIJ):

In continuous Ink-jet printing, there will be continuous pressure exerted on the nozzle so that it sprays the bio ink out. Electric field is then applied to deflect the jet of bio ink on the substrate. Whereas, the bio ink that hasn't been deflected on to the substrate to build the construct is collected and then reused but it might also cause contamination which is a demerit.

2. Drop on demand(DOD):

This method is very similar to CIJ except that the droplets are created on demand with pressure pulse but not continuously. It has several biological as well as non-biological applications. **[10]**

There is are two types of DOD technique:

- **Piezoelectric:**

Piezoelectric method has a transducer that is present in the micro-fluid chamber which creates a transient pressure for droplet actuation. It uses acoustic waves breakdown the droplets of bio ink. The rheological properties of the bio ink contributes to its spraying.

- **Thermal ink-jet:**

Thermal ink jet type operates with the help of heat that acts on the ink near nozzle to increase the material temperature to vaporize and become micro-fluid. The electric heat used in this technique is used to generate steam bubbles to make nozzle spray liquid drops. The whole process of printing takes place through digital control over a bio-paper like polymer construct or a hydrogel substrate or a culture dish. The bio ink is exposed to heat just for a few milliseconds so there is no chance of deterioration of the bio ink. **[2]**

Sterilization is a very important step in this technique to avoid any sort of contamination. It can be sterilized by exposing it to UV radiations overnight or incubating the bio-paper. Ink-jet is a technique that can be used to print directly on the body especially in the wounded area even though it is uneven through automation or manually. **[11]**

These days, ink jet can be customized using different types of bio inks to enhance speed, flow, resolution and accuracy. There are a specific kind of ink jet printers that utilize the acoustic radiation along with the ultrasonic sound to eject the bio ink. The parameters of ultrasonic sounds can be altered accordingly to regulate the flow and size of the droplets. In this method, the cells have a longer durability and viability because they are less subjected to heat, pressure and the printer heads used are nozzle-less that reduces the shear stress. The only demerits are that bio inks with high viscosity are hard to use and the frequency between 15-25Hz can cause cell damage.

MERITS:

- Quite affordable
- Contact less thus less chance of contamination
- Recently Xu along with his group used DOD technique and had built vascular like Alginate tubes with hemi branching point
- Even the heat used in some techniques is localized to the printer head but will not effect the stability of the bio ink

DEMERITS:

- Clogging
- Variable droplet sizes
- Less directional
- Poor cell encapsulation **[12]**

Extruded Biological Printing:

Extruded bioprinting can be done with two different techniques:

1. Direct ink writing (DIW): Here the material is extruded out of the nozzle and the structure is created layer by layer. The material must be thin enough to pass through the nozzle and must have good stress and strain properties because to enable good flow, a stress beyond the yield stress is applied. Once the resin is placed on the substrate, it retains it stability and rigidity. The thin flow of the resin is induced by the fillers like filler particles or nano clay particles. These rheological properties enable retention of shape

Extruding biological printing basically uses three types of cross-linking mechanisms

1. Chemical cross-linking
2. Photo cross-linking
3. Physical cross-linking

There will be several methods of cross-linking:

1. Bioplotting:

Cells are encapsulated into the gel and is squeezed into the pool of cross-linking agent. The printed stent shall be left in the pool until the complete printing is done. The density of the extruded bioink must be more when compared to the pool of cross-linking agent. The entire process of deposition and extrusion can be altered by altering the temperature and viscosity of the cross-linking pool.

2. Secondary nozzle assisted printing:

Secondary nozzle can rotate around the main nozzle. Its main function is to spray the cross-linking polymer to main nozzle to make bioink extruded from the main nozzle.

3. Coaxial nozzle system printing:

In coaxial nozzle system, the cross lining polymer is extruded from between the inner and outer tube. While the bioink extrudes from the inner tube.

4. Pre-crosslinked printing:

The utilization of pre-crosslinked alginic acid in bio-printing, along with alginic acid possessing pre-crosslinking ability but relatively low crosslinking degree, ensures sufficient material strength. Once the printing process is finished, the bio-printed stent is immersed in a crosslinking solution to achieve complete crosslinking. This printing method guarantees improved structural mechanical properties, although imbalanced bioink may result in intermittent filaments during the spinning process.

5. Atomized cross-linking printing:

Alginic acid is printed on a platform and an ultrasound humidifier is used to make the entire space full of droplets. Those drops are atomized thus are uniformly structured and distributed all over the space which is the creates cross-linking among the layers. **[2]**

There are alternate methods for solidification which include UV curing of printed layer, thermal cure or extrusion into support bath. Thermal cure holds the printed material in place until the ink solidifies. It is called Freeform reversible embedding (FRE) or Embedded 3D printing (e3D printing). The basic resolution of resin used in DIW range from hundreds of microns to sub microns usually dependent on the size of the nozzle.

In the latest research, when the rodent hepatocytes are incorporated in the gelatin hydrogels along with alginate, chitosan and fibrinogen can construct a functional liver by employing pressure assisted multi syringe deposition system. Initially, the thermal cross linking of gelatin is done as it is extruded from a syringe that is of low temperature to a warmer stage. The further strengthening is through chemical cross linking. Usually the constructs that are made with gelatin-chitosan base degrade due to enzymatic reactions but this method can help overcome these demerits. **[10]**

Fig 3 and 4 represents the Schematic diagram of printing principle for ion crosslinking materials and Schematic diagram of 3D bioprinting

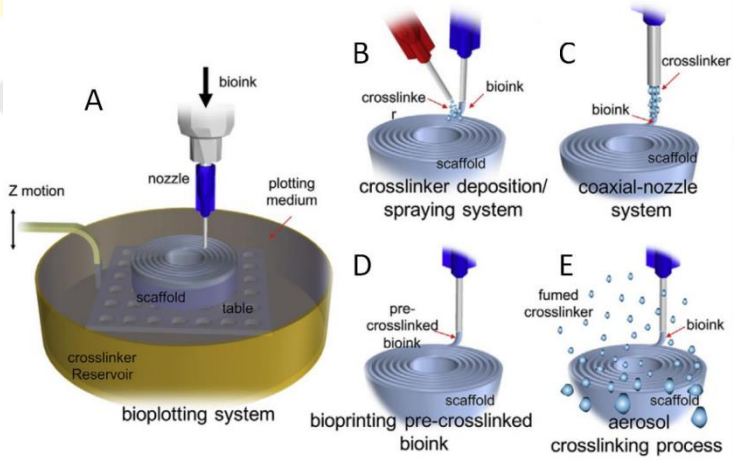

Fig 3:

A- bioplotting

B- Secondary nozzle assisted printing:

C- coaxial nozzle system printing

D- pre-crosslinked printing
E- Atomized cross-linking printing[1]

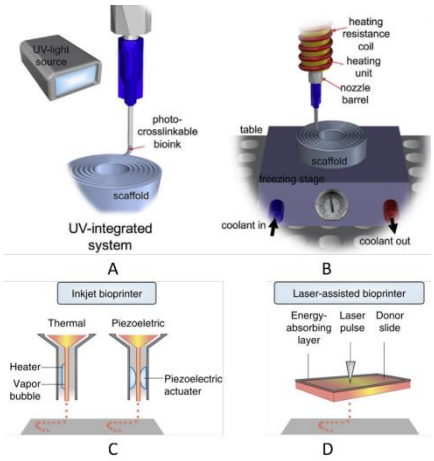

Fig 4: 3D bioprinting schematic diagram [1]

Laser Bioprinting Or Light Based Bioprinting:

The Laser Bio-printing (BioLP) device consists primarily of three main components: the laser source, the target boss, and the receiving layer. The laser source utilizes a single wavelength and pulse laser device. The target boss is composed of a transparent substrate, a light-absorbing layer, and a coating layer of biological solution. The receiving layer functions as a buffer and is typically an ordinary glass slide. The transparent substrate in the target boss is commonly made of quartz slide, which has minimal laser absorption. The laser-absorbing layers are predominantly created by applying metal or metallic oxide films, while a few use thin films of high molecular polymer. The biomaterial coating layer refers to a mixture of cells and biological materials. In this process, a pulsed laser beam is employed to deposit bio-ink, including cells onto a substrate. This approach enables a non-contact direct writing method for 3D printing using lasers and is shown in fig 5. **[2]**

The energy source is typically UV lasers or near UV wavelength laser with nanosecond pulse wavelength. The laser causes the heat-sensitive bio-ink from the ribbon to volatilize. To facilitate vial cell transfer. A laser absorbing sacrificial inter layer is placed between the bio-ink and ribbon, depending on the optical characteristics of the laser and ink. The bio-ink is applied onto a target plate. Additionally, the substrate where the ink is deposited is coated with either a natural polymer, nutrient medium or biopolymer. This coating aids in the deposition process and supports cell growth. Due to volatile nature of bio-ink, when a laser pulse is applied a high speed jet of cell-laden bio-ink is propelled onto the substrate.

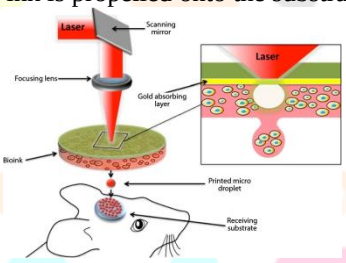

Fig 5: Schematic representation of laser assisted 3D bioprinting

Researchers have developed two techniques known as "absorbing film-assisted laser-induced forward transfer (AFALIFT)" **[13]** and "matrix-assisted pulsed laser evaporation direct writing (MAPLEDW)" **[14]**.On the other hand, the laser-induced forward transfer (LIFT) technique was initially proposed for direct writing of metal features on an optically transparent substrate using a high-energy laser pulse with direct deposition. This technique was later extended for printing biomolecules in the form of AFALIFT and BioLP **[15]**. To protect the cells from laser exposure, a laser-absorbing layer made of any metal or its oxide (e.g., Ti, TiO2, Ag, etc.) is included at the interface of the ribbon and bioink as a sacrificial layer. When a high-energy pulsed laser is applied, rapid thermal expansion of this sacrificial layer propels a small volume of bio-ink onto the substrate with minimal cell damage.

The BioLP process slightly deviates from this approach by utilizing a low-powered pulsed laser, and the sacrificial layer is a hydrogel, such as Matrigel®. In this case, the hydrogel itself acts as the binding medium for the bio-ink onto the target plate or ribbon. The entire process is computer-controlled and employs a CCD camera to enable selective cell patterning **[16]**. Cells can be printed either as encapsulated particles in ECM-like biomaterial or directly imprinted onto or within the depths of the ECM layer. Various parameters affect cell viability during the printing process, including the ECM thickness onto which the cells are deposited, the laser-pulse energy, and the viscosity of the bioink. Higher laser energy increases cell fatality, while increasing the thickness of the sacrificial layer and bioink viscosity results in greater cell viability. Researchers **[17]** have also studied the effects of printing speed on printing resolution, providing evidence that the fabrication of soft, free-form tissue, capable of hosting a high cell density in vivo, is achievable by printing blends of cells onto the ECM via LAB.

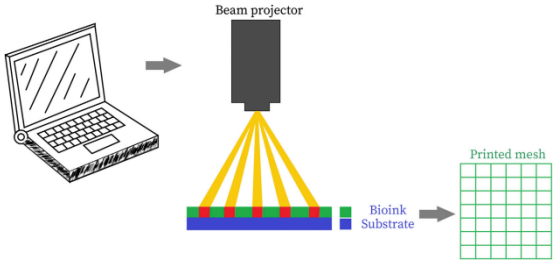

Fig 6: Schematic representation of stereolithographic 3D bioprinting [18]

Laser-assisted bioprinting (LAB) is a bioprinting method that utilizes a laser to deposit bioink onto substrate surfaces. It employs the laser-induced forward-transfer (LIFT) effect and involves three main components: a pulse laser source, a ribbon or target containing the bioink, and a substrate to receive the printed material. The ribbon or target should be made of a non-absorbing material and coated with a thin layer of absorbing metal to facilitate the deposition of the printed material. The entire LAB printing process takes approximately 3 hours. To ensure minimal contamination and improved printing efficiency, a laminar flow cabinet and incubator are required. A well-equipped laser-assisted bioprinting workstation must include specifications such as the laser wavelength, pulse duration, repetition rate, beam quality, galvanometric mirror, CAD/CAM software, and printer cartridges.

In this technique, the bioink is in liquid form, and the quality of printed cells is determined by its viscosity and cell composition. Maintaining sterilization conditions and viscoelastic properties of the ink solution during the printing process is crucial. To achieve efficient printing, analyzing the cartridges under a microscope before printing helps maintain the concentration and uniform distribution of cells for complete deposition on the substrate. Additionally, placing the cartridge and holder on ice blocks before printing can control water loss and ensure stable viscoelastic properties.

Laser-assisted bioprinting is an advanced technique that allows precise control of cell density and 3D cell organization. Moreover, it can mimic the physiological performance of actual biological components. This method offers automation in processing and high reproducibility of printed materials.**[11]**

Laser-assisted bioprinting can achieve high resolution, with the ability to print at a single cell per droplet. This technique offers precise control over tissue organization and cell population, making it a promising approach for developing tissue equivalents that closely resemble the structure and function of native tissue. Initially used for printing inorganic or organic structures with micrometer scale resolution, laser-induced forward transfer is now successfully applied to print bioinks such as DNA, cells, and peptides.

Although laser-assisted bioprinting was not widely used in the past, it has gained popularity in recent times for fabricating engineered tissues in regenerative medicine applications. The laser-assisted bioprinting system comprises a pulsed laser beam to induce bioink transfer, a focusing system to align and focus the laser, an absorbing layer (typically made of gold or platinum) called the ribbon, and a substrate for the bioink layer. During the printing process, the laser pulse is focused on the ribbon layer, generating a high-pressure bubble that transfers the bioink onto the substrate. The resolution of the laser-assisted bioprinting system depends on factors such as laser energy, the air gap between the absorbing layer and substrate, the nature of the substrate surface, and the surface tension and viscosity of the bioink. Notably, laser-assisted bioprinting is a nozzle-free printing method, eliminating the risk of bioink or cell clogging, which can occur in other bioprinting approaches

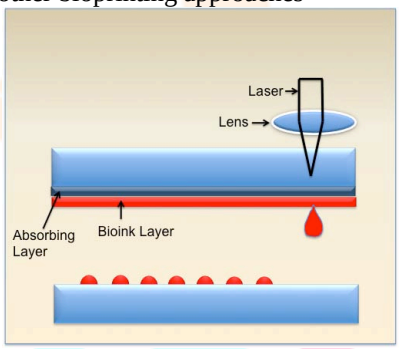

Fig 7: Laser-assisted printers use an absorbing layer to create laser pulse pressure that creates droplet ejection from the bioink layer [19]

These types of printers require bioink with fast gelation kinetics to achieve constructs with good shape fidelity, which can, in turn, hinder the flow rate during printing. Additionally, the preparation of ribbons or absorbing layers containing multiple cells is a time-consuming process. Moreover, constructs fabricated by laser-assisted bioprinting may contain traces of contamination from the absorbing layer. To address this issue, non-metallic substances are now being used to create absorbing layers as shown in fig7 .

Another challenge in laser-assisted bioprinting is the difficulty in focusing the laser spot and precisely locating cells during printing. To overcome this, the "aim and shoot" technique is employed. With this method, the laser beam scans and selects the region of interest, enabling the precise ejection of one cell per laser pulse. Notably, this printing approach has successfully fabricated bone constructs and skin with cells for implantation.

In various constructs fabricated using different bioprinting methods are presented, along with their respective advantages and disadvantages. **[12]**

Stereolithography:

The stereolithographic bioprinting method, as depicted in fig 6, relies on the height of the design rather than its complexity. It builds up the design layer by layer by adding materials through the projection of light on a photo-sensitive heat-curable bio-ink in a plane-by-plane fashion **[20]**. The ink used in this printing system must contain photocurable moieties as it utilizes light as an

agent for cross-linking **[21]**. Acrylate derivatives of Polyethylene Glycol (PEG) like PEG dimethacrylate (PEGDMA) and PEG diacrylate (PEGDA) are commonly employed for photopolymerization of tissue engineering scaffolds **[22].**

The formation of 3D tissue constructs is achieved through light-initiated polymerization **[23]**. Stereolithography has been integrated with clinical imaging techniques such as CT scan/MRI to improve diagnostic techniques, enhance the quality and design of prostheses and implants, and achieve success in complex surgeries. There are two broad categories of stereolithographic printing: the Single-photon method and the Multiphoton method. The Single-photon method can be further subdivided into:

1) Visible radiation systems
2) Conventional stereolithography
3) IR stereolithography systems
4) Stereo-thermal lithography systems.

Light projection systems can be implemented either directly by laser writing or through mask projection systems, either physically or digitally**[24]**.

In "Conventional single-photon stereolithography apparatus (SLA)," UV-sensitive fluid oligomers can be cross-linked into sol-gel polymeric networks using UV radiation photons. Photosensitive resins are employed for this purpose, facilitating polymerization through initiation, elongation, and termination steps. Various resins have been utilized for SLA purposes, such as biodegradable resins for non-toxicity, elastomeric resins for flexibility, and high-strength resins for mechanical strength. Stereolithographic bioprinting has been effectively employed in tissue engineering to fabricate biocompatible scaffolds, which prevent inflammatory responses during implantation and offer good degradability with non-toxic byproducts, allowing for absolute renal clearance and tissue regeneration. Researchers have demonstrated its success in bone regeneration by polymerizing UV-curable polymer-ceramic composites for cell-seeding **[25]** and fabricating bone regeneration scaffolds using vinyl ester resin in a rabbit model **[26]**. Stereolithography has also been used in cartilage tissue engineering by preparing a bioresorbable scaffold based on Hyaff 1, a hyaluronic acid derivative **[27].**

Furthermore, stereolithographic printing has been employed to fabricate various tissue constructs, such as a two-dimensional cardiac tissue construct using primary cardiomyocytes isolated from neonatal rats **[28]** and a tri-leaflet heart valve using polyhydroxy octanoate and poly-4-hydroxybutyrate elastomers **[29]**. Secretion of vascular endothelial growth factor (VEGF) by fibroblast cells encapsulated in Polyethylene glycol-based hydrogels, fabricated using direct "laser writing," has demonstrated neovascularization potential in tissue engineering **[30]**. Additionally, two-photon laser scanning photolithography has been utilized to fabricate a 3D liver tissue construct, functionalized with collagen seeded with rat hepatocytes **[31]**. A recent development in stereolithographic bioprinting includes a soft robotic device called "biobot," developed by Bashir and coworkers, which incorporates the auto-rhythmic and synchronous nature of cardiac tissue for locomotion purposes **[32]**. However, there are some challenges associated with stereolithographic methods, such as the fabrication of constructs using multiple materials and the spatiotemporal regulation of material deposition onto the substrate matrix. Nonetheless, stereolithographic bioprinting as shown in the Fig 8, remains a promising and versatile technique for tissue engineering applications.

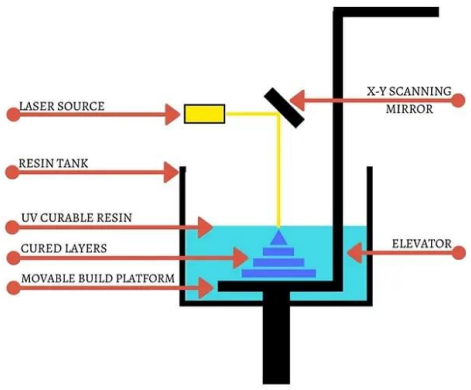

Fig 8: Stereolithography

APPLICATIONS

The combination of the internet of things (IoT) and bioprinting technology has resulted in significant advancements in surgical procedures. The ultimate objective is to eliminate the prolonged wait for donor organs by bioprinting entire organs that closely resemble both in structure and function. The primary organs targeted for bioprinting include the heart, bone, skin, cartilage, and tendon. This innovative approach aims to revolutionize organ transplantation and improve the overall success of surgical interventions.

1. In cosmetics and pharmaceutical industry:

The pharmaceutical industry usually uses animals in the clinical trails. Animal trails are not always successful due to lack of complete similarity to human body due to highdifference in the anatomy and physiology. Also there are several ethical restrictions regarding the pre clinical trails. There are several drugs that are proven toxic to humans even after successful animal trails. So, with the help of 3D bioprinting, the skin equivalents are made as an alternative to the animal models. The pharmaceutical and the cosmetics industries are in requirement of skin replicas to conduct tests of noval topical formulations. Thereby there is a great demand for the 3D bioprinted skin in the skincare companies. It's a revolutionary invention and advantageous for the testing of cosmetics and topical formulations. Prior to the clinical tests, invitro tests must be conducted to examine the safety, allergic reactions, toxic effects and side effects of the drug before its launch into the market. This method must be fully automated and standardized.

For the cosmetic testing, several types of skin models that can be fabricated like dry, oily, combination and sensitive skins. The skin constructions can aslo be used to measure the penetration of drug into the skin. This technology attracted the attention of global cosmetic leaders such as L'Oreal and Proctor & Gamble, who invested in the research and development of 3D bioprinted skin models. **[6]**

2. Drug delivery:

A drug delivery system is a method employed to transport drugs to a specific area in the body in a controlled manner. It detects the untreated area in the body and releases the drug based on the body's requirements. Figure illustrates the techniques for loading drugs into a 3D-printed hydrogel scaffold for in vitro and in vivo studies. In the pre-loading technique, the drug is introduced with the biomaterial before printing, while in the direct loading technique, the drug is introduced after the hydrogel scaffold has been printed.

Previously, uncontrolled drug release has been identified as a significant issue, leading to potential side effects and harm to consumers. Researchers have found that using biomaterial and 3D bioprinting for drug delivery can encounter problems. Three-dimensional bioprinting is preferred in this system as it allows drug modifications and faster production.

There are several mechanisms for controlled drug release, including temporal-controlled, distribution-controlled, and erosion-controlled methods. Drug delivery systems are broadly categorized into two groups: oral and transdermal delivery systems. The oral system involves administering drugs through the mouth, while the transdermal system delivers drugs through the skin. Each controlled release and drug delivery system has its roles and benefits in the pharmaceutical field.

Further studies on solid drug dosage forms have explored the effect of geometry and composition on drug dissolution. The drug's composition significantly affects its dissolution compared to its surface area and geometry. Mini tablets generated through passive diffusion exhibit smoother surface structures and fewer defects compared to those produced using hot-melt extrusion, depending on the filament quality and density. Hot-melt extrusion can achieve higher drug loadings and weights but results in lower filament homogeneity, which also complicates obtaining the desired filament diameter.

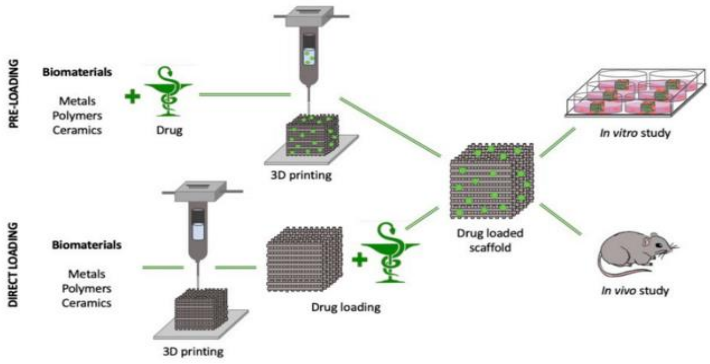

Fig 9: Loading of drug into the scaffold before and after printing for the in vitro and in vivo study. Reproduced from [33] which is licensed under a Creative Commons Attribution-(CC BY 4.0)

Recent research conducted by scientists indicates that the implementation of biomaterial and 3D bioprinting may encounter challenges concerning drug delivery processes. Three-dimensional bioprinting has gained significant popularity in this area due to its ability to modify drugs and expedite production. Controlled release systems for drugs include temporal-controlled, distribution-controlled, and erosion-controlled mechanisms. Drug delivery systems are broadly categorized into oral and transdermal methods, with the former administered through the oral system and the latter through the skin. Each controlled release and drug delivery system plays a crucial role and offers specific benefits in the pharmaceutical field.

Further investigations have been conducted on solid drug dosage forms to assess the impact of geometry and composition on drug dissolution. The composition has a more substantial influence on drug dissolution compared to surface area and geometry. Mini tablets created through passive diffusion exhibit a smoother surface structure with fewer defects compared to those produced via hot-melt extrusion, which relies on the quality and density of the filament. While hot-melt extrusion allows for higher drug loadings and weights, it results in lower filament homogeneity and complexity in achieving the desired filament diameter.**[11]**

3. Tissue engineering:

Tissue engineering has experienced rapid advancements with the emergence of three-dimensional bioprinting. This field involves the use of living cells and tissues in engineering processes. Biomaterials play a crucial role in constructing new tissues and repairing damaged organs, making tissue engineering an essential aspect of biomedical applications. It enables the functionalization of biomaterials and exploration of various medical areas, holding great promise for the future.

Fig 9 and 10 demonstrates the successful application of a 3D-printed hydrogel bone scaffold, replacing tissue in rats and rabbits. For three-dimensional bioprinting in tissue engineering to be viable for human use, it must exhibit exceptional biocompatibility without causing any rejection or harm.

Numerous studies have explored the implementation of 3D bioprinting in tissue engineering using various biomaterials. Alginate hydrogel printed through inkjet bioprinting has shown promising results in terms of process efficiency. To minimize contamination in the printed material, researchers developed a bio clean bench for their inkjet printer. Interestingly, their printer operates without electrical energy and can produce smaller printed materials, allowing them to understand the impact of bead size in 3D bioprinting. However, printing vascular tissue poses challenges related to modifying the biomaterial chemically, controlling pore size, and incorporating growth factors. Additional support, such as sacrificial ink that can be removed after printing, is necessary for printing vascular tissue.

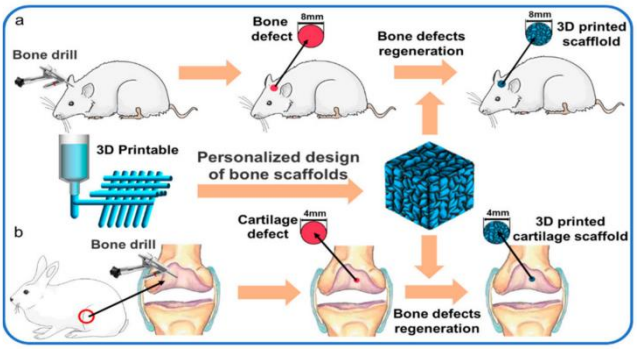

Fig 10: Printed bone scaffolds can replace the bone and cartilage defects in rat and rabbit, (a) regeneration of skull defect in rats articular cartilage regeneration in rabbits. Reproduced from [34] which is licensed under a Creative Commons Attribution-(CC BY 4.0)

More recently, conductive materials have been integrated into tissue engineering through 3D printing techniques. Using conductive material as ink leads to more functionalized printed products that reduce the risk of rejection by the human body. This advancement holds promising potential in the field of tissue engineering and opens up new possibilities for medical applications.**[11]**

4. 3D-Bioprinting for Otologic Applications:

Tissue engineering in the field of otology has primarily concentrated on cartilage regeneration to reconstruct the auricle (external ear) for patients with microtia, and occasionally for other ear components like the tympanic membrane and bony ossicles. Current methods for external ear reconstruction involve prosthetic reconstruction or the use of autologous cartilage grafts. However, prostheses may have issues like extrusion, and cartilage grafts can lead to donor site complications or size limitations.

To address these challenges, cartilage tissue regeneration offers a viable option as an alternative source of autologous cartilage for improved auricular reconstruction. The idea of tissue engineering for auricular reconstruction gained attention in 1997 with the publication of the Vacanti mouse, which explored the feasibility of growing cartilage on a biomaterial scaffold in the shape of a human auricle. While some reports have described 3D bioprinted cartilage-like scaffolds in the form of the auricle, only a few have investigated outcomes after in vivo implantation and is depicted in fig 11.

One such study by Kang et al. involved the construction of patient-specific, human-scale ear-shaped cartilage scaffolds using a combination of cell-laden composite hydrogels (gelatin, fibrinogen, hyaluronic acid, and glycerol) and PCL through extrusion-based 3D bioprinting. The resulting constructs, which included rabbit ear chondrocytes mixed with the composite hydrogel and PCL as a support scaffold for mechanical integrity, along with a Pluronic F-127 hydrogel as a sacrificial outer support layer, demonstrated the maintenance of their shape after being implanted in the dorsal subcutaneous space of athymic mice for 1 or 2 months.

Histological analysis at these time points revealed the formation of human-scale cartilage tissue scaffolds, with the presence of microchannels between the printed filaments. These microchannels hold the potential to facilitate nutrient and oxygen diffusion, suggesting the possibility of future vascularization upon implantation.

Despite the promising structural and functional characteristics observed, the study used nude mice in an ectopic site. As graft tolerance monitoring is crucial, further research is needed to investigate the regeneration process following implantation of these tissue constructs in an immunocompetent animal model that more accurately mimics the surgical correction of aural atresia or microtia.

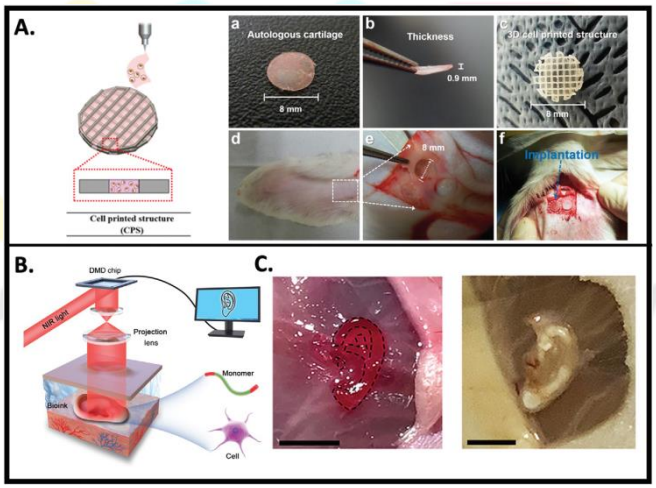

Fig 11: 3D bioprinting for otologic applications. A) Schematic of cell-printed scaffold (CPS) composed of chondrocytes encapsulated in alginate bioink printed with PCL framework. a,b) Autologous cartilage, c) CPS, d) defect site on the rabbit ear, and e) defect creation, f) implantation of the grafts into the cartilage defect of rabbit model. Reproduced with permission. Copyright 2017, Wiley Periodicals LLC. B) Schematic diagram of digital nearinfrared (NIR) photopolymerization (DNP)-based noninvasive 3D bioprinting. Adapted with permission Copyright 2020, American Association for the Advancement of Science (AAAS). C) DNP-based 3D bioprinted, ear-shaped construct printed subcutaneously in BALB/c nude mice. Adapted with permission.[115] Copyright 2020, AAAS. Scale bar, 5 mm.[8]

5. Treatment of Burn Injuries and Wound Healing

Many individuals suffer from non-healing skin wounds, typically treated using transplants from the patients' bodies or donors. 3D bioprinting offers a promising alternative to conventional methods. One major advantage of this innovative technology is the ability to create skin equivalents more quickly and cost-effectively. It has the potential to revolutionize injury and surgery treatments, particularly for healing burned skin. Specialized 3D bioprinters have been developed to print skin for injured patients.

There are two main strategies for fabricating skin for wound healing treatment: ex vivo and in situ bioprinting. In ex vivo methods, such as inkjet-, extrusion-, and laser-based bioprinting, a skin construct containing both dermis and epidermis is printed and then matured in vitro if necessary before being grafted onto the patient's wound. Among these methods, extrusion-based bioprinting is the simplest and quickest. It involves depositing all the components needed to form the dermis, such as human fibroblasts, human plasma, and calcium chloride, simultaneously. Then, human keratinocytes are placed on top of this layer to create the epidermis.

Researchers have demonstrated the feasibility of using laser-assisted bioprinting to develop skin equivalents and transplant them onto mice's wounds. In these experiments, the transplants adhered to the surrounding tissues, and the cells in the graft proliferated and differentiated. Additionally, a 3D bioprinter, along with primary human fibroblasts and keratinocytes, has been shown to produce human-plasma-derived bilayered skin suitable for treating burn injuries, traumatic wounds, and surgical wounds.Furthermore, the use of 3D printed gelatin-silk fibroin composite scaffolds has been reported to increase the rate of wound healing, and the addition of fibroblast growth factor may further enhance the treatment's effectiveness. These advancements in 3D bioprinting for skin regeneration hold great promise for the future of wound healing and personalized medicine. **[6]**

6. 3D bioprinting of bone tissue

Bone is a structurally complex and highly vascularized tissue, consisting of a ceramic phase within a gel-like matrix of proteins and polysaccharides, as illustrated in Figure 8 (Blausen.com Staff 2014; Gong et al., 2015). Incidents like bone fractures and osteodegenerative diseases resulting from trauma, diseases, or dysfunctional tissue can lead to chronic bone defects, necessitating bone regeneration to restore damaged tissue. Existing techniques have certain limitations in replicating the intricate anatomy and composition of bones at a large human scale. They often lack effective communication among different cell types (osteoblasts, osteoclasts, and endothelial cells) within a 3D environment, leading to inconsistent tissue constructs (Bodhak et al., 2018). Moreover, hydrogels used in bone tissue engineering struggle to form a mineralized matrix.

To address these challenges, 3D bioprinting has emerged as a promising approach for bone tissue engineering, offering several advantages over conventional methods. 3D bioprinted constructs provide sufficient mechanical support during tissue regeneration, ensuring maintenance of shape and chemistry in a controlled manner with interconnected porosity. Importantly, these tissue engineering constructs eliminate the risk of tissue rejection or disease transmission, as they do not require obtaining tissues from donors or other body parts. Another significant benefit of 3D bioprinting is the ability to utilize anatomically precise models based on patient-specific data acquired through clinical imaging, using computer graphics like CAD/CAM, to replicate the complex bone morphology accurately.**[10]**

Some of the applications used in regenerative medicine are listed below in the table 1.

Table 1: Applications of 3D bioprinting in regenerative medicine [12]

Applications	Bioink	Printing method	Cell type	Inference
Cartilage	Alginate-Polyethylene Glycol, Acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA)	Microextrusion Microextrusion	Bone marrow derived hMSCs Primary articular chondrocy tes and nucleus pulposus	Tougher mechanical integrity like native cartilage Porous scaffold for cartilage and intervertebral disc tissue enginee ring
Skin	Layer-by-layer assembled collagen	Microextrusion	Human skin fibroblasts and human skin keratinocytes	Skin matrix that resembles struc tural and biological features of native skin
Bone	DermaMatrix™ human all ograft with bone morphogenetic protein-2	Inkjet	Mouse C2C12 progenitor cells	Osteogenic differentiation of C2-C12 cells and promotes clavarial bone healing
Nerve	Polyurethane	Microextrusion	Neural stem cells	Recovery from CNS neural injury in zebra fish

Heart	Alginate-Gelatine	Microextrusion	Aortic root sinus smooth mus cle cells (SMC) and aortic valve leaflet interstitial cells (VIC)	Cell encapsulated aortic valve re tain anatomic complexity
Liver	Alginate	Microextrusion	Human induced pluripotent stem cells	Post-print differentiation into hep atocyte lineage

ADVANTAGES:

- **Tissue and Organ Regeneration:** 3D bioprinting is an innovative technique enabling the production of intricate and tailor-made tissues and organs. These bioengineered structures hold promising applications in transplantation and regenerative medicine, addressing the scarcity of organ donors and potentially lowering transplant rejection instances**[5]**
- **Patient-Specific Models:** 3D bioprinting allows the creation of personalized models that replicate a patient's unique anatomy. These models serve multiple purposes, including preoperative planning, medical education, and training. Surgeons can practice intricate procedures on these models, providing valuable experience before undertaking the actual surgery on the patient.**[12]**
- **Drug Testing and Development:** 3D bioprinted tissues offer superior and dependable models for drug testing and development in comparison to conventional 2D cell cultures. By closely emulating the microenvironment of human tissues, these bioprinted models enable more precise assessments of drug effectiveness and safety, leading to improved predictions of drug efficacy and potential toxicity.**[35]**
- **Disease Modeling:** 3D bioprinting enables the generation of disease models utilizing cells derived from patients. Such models serve as valuable tools to investigate disease progression, comprehend underlying mechanisms, and design specific therapies tailored to the individual's condition.**[36]**
- **Biological Complexity and Vascularization:** The versatility of 3D bioprinting permits the integration of diverse cell types and extracellular matrix components, resulting in the development of intricate and biomimetic tissues. Furthermore, advancements in bioprinting technology have made it possible to incorporate vascular networks, ensuring effective distribution of nutrients and oxygen within the printed tissues.**[37]**
- **Personalized Implants and Prosthetics:** 3D bioprinting offers the capability to create customized implants and prosthetics, precisely tailored to match the unique anatomical characteristics of each patient. This individualized approach enhances the fit, comfort, and functionality of these medical devices, leading to improved patient outcomes.**[38]**

DISADVANTAGES:

- **Limited Complexity and Functionality:** Present bioprinting methods face constraints in fully attaining the complexity and functionality of native tissues and organs. Replicating the intricate structures and cellular interactions present in natural tissues poses challenges that can impact the overall functionality of the bioprinted constructs.**[39]**
- **Bioprinting Resolution and Speed:** Bioprinting processes typically exhibit lower resolution compared to traditional 3D printing techniques, resulting in decreased accuracy when reproducing intricate structures. Moreover, the printing speed can be relatively slow, presenting challenges and time-consuming efforts when aiming to manufacture large and complex tissues**. [40]**
- **Cell Viability and Functionality:** During the bioprinting process, cells may encounter stress and damage, affecting their viability and functionality. Consequently, some cells might not survive the printing process, resulting in decreased overall cell functionality within the bioprinted constructs. **[41]**
- **Vascularization Challenges:** Developing functional blood vessel networks within bioprinted tissues poses a substantial challenge. Proper vascularization is of utmost importance to ensure the survival and functionality of larger and thicker bioprinted constructs**. [42]**
- **Regulatory and Ethical Concerns:** As 3D bioprinting progresses toward clinical applications, it necessitates addressing regulatory and ethical aspects. Safeguarding the safety and efficacy of bioprinted products for human use and tackling potential ethical concerns associated with tissue engineering and organ transplantation are of paramount importance.**[43]**
- **Material Biocompatibility and Degradation:** Bioprinting materials must possess biocompatibility and degrade gradually, allowing the patient's native tissue to replace them over time. It is crucial to ensure that these materials do not induce adverse reactions or hinder tissue integration to achieve successful bioprinting outcomes.**[44]**

It is worth acknowledging that despite the challenges and limitations, ongoing research and technological advancements are actively addressing these concerns. As the field advances, many of these drawbacks may be surmounted, paving the way for even more remarkable breakthroughs in 3D bioprinting technology.

CHALLENGES:

3D bioprinting is a cutting-edge technology with immense potential, but it also faces several challenges that need to be addressed for its widespread adoption and success.

- **Bioprinting Resolution and Accuracy:** Attaining high resolution and accuracy in bioprinting poses a challenge. The printing process must faithfully replicate the intricate structures and cellular organization present in native tissues to ensure precision.**[40]**
- **Vascularization and Perfusion:** Establishing functional blood vessel networks (vascularization) within bioprinted tissues continues to be a substantial obstacle. Ensuring proper vascularization is indispensable for the survival and optimal function of larger and more intricate tissue constructs.**[42]**

- **Biocompatible and Degradable Materials:** Identifying appropriate biocompatible materials that can effectively mimic the properties of native tissues and degrade naturally over time presents a challenge. It is essential that these materials do not trigger adverse reactions and are gradually replaced by the patient's own tissue as part of the bioprinting process.**[44]**
- **Cell Source and Viability:** Obtaining viable and functional cells for bioprinting is of utmost importance. Ensuring that the printed cells maintain their viability and functionality both during and after the printing process is critical for achieving successful tissue engineering outcomes.**[41]**
- **Ethical and Regulatory Considerations:** As 3D bioprinting progresses toward clinical applications, it is essential to thoughtfully address ethical considerations concerning tissue engineering, organ transplantation, and patient consent. Simultaneously, the development of regulatory frameworks for approving bioprinted products becomes crucial to ensure responsible and safe implementation in the medical field.**[43]**
- **Integration with Native Tissues:** Achieving seamless integration of bioprinted tissues with the host's native tissues presents a challenge. The bioprinted constructs must demonstrate proper functionality and effective communication with the surrounding tissues to ensure successful integration.**[37]**
- **Scalability and Production Cost:** Scaling up bioprinting to manufacture larger tissue constructs and organs while maintaining cost-effectiveness poses challenges. Effectively managing the expenses related to bioprinting technology and materials is crucial to ensure accessibility for widespread adoption and use.**[45]**

Overcoming these challenges necessitates interdisciplinary collaboration among scientists, engineers, biologists, and clinicians. As research progresses and technology advances, it is anticipated that many of these hurdles will be successfully surmounted, unlocking new possibilities in regenerative medicine and personalized healthcare.

BIOETHICAL AND LEGAL ISSUES OF 3D BIOPRINTING

1. CURRENT AND POTENTIAL APPROACHES FOR BIOETHICAL DEBATES IN RELATION TO THE EMERGENCE AND ADVANCEMENT OF 3D-BIOPRINTING

The development of bioprinting technologies raises profound questions regarding human nature and the ethical implications of biotechnological advancements. Concepts like "human enhancement," "technological design," youth extension, and "technological immortality" come into play. The latter involves two groups of programs: rejuvenation technologies (halting the aging process) and consciousness transfer technology (transferring human personality). Bioprinting, with its potential to create new organs and replace old ones, falls under rejuvenation technologies, leading to discussions about its indications and limits of application.

One of the primary concerns with the development of organs and tissues bioprinting is the feasibility and moral justification of these technologies. The apparent purpose is to address the critical need for organ transplantation and the global shortage of organ donations, thereby potentially saving countless lives. Governments and international organizations view this technology as morally justified when it serves therapeutic purposes. The ethical framework, particularly utilitarian philosophy, often supports human modification projects based on the individual's pursuit of self-improvement and a better life.

However, there are varying perspectives, ranging from radical transhumanism, which seeks unlimited technological improvement of human beings, to more cautious views considering social risks associated with human alteration. The lines between therapy and human enhancement are becoming increasingly blurred as medical biotechnologies expand their scope.

Ethical consideration also includes the speed at which these technologies are evolving. Identifying and studying ethical problems before therapeutic 3D bioprinting becomes widely used in patients is of utmost importance.

The cornerstone of human rights protection in biomedical research comprises two fundamental principles: informed consent and confidentiality. Confidentiality, linked to the concept of "medical secrecy," emphasizes the need to keep the circumstances of treatment and the patient's personal information confidential, respecting their privacy and ensuring trust in the healthcare process. Trust relationships built on confidentiality are crucial for effective and timely medical care.

The principle of informed consent is a critical element in the ethical and legal framework supporting medical activities. It derives from the concept of general human rights and is widely acknowledged and outlined in various international and national documents. For instance, the Nuremberg Code emphasizes the requirement for absolute voluntary consent for human participation in medical trials. This entails providing participants with knowledge about the experiment's nature, duration, purpose, methods, and associated risks. Similarly, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETS No. 164) stipulates that medical interventions can only be performed after obtaining a person's voluntary written consent, which includes information about the intervention's purpose, nature, consequences, and risks.

However, challenges might arise in 3D bioprinting when obtaining informed consent, especially in emergency situations where a patient is unable to express informed consent. Additionally, situations where participants lack the full ability to make a donation decision, such as some patients in intensive care units, may present further obstacles in obtaining informed consent.

2. LEGAL ISSUES OF 3D BIOPRINTING AND INTRODUCTION INTO CLINICAL PRACTICE:

Legal and governmental institutions worldwide face the challenge of defining the legal regulations surrounding 3D bioprinting due to its complexity and the lack of universally accepted solutions for addressing potential and uncertain risks of harm. The involvement of multiple participants in the bioprinting production chain further exacerbates these issues, necessitating collaboration among various stakeholders, including 3D model designers, medical professionals, lawyers, engineers, biologists,

ethical committee members, and insurance companies. Such multi-stakeholder cooperation is crucial in forming an acceptable pathway for the development and clinical implementation of bioprinting technology.

Currently, there is no comprehensive regulatory regime governing the entire bioprinting process. However, partial legislation exists concerning tissue engineering and regenerative medicine. The principles outlined in the European Commission (EC) and European Medicines Agency's advanced therapy medicinal products (ATMPs) regulations may be applied to different stages of 3D bioprinting production. Key aspects of bioprinting management include risk regulation and responsibility for product quality, raising important questions about the primary responsibility for bioprinted product quality, quality control, and liability in case of quality claims from recipients.

In Russia, the legal issues surrounding the creation and use of bioprinted human organs have been given special consideration. The absence of specific norms in Russian legislation regulating the creation and implantation of bioprinted human organs is seen as an impediment to the development of 3D bioprinting technologies. The current revision of the Federal law "On biomedical cellular products" does not yet regulate the utilization of biofabricated human organs, as it does not address organ transplantation matters. Similarly, the Law of the Russian Federation "On human organs and (or) tissue transplantation" does not cover the use of 3D printed organs since such bioprinted products are considered artificial.

At present, the legal relations between 3D bioprinting providers, medical organizations, and patients can be established through contracts for works or medical services. In the case of personalized biofabrication of organs or tissues for an individual order, such contracts can be utilized. However, if bioprinted organs are depersonalized, a sale-purchase agreement may be more applicable.

3. ETHICAL ISSUES OF ARTIFICIAL OVARY 3D BIOPRINTING AND INTRODUCTION INTO CLINICAL PRACTICE:

Within the realm of organ bioprinting, bio fabrication of reproductive organs stands out because the need for transplantation of artificial ovaries, testes, and uteruses is not directly related to life-threatening situations but rather to the perceived quality of life. Gonadotoxic oncological treatments can lead to primary ovarian insufficiency in women of reproductive age, necessitating various methods of fertility preservation, such as oocytes, embryos, and ovarian tissue cryopreservation. However, there are currently no options for restoring fertility after remission for patients with ovarian cancer or types of cancer that spread to the ovaries, like leukemia, neuroblastoma, and Burkitt lymphoma. Such patients face irreversible loss of reproductive and ovarian endocrine function after ovariectomy or gonadotoxic oncological treatment.

The development of artificial ovary technology offers hope to this group of patients, providing a potential means for fertility preservation and the possibility of having genetically-related children. Although the technology is still under development, successful animal experiments have been conducted. However, human clinical trials are yet to come, and at this stage, it is vital to address significant ethical and regulatory concerns associated with artificial ovary 3D bioprinting.

Apart from the general ethical and legal issues discussed in the context of 3D bioprinting, the bioprinted ovary project also involves matters pertaining to human reproduction. One major ethical issue revolves around the risk-benefit ratio. While ovariectomy and gonadotoxic chemo- and radio-therapies are essential for preserving the patient's life and can be considered beneficial, the loss of reproductive function resulting from these treatments is a harm. Therefore, if there is an opportunity to restore reproductive function without causing further harm (following the principle of primum non nocere), developing artificial ovary technology becomes a compelling argument. However, several profound conditions must be carefully considered in the pursuit of this technology.**[46]**

Ethical aspects:

Ethics is a comprehensive concept encompassing various disciplines within philosophy, and one of its branches is applied ethics. It is essential to differentiate applied ethics from other aspects of ethics, such as normative ethics, descriptive ethics, and meta-ethics. While normative ethics examines general moral principles, descriptive ethics describes the actual moral beliefs and practices of individuals or societies, and meta-ethics delves into the nature and meaning of ethical statements. On the other hand, applied ethics focuses on addressing moral questions related to specific contentious practices, such as engineering ethics, medical ethics, bioethics, and environmental ethics.

Legal aspects:

The legal considerations surrounding bioprinting demand broader and more concentrated attention. These encompass patenting and related issues, regulatory policies, government interventions, and decision-making. The primary challenge that needs to be tackled is the lack of understanding of this technology among regulatory authorities. As Trommelmans et al. [89] aptly emphasized when discussing the field of tissue engineering as a new paradigm in medicine (applicable to bioprinting as well), it is crucial to address one or two major legal aspects to ensure its successful implementation.

Social aspects:

The success of a new technology hinges on its acceptance by the general public. Take, for instance, reproductive cloning, an intriguing technology for researchers and scientists, which has faced dismissal by the majority due to concerns related to "playing God." Similarly, bioprinting might encounter a similar fate if the social aspects are not carefully considered. The softer impacts, such as their influence on the human psyche, cultural beliefs, and religious perspectives, play a pivotal role in determining the successful adoption of bioprinting technology.**[47]**

The FDA's Center for Devices and Radiological Health (CDRH) oversees the regulation of companies involved in the manufacturing, repackaging, re-labeling, and importing of medical devices sold in the United States.Similar to other medical devices, 3D-printed medical devices also undergo the same manufacturing process and adhere to regulatory requirements for obtaining approval. These requirements fall under two categories: premarket requirements that apply before marketing and post-market requirements that apply after marketing.**[48]**

Medical devices are categorized into Class I, II, and III, with regulatory control increasing from Class I to Class III. The classification also determines the specific regulatory requirements for each device type. Class I devices are exempt from Pre-market Notification 510(k); Class II devices require Pre-market Notification 510(k); and Class III devices require Pre-market Approval.

For medical devices classified under Class C or D, the manufacturing site undergoes inspection within 60 days from the date of the marketing application by regulatory entities. The purpose of this inspection is to verify compliance with the quality management system. A thorough inspection report is then prepared by the inspection team. Subsequently, the government has 45 days to decide whether to grant a license or reject the application for manufacturing or distributing the medical device.**[49]**

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