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3D Bioprinting Technology: Innovation and Application in Pharmaceutical and other Heath care sectors.

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ABSTRACT:
The field of 3D bioprinting represents a cutting-edge technology at the intersection of biomedical engineering and materials science, offering revolutionary advancements in tissue engineering and regenerative medicine. This document delves into the innovations and applications of 3D bioprinting, highlighting various techniques such as laser-assisted bioprinting and stereolithography (SLA). The process of bioprinting leverages advanced materials, including hydrogels and photoinitiators, to create complex, cell-laden structures with high precision. Key requirements for effective bioprinted scaffolds, such as biocompatibility, biodegradability, and mechanical strength, are discussed in detail. Moreover, the document examines the challenges associated with traditional tissue engineering methods and how 3D bioprinting offers viable solutions by enabling precise cell placement and reducing the use of cytotoxic materials. Through a comprehensive review of current methodologies and future prospects, this document emphasizes the transformative potential of 3D bioprinting in clinical applications, drug development, and personalized medicine.

Keywords: 3d bioprinting, biomedical engineering, materials science, tissue engineering, regenerative medicine, laser-assisted bioprinting, stereolithography (sla), hydrogels, photoinitiators, biocompatibility, biodegradability, mechanical strength, cell placement, cytotoxic materials, personalized medicine.

I. INTRODUCTION:

In the last few years, additive manufacturing or 3D printing, has become more and more important along with developing technology.[1] Due to its capacity to get around some of the technical difficulties in the field of tissue engineering, bioprinting technology has received a lot of interest lately.[2]

It acts as a prerequisite advancement for the advancement of emerging technologies such as drug testing, tissue engineering, biomimetic sensors and 3D tissue models. [3]Unlike 3D printing, 3D bioprinting technology allows for the printing of biological materials such as organs, tissues, and cells in order to produce biocompatible and biomimetic biomaterials. There are several varieties, including inkjet, extrusion-based, laser-based, and laser-supported bioprinting. Particular interest in the application of 3D bioprinting technology is shown in the medical, pharmaceutical, and biological fields. By enabling the synthesis of drug carrier systems, tissues, organs, and scaffolds appropriate for patient anatomy and patient-specificity as well as optional and instantaneous production, this technology increases bioavailability and patient compliance.[1]

The term "bioprinting" refers to the process of creating biological constructions with tissues, cells, and biomolecules that have a specific structure and biological function using materials science and production techniques.

Because bioprinting techniques have the potential to address most of the issues with traditional tissue engineering approaches, they have lately been investigated for a variety of biological applications. In traditional tissue engineering, scaffolds, cells, and substances like growth factors are combined.

Seeds of cells and substances that support tissue regeneration are placed within scaffolds. Strategies for tissue engineering have been applied to the regeneration of the skin, trachea, bone, esophagus, and heart among other organs. Despite the therapeutic efficacy of tissue engineering techniques, all scaffolds available today lack the complex and sophisticated native structure.Furthermore, the scaffolds created through tissue engineering do not replicate the natural structure of the tissues.

Essential requirements to fabricate the ideal scaffolds for tissue regeneration are biocompatibility, biodegradability, mechanical strength, biomimetic structure, sufficient porosity, therapeutic activity. To create tissue scaffolds, a

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variety of fabrication techniques have been devised, including electrospinning, freeze-drying, phase separation, gas foaming, particle leaching, and solvent casting.Tissue-engineered scaffolds, however, have limitations in supporting cell growth in three dimensions, do not perfectly replicate the original architecture of tissues, and have trouble depositing various cell types in the scaffolds at specific locations. Furthermore, a lot of these manufacturing techniques use organic solvents, which hinder cellular development. However, bioprinting provides a different strategy to address the majority of issues related to the tissue engineering techniques used today. Creating scaffolds to aid in tissue regeneration and repair is the primary goal of tissue engineering techniques. Although whole or partial organs can also be developed using 3D bioprinting techniques, the primary benefit is in the ability to manufacture entire organs for the purpose of transplantation. By combining scaffolds with cells and bioactive chemicals, bioprinting can be utilized to create biological constructions with precise micro/nano architectures. [3]

Above all, utilizing CAD models (Chua et al., 2003), 3D printing can easily produce intricate designs and complex structures.By using an initial CAD model of the finished object or design, bioprinting directly prints living cells and biomaterials layer by layer. Therefore, bioprinting can enable the exact positioning needed to produce tissue models with a high cell density that resemble actual human body architecture.[4]

Current challenges in drug delivery

Drug development in the clinical phase and drug discovery in the preclinical phase are the two main phases in the process of introducing a new drug. In the former, a huge number of potential compounds are identified as promising molecular candidates that will react with a biological target. The interaction of the drug with the target must be studied over a number of phases and testing cycles.

The next stage of development focuses on confirming the candidates safety and effectiveness through phase I–III and phase IV studies, which are conducted prior to and following market approval, respectively. Early in the pipeline, it is thought to be crucial to integrate drug delivery strategies with drug research and development procedures by employing models that would simulate their future. Despite the fact that the pharmaceutical business has made significant strides, the primary cause of the enormous time and expense spent on pharmaceutical research is still a high attrition rate.

Out of thousands of compounds, one to three candidates are chosen during the drug discovery phase, and out of about twenty-four candidates in development, only one novel molecular entity (NME) is eventually introduced . Reducing the attrition rate of drug candidates in clinical developmentmainly Phase II and Phase IIIpresents the biggest challenge and opportunity for pharmaceutical research and development (R&D) since clinical development accounts for nearly 60% of the overall cost and most of the discovery cycle time.

Furthermore, non-clinical toxicity was the leading source of attrition among the 605 terminated compounds out of 808 proposed compounds, accounting for 240 (40%) of the failures, according to a review of combined 2000–2010 data from four major pharmaceutical corporations.

The persistently high proportion of non-clinical toxicological failures could be caused by more predictive toxicity assays, which would be necessary for mechanisms that are more difficult to infer from in vitro data. Creating new methods to boost the utility of in vitro research can save pre-human trial expenses and greatly improve the early detection of a compound's toxicity.[5]

The majority of traditional in vitro drug discovery tests are conducted in two-dimensional (2D) monolayer cell culture methods, which are not ideal for accurately simulating invivo circumstances for evaluating cellular responses to medicines , as will be discussed in more detail in the following section. Drug effects are often modified in 2D cell culture systems, leading to unexpected or deceptive outcomes. Thus, the development of in vitro cell-based systems that can precisely predict safety and efficacy in vivo is essential. The advancement of technologies for creating three-dimensional (3D) structures could offer prospective remedies for the issues surrounding medication research.[5]

3D models in Pharmaceutics: Applications and limitations

To overcome above limitations and enhance the outcomes of cell experiments, two significant 3D culture systems that is 3D scaffold-based and scaffold-free systems have been created in the last ten years. [5]

Cell sheets are stacked, single or aggregated cells are seeded on a scaffold that has

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already been constructed, or the cells are embedded in matrixes that resemble extracellular gel (ECM) prior to polymerization or solidification to create scaffold-based models.[6] Supporting cell adhesion, growth, differentiation, and migration are the functions of scaffolds. Decellularized extracellular matrix (dECM) and a variety of natural or synthetic polymers with a range of mechanical, biocompatibility, and toxicological characteristics are frequently utilized scaffold materials.[7]

Cells are free to multiply in scaffold-free settings without the assistance of any external structures. In this mode, the 3D structures are formed by cellular self-assembly via cadherin-mediated adhesion.[5]

Furthermore, high-throughput microarrays can be used to microfabricate 3D models by a variety of techniques, such as cell printing, surface patterning, microfluidics, and micro-well technology. Numerous 3D constructs or microarrays of physiological organs, including the skin, heart, kidney, liver, lung, and disease models, such as pulmonary edema, have been developed thus far for high-throughput screening (HTS) of lead compounds for the development of pharmaceuticals and cosmetics as well as acute or chronic drug assays.[5]

Many 3D culture methods, including microarrays, with diverse ECM compositions have been created for various pharmaceutical applications, including as targeted drug delivery, therapeutic efficacy or toxicity studies, and HTS, due to the many benefits. Nonetheless, the development of biomimetic structures with suitable topological and mechanical modeling remains a major production problem due to the intricacy and specificity of 3D biological niches. Furthermore, not all 3D tissue culture models will be suitable for routine drug testing by the pharmaceutical industry due to the complexity of the unique setup needed for the creation of 3D models.[5]

Even with their advantages, 3D models nevertheless have a number of drawbacks. One of the main drawbacks is that, in comparison to 2D models, the inclusion of various cell types leads to greater heterogeneity and data unpredictability. Moreover, the volume and size of 3D models are not standardized. Secondly, biological features of some naturally-derived ECM matrices vary significantly from batch to batch, leading to inconsistent experimental outcomes.[5]

For large-scale research or high-throughput tests, 3D culture is more costly and time-consuming than conventional 2D culture. But the biggest drawback of 3D culture is its lack of vascularization, which has a significant impact on how cells behave because there is less oxygen, nutrition, medication, and intercellular material transported throughout the 3D structure.[5]

Lastly, from in vitro grown cells, functional 3D tissue models are unable to build hierarchical, organized architectures and structures that replicate the organization of native tissues.[5]

Basic Steps of 3D Bioprinting

The overall process of 3D bioprinting can be achieved via three distinct steps; pre-bioprinting, bioprinting, and post-bioprinting.

Pre-bioprinting

Creating a model that the printer will use and selecting the materials to be utilised are the initial steps in the pre-bioprinting process.

The process starts with the biopsy of a tissue sample, which yields a biological model that the 3D bioprinting technique will use to replicate.

In this step, technologies such as magnetic resonance imaging (MRI) scans and computed tomography (CT) are used.

To create 2D images, the images acquired using these techniques are tomographically rebuilt.

Next, the cells required for the procedure are chosen and multiplied. To maintain their viability, the resulting mass of cells is combined with oxygen and other nutrients.[8]

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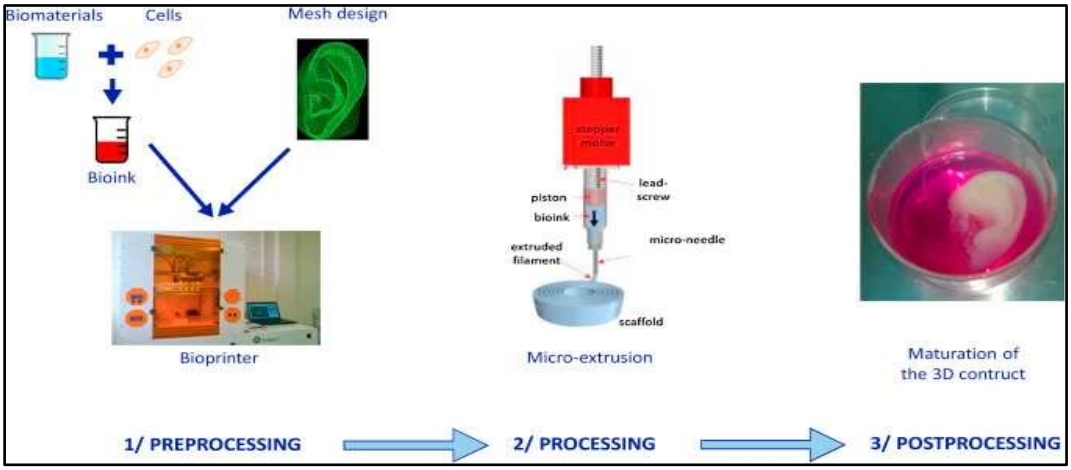

Fig no 1: A biological model used to replicate and involving MRI Scans to acquire 2D images in 3D bioprinting technology

Bioprinting process

Placing the bioink in the printer to create a three-dimensional structure is the second step in the printing process.

After the cells, nutrients, and matrix are combined to make bioink, the combination is put onto the printer cartridge, which deposits the material according to the predefined digital model.

In order to create a three-dimensional tissue structure, bioink is deposited onto the scaffold layer by layer during the development of biological constructions.

Because it calls for the development of various cell types according to the kind of tissues and organs to be generated, this step of the bioprinting process is complicated.[9]

Post bioprinting

The final stage of the bioprinting process, known as postbioprinting, is crucial for giving the printed structure solidity. Chemical and physical stimulations are needed to keep biological matter in its proper structure and functioning.

These stimuli give cells instructions on how to reorganise and continue growing tissues.
If this phase is skipped, the material's mechanical structure may be damaged, which would then impair the material's ability to function.[9]

3D Bioprinting Technology Methods

The process of bioprinting a tissue or organ is intricate and is dependent on the cellular systems, printing methods, and intrinsic qualities of the bioinks. In addition, variables including temperature, humidity, the surface tension and viscosity of the bioink, and needle orifice size affect the printed structure's resolution. Using a cartridge or syringe, a standard bioprinting device can distribute bioinks onto a suitable substrate of choice.

More sophisticated bioprinting systems have numerous print heads that can hold separate or identical bioinks. With the use of computer-aided tools like CAD (Computer Aided Design), printing patterns may be created, altered, and printed. This procedure is simple and straightforward to use because it only takes a few seconds to minutes to make changes to the CAD files.[3]Depending on how they function, the bioprinting techniques can be further divided into basic categories: orifice-based or orifice-free technologies.[4]

Inkjet based Bioprinting

Initially, commercially accessible inkjet printers that were appropriately customised were used to print living cells. When inkjet bioprinting was first being developed, one issue that arose was that the cells would instantly dry out on the substrate, which would cause them to die during printing.[10]Cell-loaded hydrogels were created by encasing the cells in a highly hydrated polymer in order to solve the issue. Cells may be precisely positioned using inkjet bioprinting; some research have even managed to print as few as one single cell per printed droplet. Droplets expelled by heat or piezoelectric methods are used to pattern cells and biomaterials into the desired pattern.[11]

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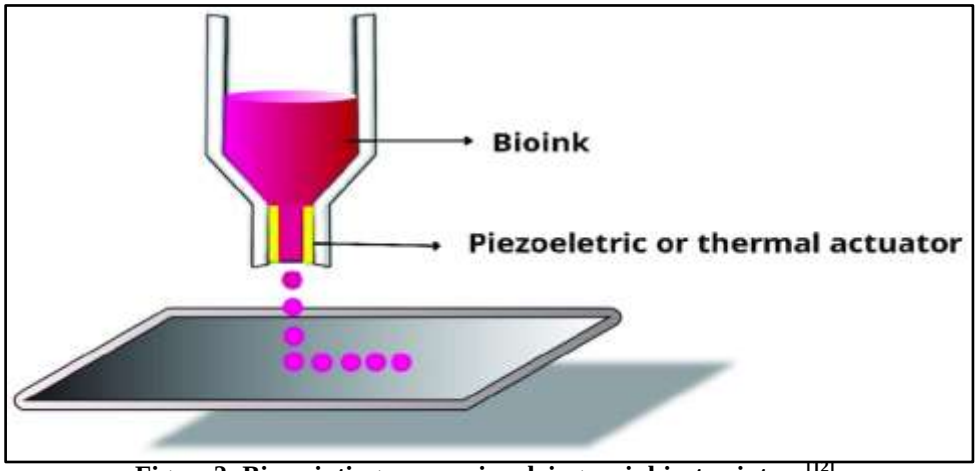

Fig no 2: Bio printing process involving an inkject printer. [12]

Diagram of bioprinting using an inkjet printer. The bioink is forced through the micro-nozzle by heat-induced bubble nucleation in thermal inkjet technology. The bioink is forced through the micro-nozzle by the acoustic waves produced by the piezoelectric actuator.

A heated element is used in thermal-based inkjet printing to create a bubble. A droplet is expelled from the printer as a result of the bubble creating internal pressure. The temperature range that the thermal element may attain is 100 °C to 300 °C. Research has demonstrated that the high temperatures are localised and only last for a brief period of time, allaying initial fears that they harm.

Acoustic waves are used by a piezoelectric device to expel the bioink. Because the viscosity of the highly concentrated and viscous bioinks dampens the applied acoustic/pressure waves and prevents a droplet from being ejected, this process restricts the application of these bioinks. Using low concentration solutions results in a low viscosity, which is a barrier to the production of three-dimensional structures.

Up to 50 µm of high resolution is possible with inkjet printing. Although there is a chance that produced shear stresses could harm the cells, the majority of research suggests that this is not the case. Most inkjet bioprinters offer good cell viability. High print speeds, low costs, and broad availability are benefits of inkjet-based bioprinting; nevertheless, drawbacks include inaccurate cell encapsulation because of the ink's low concentration and poor droplet directionality.

Excellent cell viability and the capacity to create a neural network in printed organs have been proven by inkjet bioprinting. Using a piezoelectric inkjet printer.

Immediately following printing, 86% and 90% of the neuronal and glial cell viabilities were found. The printed cells proliferated at a pace that was comparable to that of the unprinted cells. After seven days, the printed cells appeared to have formed neurites that lengthened.

Aho et al. used an alginate hydrogel with feline cardiomyocytes cardiac muscle cells to create cardiac tissue with a beating cell response. In order to promote crosslinking, layers of CaCl2 were printed into an alginate hydrogel precursor solution to create the tissue. According to the findings, cardiac cells adhered to the alginate successfully imitated the original heart extracellular matrix. Under mild electrical shocks, the printed heart tissues demonstrated contractile characteristics.

Because inkjet bioprinting has high resolution and cell survival, it is quite interesting. Multiple cell types can be accurately positioned using this approach . For further advancements, inkjet bioprinting might need to be integrated with other printing methods due to the constraints of restricted viscosities and vertical printing.[11]

Laser Based Bioprinting

Stereolithography (SLA) is an AM technique that cure photosensitive polymers layer by layer using ultraviolet (UV) or visible light. The drawbacks of shear pressure that come with nozzle-based bioprinting are eliminated by this nozzle-free method. With resolutions ranging from 5 to 300 µm, it provides a quick and precise production. At the top of the bioink vat, where the biomaterial is exposed to light energy, polymerization takes place. Theplatform holding up the structure will be lowered in the vat so that a fresh layer can be

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photopolymerized on top after each layer has completed polymerization.

On the vat's surface, where light energy is applied to the light-sensitive bioink, photopolymerization takes place. During fabrication, the axial platform slides along the Z-axis downward. This layer-by-layer method is based more on the design's height than its complexity.Chemical compounds known as photoinitiators react with a material's monomers to form reactive agents, which in turn start the production of polymer chains. Different wavelength ranges can activate different photoinitiators; some are triggered by UV light, while others are triggered by visible light. The concentration of the photointiator determines the stiffness and network density of the cured resin; however, larger concentrations may have unfavourable cytotoxic consequences. Nonetheless, the degree of cytotoxicity varies with photoinitiators. Eosin Y for visible light and Irgacure 2959 for UV cross-linkage are the most widely utilised and least cytotoxic photoinitiators. In fact, studies have indicated that Eosin Y is less harmful than Irgacure 2959.[11]

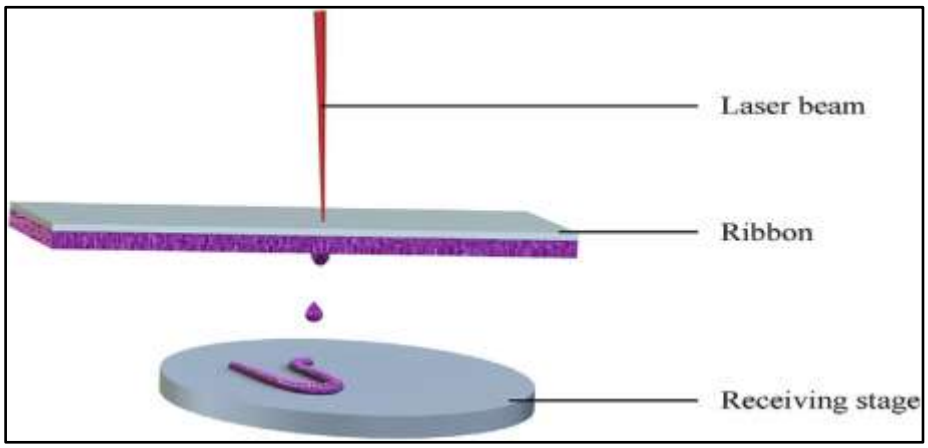

Fig no 3: The diagram representing a flow of laser beam through a ribbon of laser based bioprinting device. [13]

A number of researchers have looked into different ways to enable photopolymerization of bioinks because using UV light or photoinitiators that are cytotoxic can harm cells. A thiol-ene reaction was used to create a family of materials that crosslink without the need for a photoinitiator. Two types of monomers with at least two alkene or thiol groups make up the utilised monomers. When exposed to ultraviolet (UV) light, these two substances react spontaneously at a wavelength of about 266 nm. After three days, a 1:1 ratio of thiol to alkene showed high cell viability, approximately 95%. Even though this number of thiol groups produces high amounts of surface functional groups, allowing greater subsequent surface modification, doubling the thiol concentration had a deleterious effect.[14]

Despite the fact that mammalian cells may be harmed by near-UV blue light, which has a wavelength of 400–490 nm ,the human Adipose-Derived Stem Cells (hADSCs) showed considerable metabolic activity after creation, by 75% and 50% in 5 to 7 days, respectively. Camphorquinone and eosin Y are further photoinitiators that have the ability to absorb visible light. They crosslink at wavelengths of 400–700 nm and 514 nm, respectively . PEG was combined with eosin Y and methacrylatedgelatin (GelMA) . Samples lacking GelMA showed lower cell viability after five days than samples with 5% and 7.5% GelMA, which kept cell viabilities at about 80% . The non-adhesive nature of PEG, which kills anchorage-dependent cells, may be connected to the somewhat lower cell survival .

The field of bioprinting can greatly benefit from the application of stereolithography. Because there is no shear stress and no restriction on the viscosity of the bioink, it is a desirable option for enclosing cells in scaffolds. The damage that UV and near UV light do to cell DNA, the restricted selection of photosensitive biomaterials, and the cytotoxicity of additional materials are some of the drawbacks of SLA. Alternatives such as the use of materials free of photoinitiators or photoinitiators that absorb visible light have previously been explored by several researchers. [11]

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Laser assisted Bioprinting

The original purpose of developing laser-assisted printing was to deposit metals onto receiver sheets. subsequently create the method for printing spinal cord cells from embryonic chick embryos . Three components make up laser-assisted bioprinting (LAB): a laser pulse, a receiver slide, and a donor slide (also known as a ribbon). A layer of clear glass, a thin coating of metal, and a layer of bioink combine to form a ribbon. As shown in Figure 4, the hydrogel's metal layer is vaporised by a laser pulse, transferring the bioink from the ribbon onto the receiver slide. This scaffold-free method offers a resolution of 10–50 µm and exceptionally high cell viabilities (>95[15]). A single cell per droplet precision has been shown in certain LAB studies.

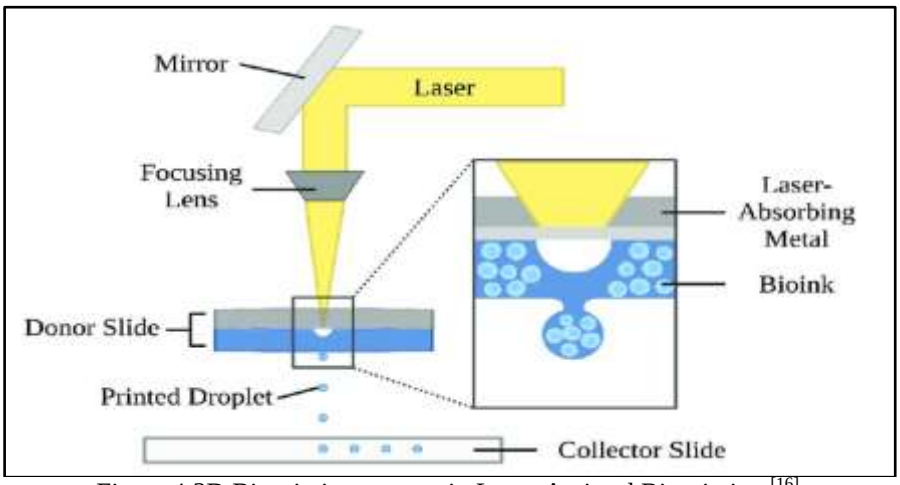

Fig no 4:3D Bioprinting process in Laser-Assisted Bioprinting.[16]

Diagram of Bioprinting Assisted by Laser. clear glass; a thin layer of metal; and a bubble created by vaporisation. Laser-induced bubble nucleation drives bioink droplets towards the substrate. Cell viability is not significantly impacted by this approach. A scaffold, polymer sheet, or biopaper can be used as a receiver-slide.
Researchers looked at how printed mesenchymal stem cells (MSCs) responded to the LAB laser pulse. The results showed that the laser pulse had very little impact. After five days in cell culture, cell proliferation rates were comparable to the control group of non-printed cells, and there were no documented alterations in gene expression brought on by the heat shock of the laser pulse. After three weeks in osteogenic media, the expression of alkaline phosphatase (ALP) and the buildup of calcium were comparable to those of non-printed MSCs.

It has been shown in several investigations that LAB can accurately position different types of cells, and that it can even put a single cell per droplet. Nevertheless, it is a costly procedure with limited scalability and stability. When paired with other biofabrication techniques, it has demonstrated considerable potential.[11]

Extrusion based Bioprinting

Printing via extrusion is a pressure-driven method. As shown, the bioink is extruded through a nozzle and deposited in a prefabricated structure under mechanical or pneumatic pressure. The capacity to print at extremely high cell densities is the primary benefit of extrusion bioprinting. When compared to other technologies, it has some drawbacks despite its benefits and versatility. The resolution is far lower than that of other bioprinting processes, with a minimum feature size typically exceeding 100 µm. This may restrict its use in some soft tissue applications where smaller pore diameters are necessary to enhance tissue responsiveness.[17]

But might still be useful for hard tissues bigger than 10 mm in size. Although numerous studies have revealed, the pressure utilised to extrude the material has the ability to change the shape and function of the cells. In summary, a thorough investigation involving several process variables, such as viscosity, nozzle diameter, and the associated shear stress, must be assessed prior to hydrogel printing. Highly viscous hydrogel is used in this production process, and chemical additives are not always needed for the printed structure to cure.

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For extrusion-based bioprinting, the hydrogel ink's rheological behaviour is crucial. Most hydrogels are non-Newtonian fluids, which means that shear rate affects how viscous they are. On the other hand, greater viscosity of the bioink leads to increased induced shear-stress during printing, which in turn causes increased apoptotic activity in cells. The phenomena of shear thinning, or the viscosity dropping with an applied shear force, is significant in non-Newtonian fluids. This directly affects print quality by making it possible to create a plug-like flow that gives the extrusion process more control over when to start and stop. While low viscosities lead to less dense networks that may facilitate greater cellular infiltration, excessively low viscosities will result in a poorly defined structure that will ultimately compromise print fidelity.[11]

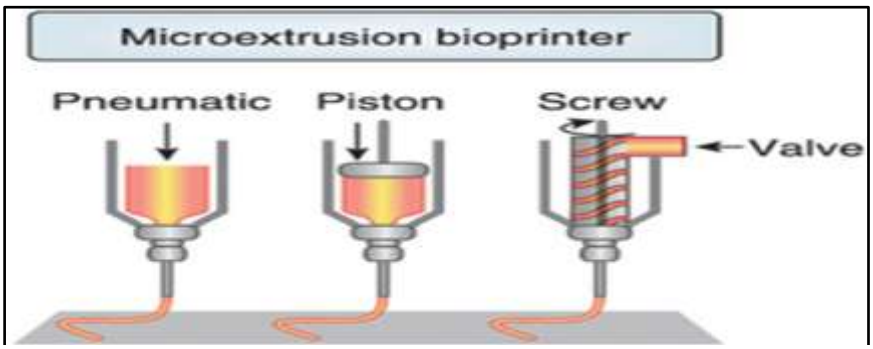

Fig no 5:Schematic representation of an Extrusion-oriented Bioprinting

Structures with excellent mechanical qualities and print fidelity can be created using extrusion-based approaches.

A trade-off between rigidity and softness needs to be considered when selecting a hydrogel to utilise as the basis material in order to have a robust supporting structure that permits nutrition infiltration and the capacity to encapsulate cells. To maintain acceptable printing fidelity, high concentrations or crosslink densities are required, however this restricts cell movement. Low concentrations, however, typically offer poor mechanical qualities and printability. Reinforcing fibres such as PCL can be employed to enhance the hydrogel's mechanical properties . Because photopolymerization allows hydrogels to form quickly after printing and retain print fidelity by incising light energy at the right wavelengths, it is emerging as a viable crosslinking reaction for bioprinting.

The hydrogel's ability to retain its mechanical qualities after printing is one of its key characteristics. The hydrogel is subjected to various stresses during printing. Extrusion- and inkjet-based printing technologies, for example, can break or disturb the hydrogel molecular network's interlinking connections due to significant shear stresses. The hydrogel crosslinking damage may result in a decrease in viscosity and a decrease in print fidelity. Research on self-healing hydrogels has been done to address this problem. Because its non-covalent reversible bonds allow it to maintain its printed structure, a self-healing hydrogel

The process of extrusion can potentially have an adverse effect on the viability and functionality of cells. Shear pressures can lead to cell apoptosis and a decrease in the number of live cells in highly concentrated bioinks . The shape, metabolic activity, and adhesiveness of the cells to the substrate can all be impacted by shear stress. However, because some cells are more resistant than others, the overall cellular response depends on the kind of cell.

A promising method for producing biomimetic structures to replace tissues and organs is extrusion bioprinting. Moreover, this method worked well for making microfluidic chips for scientific uses. Extrusion-based bioprinting, while very versatile and feasible for vertical printing, has a relatively low resolution that prevents cell positioning and necessitates the use of an advanced hydrogel bioink that preserves both mechanical integrity and cell viability. This hasled to the development and application of self-healing hydrogels and interpenetrating polymer networks.[11]

In vivo Bioprinting Applications

Due to its capacity to regulate both the amount of biomaterial utilized in the construct's fabrication and its geometry, 3D bioprinting has emerged as the most promising technique in tissue

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engineering. Some concerns, like cell survival and the vascularization of printed tissues that are to be arranged into more substantial and adaptable tissues or organs, remain unresolved.Since the primary barrier to creating humanscale tissue is vascularization, in vivo printing is only feasible for small-scale (few mm3) nutrition diffusion that has practical applications.[19]

Skin tissue

The skin is a complex tissue made up of various cell types and structural elements including blood arteries and sweat glands. Numerous attempts at 3D bioprinting of skin tissue have been made, and they have been successful in improving the skin's growth and/or recovery when assessed in vitro and in vivo.[19]

Its thickness is limited, nevertheless, by the lack of vascularization, which gives cells the oxygen and nutrition they require. Additionally, there are patient morbidity, low native tissue adhesion, and high fabrication one of the constraints that needs to be addressed is cost.

To print and preserve the viability of large skin tissue constructs, vascular network integration must be achieved by printing endothelial cells within the printed skin tissue construct or by using some other method.

Skardal et al. investigated in vivo bioprinting of skin tissues to repair a major wound in a mouse model, similar to in vivo bioprinting of bone tissues. To bioprint on an immunodeficient mouse's skin wound, mesenchymal stem cells (MSC) or amniotic fluid-derived stem cells (AFS) were suspended in fibrin/collagen gels.

The locations treated with MSCs and AFS cells exhibited thicker tissue regeneration and vascular development in comparison to the control (gel-only) group. In vitro data indicated endothelial cell migration, however in vivo results revealed none, and the construct treated with AFS cells released more growth factors than the ones treated with MSCs.

To ensure that bio-electrospraying would not compromise cell survival and functionality, a subcutaneous implant of an electrosprayed skin tissue construct was also made in the dorsal flank of mice.After two days of implantation, the implanted construct's H&E staining findings showed no appreciable cell damage.[19]

Cartilage Tissue

The repair of osteochondral abnormalities, including those in the knee joints, is typically the focus of research on cartilage tissue bioprinting. To create heterogeneous tissue, Shim et al. used a specially designed multi-head tissue/organ building system (MtoBS).On day 1, osteoblasts and chondrocytes with high cell viability (90–94%) were printed onto a Posterior Cruciate Ligament (PCL) framework. Cells were able to proliferate at a centimeter-scale size without diffusion thanks to pores in the construct that carried nutrients and oxygen.

Kundu et al. printed cartilage tissue constructs using a multi-head extrusion machine, which they then subcutaneously implanted in mice and left there for four weeks. A histological examination of the recovered construct revealed improved production of type II collagen fibrils and cartilage tissue. While GAGs and collagen fibril structure guaranteed cell proliferation and the creation of a healthy cartilage tissue construct, the PCL framework increased the mechanical stability of the build. Nevertheless, there is a problem that has to be fixed because the layered build's cell viability (85%) was somewhat lower than that of a single layered construct (95–97%).

Shim et al. created multilayered heterogeneous joint tissue constructs using an extrusion technology known as the multi head tissue/organ building system (MtoBS).A solution of atelocollagen with human turbinate-derived mesenchymal stromal cells (hTMSCs) and growth factor for osteogenesis and a solution of CB[6]-HA with hTMSCs and growth factor for chondrogenesis, respectively, were used to clearly create a subchondral bone layer and a superficial cartilage layer. Following eight weeks of implantation in the rabbit's joints, histological staining of the construct revealed freshly produced tissue throughout, merging with surrounding native tissues.[19]

Pancreatic tissues

Creating bioink produced from pancreatic tissue by micro-extrusion printing and the decellularization procedure

Examining the possibility of employing extrusion-based bioprinting to manufacture scaffold-free tissue standards on a larger scale

Testing controlled release of anticancer medication against pancreatic cancer using extrusion-based printing

Resulting in an improvement in cell viability augmentation of pancreatic functions and able to regenerate pancreatic tissue following hybrid manufacturing and Rapid fusion capability and

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high viability and also Possibility of using 3D printing to distribute medications locally.[20]

Others Tissues

The majority of bioprinting research focuses on creating structures for the regeneration of skin, cartilage, and bone. Table IV provides a summary of the others. Numerous artificial blood artery designs are proof-of-concepts that raise the prospect of printing biomaterial floating in cells into the required shape. Although there is still much work to be done on in vivo assessment, it is challenging to replicate the mechanical and biological characteristics of natural blood arteries.

The technique of bioprinting tissue or organ can be enhanced via post-printing fabrication to allow printed cells to develop into the desired cell types. Human embryonic stem cells (hESCs), which have developed into cells resembling hepatocytes, and human-induced pluripotent stem cells (hiPSCs) have both been used to create a mini-liver by Faulkner-Jones et al. A valve-based bioprinting system30 was used for this, and cell viability (84%), was not negatively impacted. They created a three-dimensional alginate matrix. Peak albumin secretion was found during an assay conducted following a 21-day differentiation regimen, indicating that the construct was hepatic in origin.

Due to their capacity for neuroprotection and regeneration, neural stem cells can be used to replace damaged or malfunctioning brain tissues at the desired location. Cell-to-cell communication, which is made possible by morphological structure, is an essential feature of brain tissue. One important aspect of creating nerve tissue is the ability of a 3D bioprinting technology to produce biomaterials in highly regulated geometries.[19]

Biopreservation

The challenges associated with traditional cryopreservation (such as ice crystal formation) can be addressed by using bioprinting technology to convert a bulk sample of cells into micro- and nano-liter droplets. Conventional cryopreservation methods use bulk volumes that impede proper cooling and rewarming, causing detrimental alteration of cells during biopreservation .[21]

Recently, red blood cells (RBCs) were vitrified using a bioprinting technique and cutting-edge bio-inspired cryo-inks.[22]

With the goal of lessening the detrimental effects on red blood cells (RBCs), the bioprinter produces significantly smaller volumes of cell-encapsulated droplets (<0.15 nl). It also offers ultrahigh cooling and rewarming rates that enable cell preservation while minimizing ice crystal formation. Additionally, the cells were loaded with a bio-inspired ectoine-based cryo-ink, which functions as a non-toxic cryoprotectant and removes the hazardous effects linked to traditional cryoprotectants (such as glycerol and dimethyl sulfoxide).[22][23]

The biopreserved cells retained their distinct shape, mechanics, and function after being rewarm. The bio-inspired bioprinting method may open up new ways to biopreserve cells for use in regenerative and reproductive medicine, such as stem cells, lymphocytes, and oocytes.[24]

II. CONCLUSION:

The use of 3D bioprinting has led to notable advancements in tissue engineering; nevertheless, additional work is still required to create more complex organs and enhance the biological capabilities of the bioprinting devices already on the market. Changes in bio-printers and printing circumstances are anticipated to better adapt biological properties of cells and organs to meet these objectives. In particular, appropriate culture and nutritional conditions are predicted to enhance cell survival and preserve cell activities during printing. Blood vascular networks have to be a prerequisite for creating complicated organs, and strong cooperation between biologists and engineers is also crucial.

A major problem in 3D bioprinting is not only to increase the biological functionality and complexity of printed tissues/organs, but also to figure out how to produce human-scale tissues/organs. First off all, it is difficult to popularize under the existing circumstances due to the vast number of cells required for printing human-scale tissues and organs, which is both labor- and money-intensive. In order to preserve the viability of printed human-scale tissues/organs, second, technical challenges pertaining to the printing techniques and the culture conditions need to be resolved. Third, it is very hard to restore the correct functioning of printed tissues/organs; this involves lining up printed cell kinds, directions, connections, blood arteries, nerve fibers, etc. in the right order. Fourth, appropriate regulations on the production and use of printed human-scale materials must take ethics into consideration.

3D bioprinting remains a viable and promising approach for regenerative medicine in spite of these obstacles. We anticipate seeing a

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great deal more functional tissues and organs created via 3D bioprinting in the near future. These might potentially be used as novel platforms for basic biology research, tailored drug screens, and medication effect testing. Above all, the creation of functioning organs and tissues will have a significant influence on medical care; hopefully, those perfect times will arrive soon.[25]

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