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3D BIOPRINTING AND BEYOND: A TECHNOLOGICAL TRANSFORMATION FOR PHARMACEUTICAL AND BIOMEDICAL APPLICATIONS

S. S. Khatake, A. R. Shirode *, S. N. Salvi, A. A. James and V. J. Kadam

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai - 400614, Maharashtra, India.

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3D Bioprinting, Boink, Fused Deposition Modeling (FDM), Direct Ink Writing (DIW), Laser-Induced Forward Transfer (LIFT), Inkjet Bioprinting, Stereolithography, 4D Bioprinting

Correspondence to Author:
Abhay Ramchandra Shirode

Assistant Professor,
Department of Quality Assurance,
Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai - 400614, Maharashtra, India.

E-mail: abhay.shirode@bvcop.in

ABSTRACT: 3D Bioprinting is a transformational technology that is an indispensable part of the pharmaceutical and biomedical fields. It has been used globally as a cutting-edge tool to improve future prospects. This review encompasses a brief explanation of what exactly 3d bioprinting is, its processing, and how it differs from 3d printing. The elemental segment of bioprinting is bioinks which are biomaterial and human cells pledged to print biomedical products, their manufacturers, and modernistic breakthroughs. The hot-off-the-press technologies comprise fused deposition modeling (FDM), direct ink writing (DIW), laser-induced forward transfer (LIFT), Inkjet bioprinting, and stereolithography from a pharmaceutical perspective. The other half of the paper deals with pharmaceutical and biomedical applications, introduces the need for 4D bioprinting, and sheds light on the future prospectus of this far-reaching technology. To conclude, 3D and 4D bioprinting uses bioinks and some modernistic methods for pharmaceutical and biomedical applications. 3DP could emerge as a transformative technology with the potential to become an essential component of industrial manufacturing and household appliances. The majority of hospitals and pharmaceutical businesses will most likely install a 3D printer for quick and customized products such as formulations, implants, and prosthetics.

INTRODUCTION: An automated computer-assisted layer-by-layer deposition of biological material called "bioprinting" is used to create functional human organs. It aims to recreate the complex tissues' normal structure and functionality by depositing materials and cells in a specific manner that mimics the native cellular architecture. 3D bioprinting involves stacking living cells to create biological tissues and organs in three dimensions biocompatible structures can be created with 3D bioprinting in a shorter amount of time than traditional methods. When using the technique to treat acute wounds, a shorter production period can be critical. Bioprinting could be a subtle method throughout design wherever bioink composition, bioprinting approach, and bioprinter sort should be taken under consideration. 3D bioprinting provides greater control and allows for the precise placement of various cell types at the required sites [2].

3D bioprinting innovation is not only backed up by its use in human health but also in veterinary applicability. Like fabrication of bone, cardiovascular organ, cartilage, corneal, and neurological constructs. The veterinarian translational capacity is well supported by existing research on animal-derived cells and animal models

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in human research Computer modeling, making of bioink, depositing of bioink, and maturing of printed goods are all steps in the bioprinting process [3, 4].

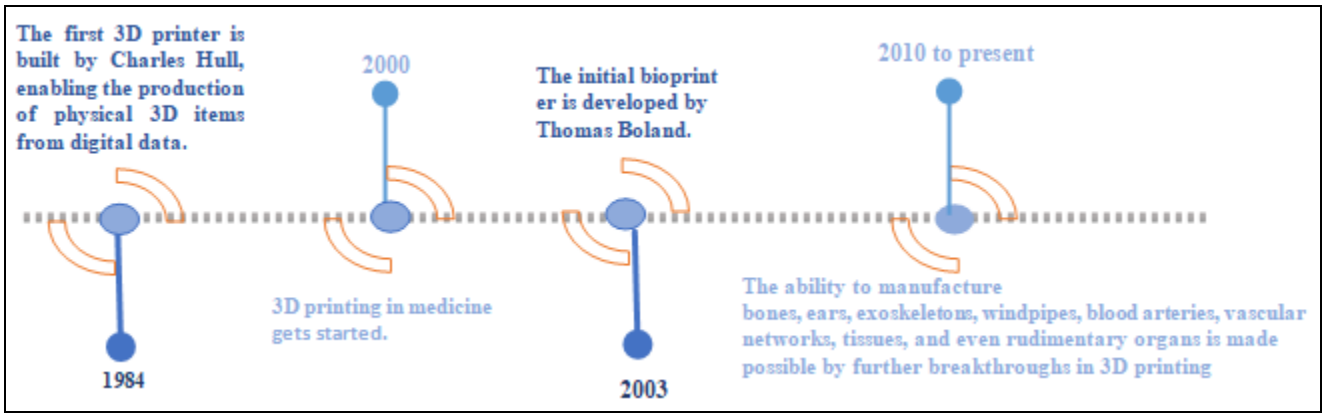

FIG. 1: HISTORY OF BIOPRINTING 1

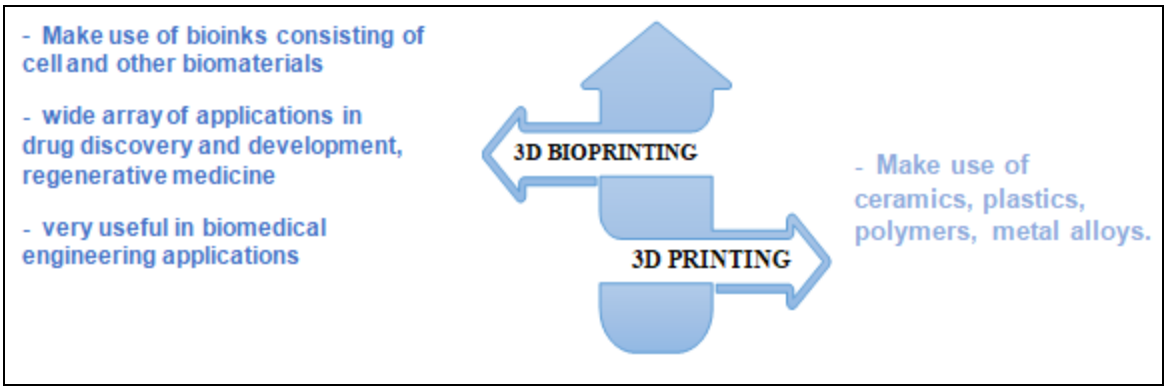

FIG. 2: 3D BIOPRINTING VS. 3D PRINTING

MATERIALS & METHODS:

Bioinks:

- Printable material or hydrogels used for 3d bioprinting.
- Bioinks can be characterized based on their cytocompatibility (by which the viability of cells, emigration, reproduction, differentiation, and formation of tissues are all governed) and printability (which has an impact on form integrity and strength/firmness) [5].
- Bioinks must demonstrate Mechanical qualities, functionality alterations, controlled biodegradability, and non-toxicity of cells (which allows them to obtain nutrients for development while also increasing metabolic activity during tissue repair) [6, 7].

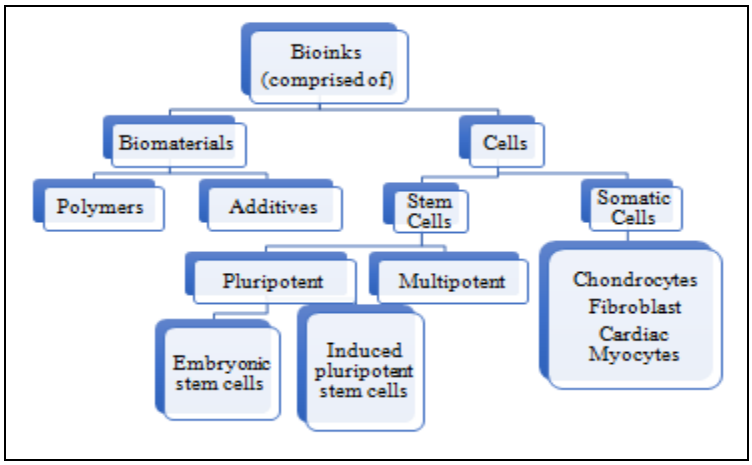

FIG. 3: BIOINKS – BIOMATERIALS AND CELLS

Biomaterials:

- Several biomaterials have been reported for use in 3D bioprinting. Important requirements for choosing are mentioned below in **Fig. 4.**
- Biomaterials can be further subdivided into:

1) Natural biomaterials

2) Synthetic biomaterials [8]

- Fibrin, cellulose, silk, ECM-derived bioinks, cell aggregates, cell spheroids, *etc* are a vital part of bioink composition.

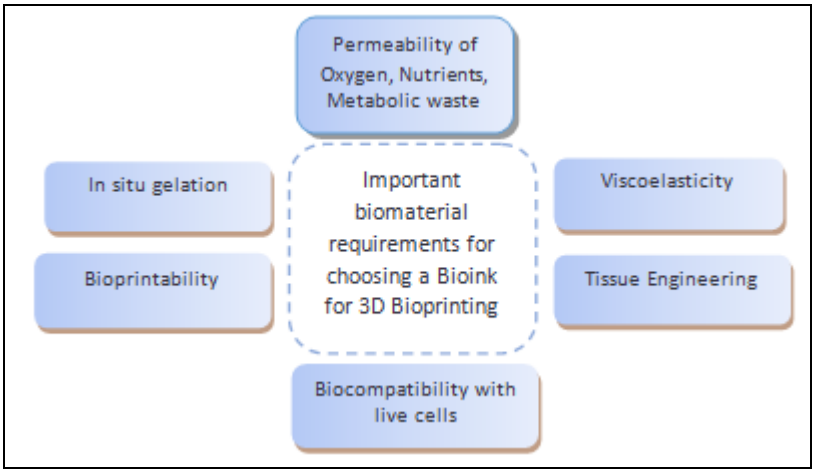

FIG. 4: BIOMATERIAL REQUIREMENTS FOR CHOOSING BIOINK

Cells: Bioprinting takes use of these stem cells ability to self-renew and differentiate in order to regulate tissue development and, eventually, construct bioprinted tissues.

Pluripotent stem cells have the potential to develop into any form of cell in the body. ESCs are formed from the blastocyst's inner cell mass. Their seclusion is linked to the annihilation of an embryo, however, in most cases, a rejected embryo from an in-vitro fertilization clinic. They need minimal genetic modification and have a low chance of developing into tumors. iPSCs may be created from adult cells, they sidestep ethical issues. Reprogramming cells to a pluripotent state, on the other hand, can be difficult to obtain and sustain [10, 11].

Multipotent stem cells/adult stem cells may be acquired ethically from a wide range of tissues. Invasive treatments such as bone marrow aspiration or liposuction-based approaches may be required to get these cells. Benefits include-

Give Birth to Offspring Linked To Their Original Tissue,

Lineage Reprogramming, When Compared To Ipscs, Their Usage Is Connected With Lower Cancer Risk [5].

TABLE 1: BIOMATERIALS CLASSIFICATION [5]

Natural biomaterials	Synthetic biomaterials
Polymers derived from natural resources. Advantages: Biocompatibility Biomimicking Of ECM Composition Self-Assembling Ability, Biodegradability. Examples: Agarose, Alginate, Collagen, Hyaluronic acid, Silk, Chitosan, Matrigel.	Polymers generated from synthetic resources Advantages: Mechanical Stability Controllability Picture Cross-Linking Capability Temperature And Ph Reactions Examples: Pluronic and polyethylene glycol, PVA, PLA, and PLGA.

Recent Advances in Bioinks:

- Development of exosomes instead of stem cells is a brand new link pointing toward bioink formulation.
- Exosomes with stem cells form membrane-bound extracellular vesicles that can influence cell growth and development.
- Exosomes that have been bioprinted can establish specific microenvironments that can assist repair abnormal cellular activity and influence the development of nearby host tissue [9, 12].

- Applicable in mitochondrial deterioration, attenuating chondrocyte degeneration and stimulating osteochondral defect repair [13].

Commercially Available Bioinks

- Alfatek PCL liquid ink, Peptide Gels: ALFATEK(India)
- Biogelx™-INK-S, Biogelx™-INKRGD:BIOGELX(UK)
- Cellink rg, dcellink fibrin: CELLINK(US)
- Gel4Cell®BMP, Gel4Cell®VEGF: INNOREGEN(Korea)

Process of 3d Bioprinting:

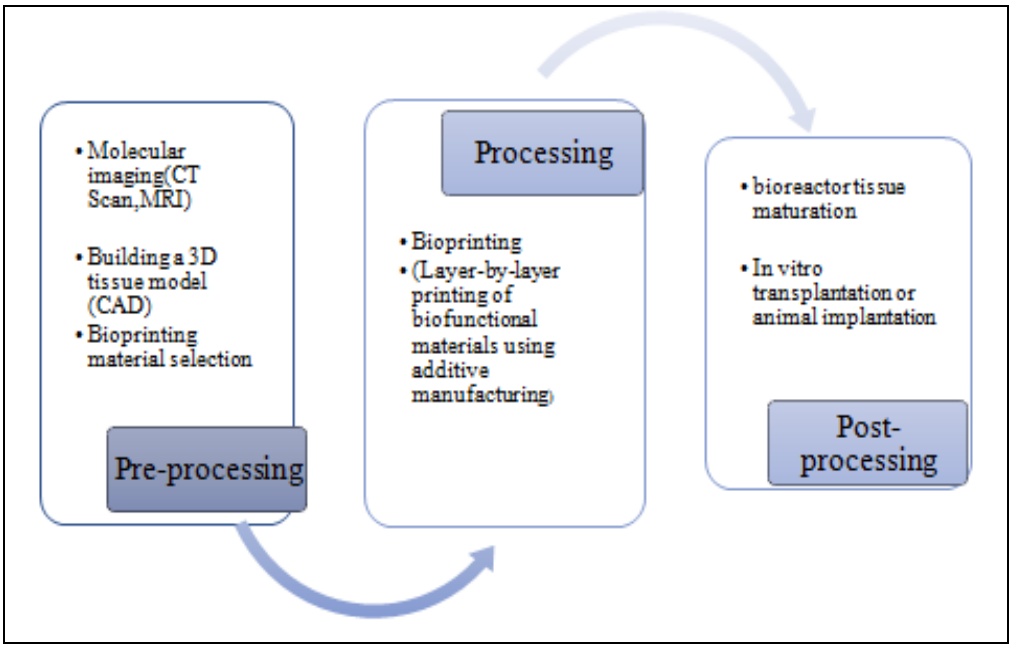

FIG. 5: PROCESS OF 3D BIOPRINTING

- Creating anatomically correct 3D models using computer graphics tools constitutes the preparatory phase.
- The choice of the bio-ink material was also made in this step.
- The tissues/medicines are actually printed throughout the processing stage.
- The term "post-processing" describes how the manufactured construct ages in a bioreaction and is then characterized in terms of its structural and functional properties.

3D Bioprinting Technology:

- Commonly, industries use older techniques such as scaffold-based, extrusion, and droplet bioprinting, but nowadays advancement came into the picture, so currently employed techniques are being used worldwide listed in the following **Fig. 6.**

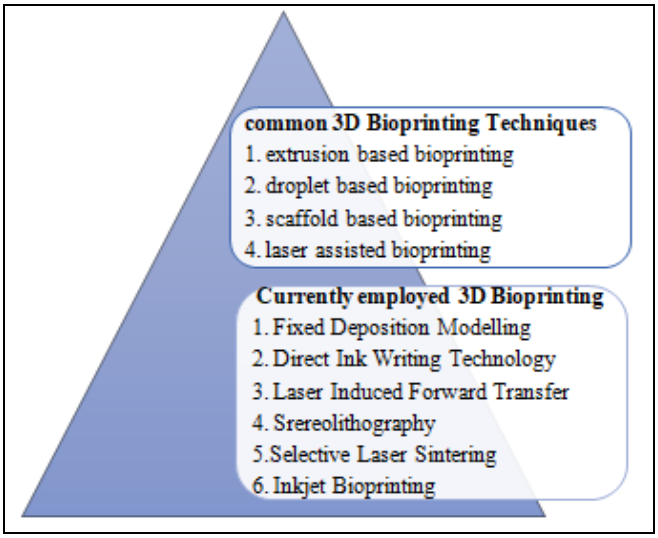

FIG. 6: TECHNOLOGIES OF 3D BIOPRINTING

Currently Employed Techniques:
Fused Deposition Modeling (FDM):
Introduction:

- Also referred to as SFF (solid freeform fabrication) techniques.

- Scott Crump pioneered the method within the Nineteen Eighties beneath the registered term united deposition modeling (FDM)
- FDM is a form of the nozzle-based deposition system. This process is based on the extrusion of molten material via a nozzle of exact diameter, which constructs three-dimensional objects by depositing successive layers of material on a heated plate according to a computer-generated design [13].
- Fused filament fabrication (FFF), typically referred to as FDM a material extrusion methodology. FDM is the most extensively used 3D printing process, with the greatest number of 3D printer users worldwide.
- The trademark fused deposition modeling (FDM) and its acronym FDM are owned by Stratasys Inc, a company co-founded by Scott Crump [16].
- Temperature and build-up, Build volume, warping, Layer height, Layer adhesion, Infill, and shell thickness, and Support structure are some of the characteristics of FDM Bioprinting [14, 15].
- Materials used in FDM bioprinting refer to **Table 2.**

FDM Working:

- In 3D printing/bioprinting, the user first builds a 3D model in a 3D modeling tool, stores it as an STL file, and then the printer's interface software transforms the file, splits the model into parts, and determines how the layers will be printed.
- FDM is a 3D printing/bioprinting technique that uses a continuous composite/thermoplastic material thread in the form of a filament
- When the model is transferred to the 3D printer/bioprinter, the construction material is extruded layer by layer through a heated nozzle until the item is finished.
- Extruder feeds the plastic filament into an extruding nozzle, melts it, and then robotically deposits it in a stratified manner onto the platform [14].
- Two linear slides the former one holding extruding nozzles and the latter one holding build platform, support the spools of plastic filament.
- They can either have an identical material as in amateur bioprinters or totally different materials.
- Model and manufacturer of the FDM printer decide whether the extruder and build platform are involved in XYZ movements. In which the X and Y axes relate to the extruder head gantry, Z axis to the build platform, and in other versions the print head to the X and Z and the build platform to Y [14].
- The Cube, Mojo, Buccaneer®, and MakerBot Replicator 2X are examples of 3D printers/bioprinters that utilize FDM technology.
- Processing stages of FDM

Stage 1:
Preparation for Part: Building options such as layer height, orientation, and infill percentage is selected. The software computes portions and Split parts and layers the part. The computer then generates building instructions and extruder trajectories.

Stage 2:
Setting up the FDM Machine: A spoolof thermoplastic filament is inserted into the printer's model and support extruder. When the nozzle of an extruder reaches the necessary temperature, the heating of the extruder starts. The filament will begin to push and melt into a tiny ribbon.

Stage 3:
Step for FDM Printing: Layer by layer, the material will be deposited by the extruder in predetermined locations to cool and harden.

When a layer is finished by the gantry (connected by a three-axis system), the Z-axis is glided over by either the head or the build platform according to the layer height. The above process is carried out for a fresh deposition.

Stage 4:

Eliminate the FDM Component: Disassembled all supports and cleaned the item after it has been taken off the build platform.

Stage 5:
Post-proceduring: After that, the part can be handled further to remove any supports that might still be there and complete it for the intended use [14].

Advantages of FDM:

- The lead times square measure bottom and cheaper than different additive-producing processes.
- FDM is being the most cost efficient technique among the others produces excellent thermoplastic parts and prototypes.
- The mechanical and environmental stability square measure is furnished by supporting the production-grade thermoplastics.
- Voids produced by this technology dont favour the potential
- Disadvantages of FDM
- FDM is not preferred for delicate thing as it lacks dimensional accuracy and backbone required .
- FDM units are not fully furnished hence , post-processing is crucial.
- Layer adhesion technique, imparts the fdm elements area unit a basic part [14]

TABLE 2: MATERIALS OF FDM BIOPRINTING

Material	Description	Strengths	Shortcomings
ABS	Abs-M30 is a Top-Notch Substance Used For Model Conceptualization, Functional Prototype Development, Producing Equipment, And Final Components	Good Thermoresistance Brings Strong Qualities (High Impact, Flexural, And Tensile Strength)	More Sensitive to Warping
PC	Polycarbonate, Frequently Employed in a Variety of Fields Including the Automotive, Aerospace, and Medical Ones	High Tensile and Flexural Strength Transparency, Rigidity, and Accuracy	Not Commonly Accessible More Expensive
PC-ABS	It is just a combination of Pc and Abs Materials, each one imparting its own strengths heat resistance and flexibility respectively.	High Intensity and flame-retardant	Not obtainable, expensive.
NYLON	Combination of Tensile Strength and Toughness. Hence, significant in Rapid Prototyping Concept Validation Models	High Strength, Excellent Wear, And Chemical Resistance	Low Humidity Resistance
TPU	Tpu 92A FDM Elastomer being a Thermoplastic Polyurethane Substance accustomed create durable stuff Elements.	Flexible, Toughness, enduring, scrape, Resistance.	Printing difficulty
PLA	Pla Is a standard Bioplastic material inclusive of Abs.	visually appealing, Easy to Print	Low Impact Strength

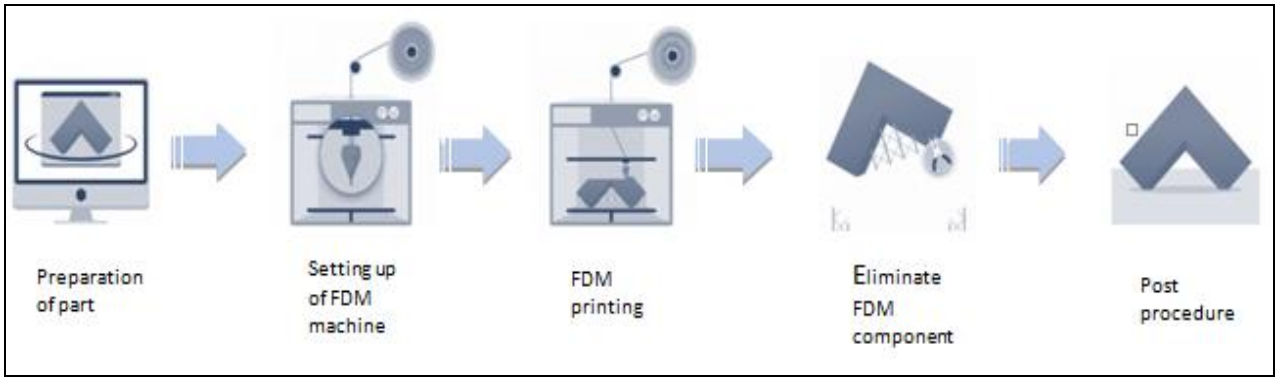

FIG. 7: PROCESS OF FDM

Challenges of FDM:

- FDM has many challenges to overcome, which are classified into three categories

Material specific:

- Filament formulation

- Filament diameter
- Thermal properties
- Mechanical properties
- Rheological properties

Operation Specific:

- Temperature
- speed
- Infill pattern and density
- Geometry

Machine-specific:

- Number of heads
- Nozzle diameter
- Gears force [13]

Direct Ink Writing:
Introduction:

- Any method that can directly print 2D/3D functional structures in complex patterns/ in intricate forms onto flat or conformal surfaces without the use of tooling or masks is referred to as "Direct Write" (DW) [17].
- Despite the fact that in some circumstances direct write can refer to freeform surface manipulation utilizing lasers and other procedures.

Categories of the DW Method:

1. Ink-Based
2. Laser Transfer
3. Thermal Spray
4. Beam Deposition
5. Liquid-Phase
6. Beam Tracing process

❖ DW is successfully recognized as a tool that can perform non-verbal written form and printed form of electronic components (conductors, insulators, batteries, capacitors, antennae, etc.) precisely from a computer file excluding the use of tooling or masks [18].

❖ Building structures with specific thermal, electrical, chemical, and biological.

❖ Reactions requires the employment of DW devices, among other things, and have a broad range of additional uses as well.

❖ Direct ink writing is a term used to describe fabrication techniques, that use a translation stage controlled by a computer, which operates an ink-deposition nozzle, a pattern-generating device.

❖ It produces materials with well-regulated composition and architecture.

❖ A variety of 3D structures can be built by DIW using direct material extrusion and deposition in a very competent manner [19].

❖ A controlled selective deposition of material in accordance with a pattern is required for Direct Ink Write (DIW).

Ink Designs used in DIW [19, 20]:

1. highly shear thinning colloidal suspensions,
2. colloidal gels,
3. polymer melts,
4. dilute colloidal fluids,
5. waxes,
6. concentrated polyelectrolyte complexes

Factors Influencing DIW:

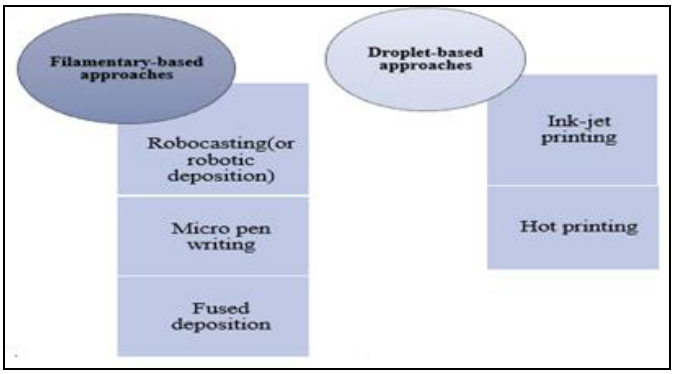

FIG. 8: FACTORS INFLUENCING DIRECT INK WRITING

Methods: This can be done in a variety of methods, including by;

1. Aerosol jetting,
2. Extruding liquid ink and
3. Ejecting material droplets onto a surface

A number of direct ink writing methods have been developed that enable the three-dimensional patterning of materials.

Advantages:

- The viscoelastic materials used by DIW are prone to collapsing while being printed. *In-situ* curing techniques which raise the yield strength of the finished structures raise the bar for the said materials.
- DIW facilitates the creation of embedded circuits and quick sensor production.
- Eliminating the masking and etching phases give this method of fabricating circuits an advantage over conventional methods.

Disadvantages:

- Need a lot of cleaning and upkeep to stay in great condition.
- Clogging is a genuine risk for substances like solder paste and it's extremely vulnerable to changes in viscosity, and because it applies pressure using air pulses
- All conductive inks and solder paste are non-Newtonian fluids, and they are thixotropic, meaning that when they are sheared, their viscosity decreases and it takes time for it to increase again. This may result in the aggregation of particles and unpleasant blockage.
- It employs a set volume conductive ink reservoir, so occasionally you'll run out of ink and need to reload your cartridge. For material replacement, entails downtime.

Challenges:

- The adjustment of ink rheology to obtain adequate printability and desirable functional qualities.
- Since the structures contain self-supporting features that must cross gaps in the underlying layer, they present the greatest barrier for ink design (s).

Laser-Induced Forward Transfer:
Introduction:

- Bohandy *et al.* for the first time, created and outlined this methodology.
- To achieve the laser direct-write of patterns regardless of surface type or material form, LIFT refers to a wide range of straightforward yet effective techniques that use a pulsed laser to locally transfer material from a source film onto a substrate that is in close proximity to or in contact with the film [25-27].
- A printing technique called laser-induced forward transfer (LIFT) enables the high-resolution deposition of a small amount of material in either the solid or liquid phase [40].
- It is a nozzle-free printing process that uses laser radiation as the material transfer driving force. It can produce prints with the similar printing quality, resolutions, and speeds to the majority of the technologies discussed above.
- The donor film is directly irradiated, either at normal incidence through a clear receiving substrate or at oblique incidence with flexible receiving substrates, and the irradiated material is propelled in the opposite direction from the incidence of the laser beam.
- However, it has shown to be particularly efficient for applications requiring high throughput and wide surface areas when employed for the printing of liquid inks.
- From the initial LIFT concept, some of these methods have developed such as

1. Matrix Assisted Pulsed Laser Evaporation-Direct Write (MAPLE-DW).
2. Laser Ablative Transfer(LAT).[33]
3. Laser-Induced Thermal Imaging or laser-induced thermal imaging (LITI)
4. Laser-Induced Pattern wise Sublimation or LIPS, for printing the RGB pixels found in their AMOLED displays.

5. Laser molecular implantation (LMI) is used to stably introduce fluorescent compounds into polymer sheets at specific depths [36].

Advantages:

- Offers good control of the propelled quantity and prevents substrate spoilage [25].
- Due to the fact that the LIFT printing process is performed outside of a clean room and without the use of special equipment, it is extremely simple and compatible with a variety of materials and substrates.

Disadvantages:

- Due to the great sensitivity of its control parameters [25].
- Cellular viability is less than those of other bioprinting techniques.
- It takes a lot of time, which inhibits the expansion of the laser-induced forward transfer industry.

Challenges:

- High-resolution printing of thin films in the solid state or small volumes of liquid droplets.

INKJET Bioprinting:
Introduction:

- Print biological materials with high preciseness, speed, and determination.
- The principle behind inkjet bioprinting is the use of heat or acoustic forces to discharge liquid droplets onto a substrate.

Classification is mentioned below in **Fig. 9.**

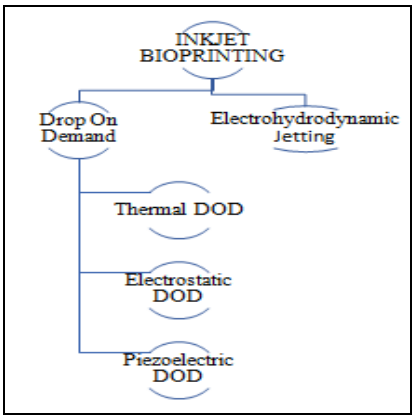

FIG. 9: CLASSIFICATION OF INKJET BIOPRINTING

Drop On Demand:

1. The drop-on-demand inkjet nozzle produces droplets only when the ejection signal is reached. This results in higher accuracy and higher ink utilization efficiency.
2. Drop-on-demand inkjet printers omit the units for droplet charging, electrostatic field generation, and ink circulation, making the overall structure simpler.
3. Usually in drop-on-demand inkjet printing, the ink in the chamber is displaced by a controlled pressure pulse. Once the pulse energy exceeds the threshold, a droplet is ejected from the nozzle.

Thermal Inkjet Bioprinting:

1. It can be done by electrically heating the print head to create pressure that triggers the discharge of droplets from the nozzle.
2. A hot element is used in printing to create a bubble nucleus. The bubble increases the pressure inside the print head, which prompts a droplet to be expelled.
3. The temperature range of the thermal element is 100 °C to 300 °C.
4. High temperatures are spotty and only last a short while [30].

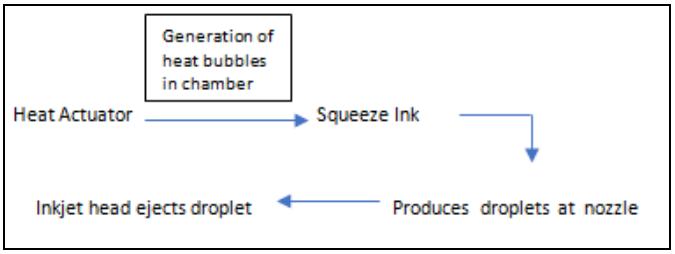

FIG. 10: MECHANISM OF THERMAL INKJET BIOPRINTING

Acoustic Inkjet Bioprinting:

1. An acoustic wave generated by a piezoelectric crystal inside the print head causes the liquid to condense into droplets.
2. A quick change in shape is caused when a voltage is given to a piezoelectric substance. In

turn, this produces the pressure needed to push the droplets through the nozzle.

3. Due to the fact that their viscosity dampens the applied acoustic/pressure waves and prevents the ejection of a droplet, extremely concentrated and viscous bioinks cannot be used [31].

Piezoelectric Inkjet Bioprinting:

1. The demitasse selectors in piezo DOD printing systems offer the advantage of ejecting variable-sized essay driblets,
2. It can publish different essay consistency on the printing face for advanced resolution printing.
3. Piezo publishing systems dominate the request f or the printing of Paper, fabrics, signage, and home scenery similar to wallpaper and ceramic pipe.
4. One to six bioinks can be handled, which offer the capability to publish/print via either single-pass or scanning printing.
5. A wide range of printing needs are supported by piezo technology such as CMYK(Cyan, Magenta, Yellow, Key/Black) colour models, cardboard, Billboards, and different wide-format aggregation, Putting labels directly on products.

Electrostatic Inkjet Bioprinting:

1. The ink is squeezed by the deformation of the chamber wall. Once the circuit is connected, the pressure plate is drawn to the conductor plate under the action of electricity.
2. The chamber will increase its volume and is replenished with ink. The circuit is then disconnected, and also the plate instantly comes to its original position
3. During this case, the chamber volume is reduced and the ink is squeezed out as droplets.
4. The resolution will be 2880 × 1440 dpi, and the separation between two neighboring droplets is 8.8-17.6 μm.

Electrohydrodynamic Jet Bioprinting:

1. Produces droplets through associate degree electric field instead of compressing ink with thermal energy or chamber deformation.
2. Bound pressure is applied to the ink within the chamber to create a conic ink meniscus at the nozzle passageway, which is termed a “Taylor cone”.
3. The printing nozzles area unit ordinarily metal-coated capillary tubes [32].
4. Once a particular voltage is applied between the nozzle and the substrate, droplets area unit ejected.
5. The significant blessings of the electro-hydrodynamic jet printing area unit are its high resolution and printing capability.

Advantages:

- High resolution
- Concentration gradient of cells, and also the growth factors within the construct.
- Electronic management of drop size and ejection rate

Disadvantages:

- Low droplet directionality and unstable cell encapsulation as a result of the low ink concentration [33].

Challenges:

- Some nanostructured materials, such as nanowires, nanofibers, or nanotubes, are challenging for inkjet processing despite being very promising in many electronic applications. Without changing distributed images' aspect ratios while printing the structures that give them their particular characteristics.
- Significant constraints concerning the rheological properties of the ink to be used limit the scope of the technique.
- One of the constraints of inkjet bioprinting is that the biological materials ought to be the liquid type to modify drop formation.

Stereo-lithography:
Introduction:

- With this technique, light is used to layer-by-layer for crosslinking the bio-inks in the reservoir [36].
- Charles W. Hull first popularized stereolithography in 1986It was defined as a method for making solid objects by successively "printing" thin layers of the ultraviolet curable material one on top of the other [37].
- The key to the strength of the SLA is its ability to quickly direct targeted radiation of applicable power and wavelength onto the surface of the liquid photopolymer resin, forming patterns of solidified photopolymer according to the cross-sectional data generated by the computer [38].
- Classification is given in the following **Fig. 11.**
- The basis of stereolithography is the resin's curing process.
- The primary step in the SLA 3D bioprinting pro cess is a photocrosslinking reaction illustrated in **Fig. 12.**

Materials used: When using SLA bioprinting, a prepolymer solution that can crosslink when exposed to light should be used to create the hydrogel.

- ❖ Methacryloyl-based photo-crosslinkable hydrogels
- ❖ PEG Dimethacrylate (PEGDMA)
- ❖ PEG Diacrylate (PEGDA)
- ❖ Gelatin Methacryloyl (Gelma)
- ❖ Dextran Methacrylate (Dexma)
- ❖ Norbornene hydrogels
- ❖ Mechanisms for photocrosslinking include
- ❖ Acryloyl-based photocrosslinking
- ❖ (Acryloyl functional group as a component of hydrogel macromers)
- ❖ thiol–ene click reaction
- ❖ (Alkenyl functional group as a component of hydrogel macromer).

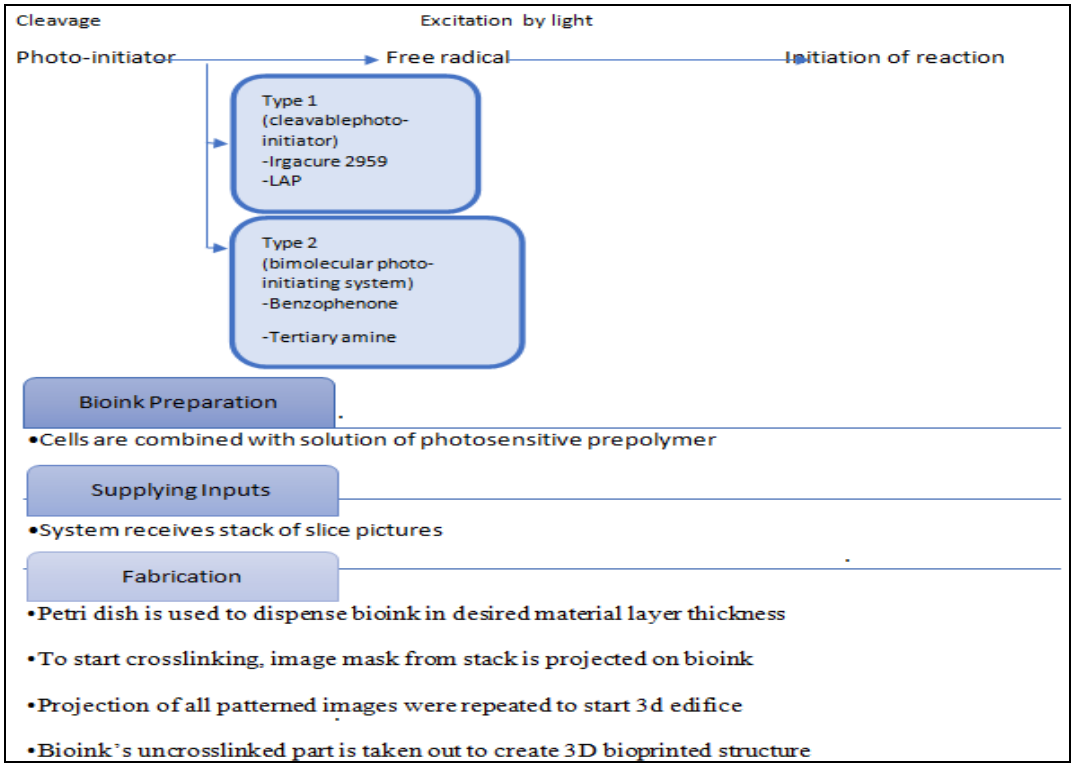

FIG. 11: CLASSIFICATION OF STEREOLITHOGRAPHY

Advantages:

- **Highly Precise:** good thinness up to 0.04 mm, and therefore the fine irradiation, it's attainable to get sophisticated geometry and prototypes.
- **Quality:** sensible useful Surface quality.
- **Smoothness:** sleek end elements, it can done with the variety of resins for various renderings.
- Attainable to make tiny elements as well as larger to 2 meter very precisely.
- On other hand, price is affordable even SLA is the process of material addition [70].

Disadvantages:

- Technique is limited to light-responsive bio-inks, typically including gelatin methacrylamide (gelma) and polyethylene glycol diacrylate (PEGDA) [71].
- Use of photopolymers leads to material waste and a costly experimentation [64].
- Number of projections decides The resolution and time required for fabrication hence there could be an extension leading to lower cell survival rate.

Victimization ultraviolet radiation light-based photoinitiation than visible light-based photo-initiation.

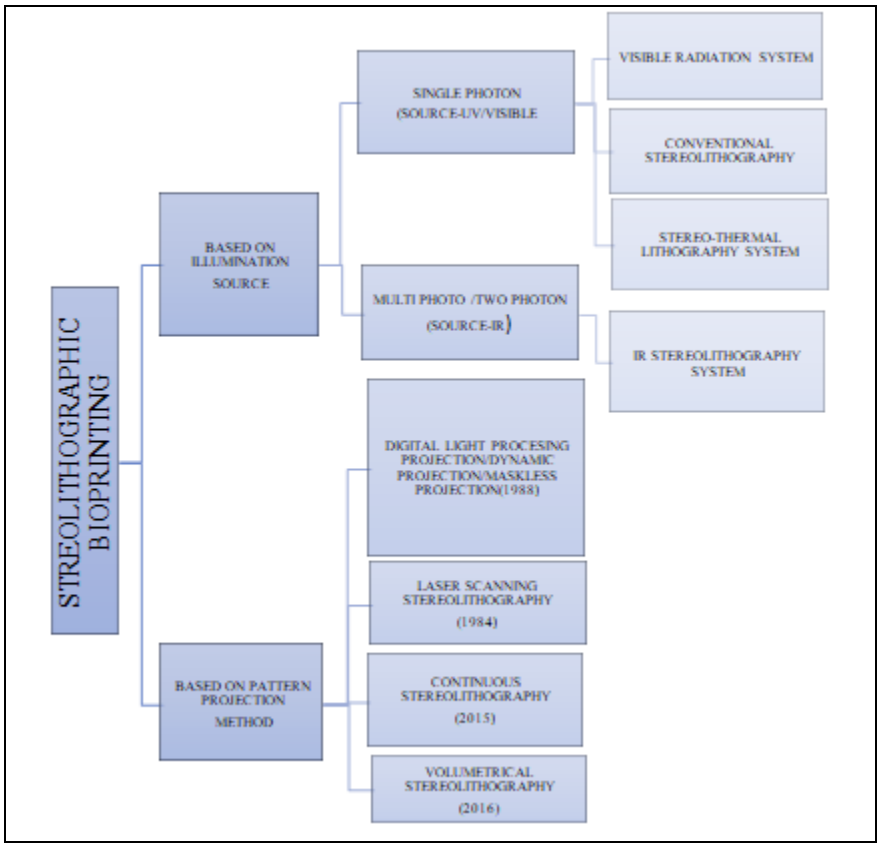

FIG. 12: CONVENTIONAL METHOD OF STEREOLITHOGRAPHY

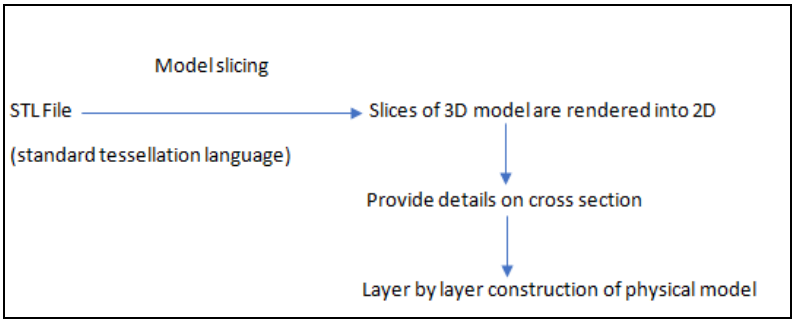

FIG. 13: ADVANCED METHOD OF STEREOLITHOGRAPHY

Challenges: Spatiotemporal regulation of deposition of materials onto the substrate matrix.

TABLE 3: APPLICATIONS OF TECHNOLOGIES

Sr. no.	Technology	Applications	References
1	Fused Deposition Modeling	1. Though the choices for product creation and manufacturing area units are limitless, the bulk of applications make up four broad categories: a)purposeful prototypes b)Production and producing tools c)Idea models d) Production quality elements 2. The creation of idea models utilized in the early stages of development. FDM models cut back prices and shorten development timelines 3. The creation of purposeful prototypes for testing functions. 4. Fabrication of producing tools.	14
2	Direct Ink Writing	1. New photopolymers with ultraviolet-assisted direct ink writing (UV-DIW). In this procedure, the ink is extruded using a DIW and then exposed to UV radiation to cure and solidify it. 2. tremendous promise for the environmentally friendly production of energy devices with arbitrary architectures. 3. Exceptional machining accuracy and controllability	19
3	Laser-induced forward transfer	1. Laser Printing of cells, Biomolecules, biological and chemical sensors, and materials for power storage 2. Laser Transfer of Intact Structures and Functional Devices 3. LIFT in Industry	26, 27, 29
4	Inkjet Bioprinting	1. The regeneration of purposeful skin and gristle tissues. 2. The high printing speed of this system permits the direct deposition of cells and biomaterials onto skin and gristle lesions. 3. Permits the deposition of primary or stem cells with uniform density onto lesions whereas maintaining cell viability and performance. 4. Layered cartilage constructs have also been developed using a combination of inkjet bioprinting and electrospinning technology. 5. Excellent cell viability and the capacity to build a neural network in printed organs. 6. The osteogenic lineage of stem cells was promoted by the bio ceramic nanoparticles, which mimicked the natural bone tissue microenvironment. With a piezoelectric inkjet printer, porcine Schwann cells and neuronal NG 108-15 cells can be used to bioprint neural tissue.	32-34
5	Stereo-lithography	1. Clinical imaging techniques such as CT scan/MRI for improvement of diagnostic techniques, quality and design of prosthesis and implants, and useful achievement of complex surgeries. 2. The tissue engineering for the fabrication of a biocompatible scaffold during which resins facilitate to stop inflammatory responses throughout implantation. 3. Sensible degradability with nontoxic byproducts that results in absolute excretory organ clearance with tissue regeneration. 4. High chondrocyte adhesion on scaffold target-hunting by pure mathematics. 5. To develop models for studying the cell behavior in 3D microenvironments. 6. In combination with novel biomaterials have been used to develop tissue constructs and study cell behavior and interaction in 3D microenvironments.	42-44

Applications of 3D Bioprinting:

- 3D bioprinting is a rapidly evolving industry that has been utilized for a variety of biomedical applications.
- Biomedical and pharmaceutical applications
- The application of bioprinting to veterinary care has important ramifications as well.
- Research efforts targeting human applications have utilized companion animal models to

investigate the safety and efficacy of bioprinted tissues [5].

Biomedical Applications [43, 7]:

- Skin tissues
- Cardiac tissue
- Cartilage tissue
- Bone tissues
- Blood vessels

Pharmaceutical Applications:

- Pharmaceutical companies have a variety of healthcare goods on the market, such as ibuprofen hydrogels, progesterone, pseudoephedrine drug delivery devices, guaifenesin polypills, and nifedipine, captopril, and glipizide [46, 49-57].
- The following dosage forms have been successfully making use of 3D Bioprinting.

1. Immediate-release tablets
2. Bilayer tablets(sustained and immediate release layers of tablets)
3. Implants
4. Capsules
5. Polypills [47]

Future of 3d Bioprinting:

- Challenges in 3D bioprinting includes inventing bioinks that are able to be distributed, that may stay stable once written, that are biocompatible and nontoxic, which will be degraded over time because the healing method takes over, isn't any tiny task.
- 3DP may emerge as a transformational technology that has the potential to become a necessary part of industrial manufacturing and households. The majority of hospitals and pharmaceutical companies will probably install a 3D printer for quick and tailormade products, including a variety of formulations, implants, and prosthetics. On-demand fabrication of products is certain to decrease the total expenditure of hospitals on healthcare products. Novel techniques will possibly evolve for customized medicine such that the entire formulation can be produced according to the requirement of a particular patient. 3D bio-printing of human anatomical structures can serve as models for physiological and toxicological studies involving animals, thushelping the drug discovery process. Comprehensive experimentation in the manufacture of artificial tissues and organs, including artificial hearts, blood vessels, kidneys, skin grafts, artificial bones, etc., ignites hope for managing organ failure ailments [47].
- The potential applications of 3D bioprinting for patients' replacement of lost or failing organs are enormous.
- Countries using 3d bioprinting**:** In order to spotlight countries and establishments presently concerned with 3D bioprinting analysis, the geographical distribution of affiliations declared within the publications was analyzed.
- The aggregated data that was collected from SciVal underwent a preliminary analysis. The countries where 3D bioprinting research is now being conducted that were deemed to be the most pertinent were the United States (USA), China, South Korea, Germany, the United Kingdom (UK), and Canada.
- In terms of absolute performance (the number of authors and institutions engaged in bioprinting research), the US holds the top spot, indicating a more diffused interest in this subject [46].

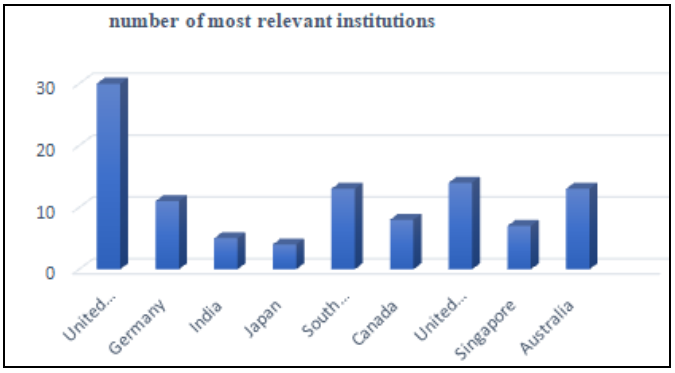

FIG. 14: COUNTRIES AND RELEVANT INSTITUTIONS CONSIDERING CURRENT AFFILIATIONS IN PAPER

TABLE 4: PHARMACEUTICAL APPLICATIONS OF 3D BIOPRINTING

Sr. no.	Drug Delivery System	Significance	Technique	Advantages	Limitations
1	Bilayer polypills	Controlled release	Molding with semisolid extrusion (SSE)	prevents issues with thermal deterioration	the need for organic solvents and the intricate instructions for making the paste The need for heavy machinery.
2	Tablets with high porosity	Layer-by-layer binding of powder deposited on the build platform to the intended results.	Binder jetting or DOP printing	It is inexpensive and simple to scale up.	The requirements for post-processing, in which any remaining solvent must be removed unprocessed powder recovered with high fragility and low resolution
3	powder	selective sintering to selected regions of layers of powders	SLS, a kind of powder bed fusion	control the micro-structures of the manufactured medicinal items to a great extent	low speed and the risk of drug product degradation due to the laser's heat production
4	Micro-needle patches, hydrogels, and oral solid doses	Polymerize photosensitive resins in layers	SLA is a form of vat photopoly-merization.	The best resolution of technologies Facilitate precise structures	the equipment is expensive, the resin toxicity must be removed through post-processing, and
5	fibrous materials, creating drug products	Micro- to nanoscale fiber engineering enables the formation of complex structures with customized geometry.	EHD	individual dosage form specifications or API release	limited effectiveness, the possibility of solvent lingering in dosage forms, and the stringent requirements for solution qualities

Way to 4D Bioprinting:

- When you 3D bioprint cells and hydrogels to try and create a bodily tissue or organ, it remains in that static form and does not have any real added value or complexity like what we see in the human body, and there exists the way to go for 4d bioprinting [58-64].
- The fourth dimension is when you biofabricate a tissue with advanced electric, magnetic, light, or acoustic technologies, with the resulting tissue not being static but dynamic. Stimuli-responsive bioinks which are considered potential bioinks are used in 4d bioprinting. These stimuli referred to the temperature, electric field, light, pH, magnetic field, acoustic, humidity, and multiple stimuli.
- Effect of the printing process on the ability of cell-laden biomaterial to respond to stimuli. Recent innovations in 4Dbioprinting released were a surgical robot that can respond to pH and stimuli with the potential to target and kill cancer and the A.I.-designed Xenobots with an ability to self-replicate [65-69].

TABLE 5: 3D BIOPRINTING VS. 4D BIOPRINTING

Parameter	3d Bioprinting	4d Bioprinting
Definition	3D bioprinting is the process of creating a three-dimensional (3D) structure by laying down successive sheets of living cells that build upon each other	4D bioprinting is “the printing of smart, environmentally responsive biological structures, tissues, and organs
Bioinks	Polymers derived from natural or synthetic resources	Stimuli-responsive bioinks(potential bioinks)
Applications	Tissue engineering Regenerative medicine Clinical transplantatio	Tissue engineering Biosensors Bio actuators biorobotics
Companies	SunP Biotech BICO Xpect-INX Allegro 3D	Ourobionics BeFC NanoRegMed

DISCUSSION: The article gives special emphasis on 3D Bioprinting used in pharmaceutical and biomedical fields. The materials used for bioprinting and the technologies being used are well discussed. The further article covers the scope for more advancement that's 4D Bioprinting.

CONCLUSION: 3D bioprinting is a transformational technology that is an indispensable part of the pharmaceutical and biomedical fields. Bioinks are biomaterial and human cells pledged to print biomedical products, their manufacturers, and modernistic breakthroughs. The hot-off-the-press technologies comprise fused deposition modeling (FDM), direct ink writing (DIW), laser-induced forward transfer (LIFT), and stereolithography.

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