

Recent Advances in 3D Printing Technologies for Scaffold Fabrication and Bioprinting

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Abstract – This article reviews various Additive Manufacturing (AM) techniques, including Stereolithography (SLA), Three-Dimensional Printing (3DP), Fused Deposition Modeling (FDM), and Selective Laser Sintering (SLS), for the fabrication of tissue engineering scaffolds. It discusses the advantages and limitations of each method while highlighting recent advancements in their development. The study emphasizes the capability of 3DP to fabricate complex structures with intricate internal geometries and channels, including those involving temperature-sensitive materials. SLA is noted for its high resolution and ability to process multi-resin models, whereas FDM is recognized for its cost-effectiveness and suitability for polymer-based applications. Furthermore, emerging research on SLS and bioprinting techniques is explored, particularly their potential to fabricate scaffolds with direct cell integration and enhanced flexibility. Overall, the article provides a comprehensive overview of current challenges and future prospects of AM technologies in advancing tissue engineering applications.

Keywords – Bioprinting, Three-Dimensional Printing (3DP), Stereolithography (SLA), Selective Laser Sintering (SLS), Fused Deposition Modeling (FDM).

I. INTRODUCTION

Different approaches have been recommended since the introduction of tissue engineering in fields such as medicine. These approaches range from simple ones such as salt crystals or sugar leaching from solid structures to sophisticated ones such as rapid manufacturing, and rapid prototyping [1]. The techniques of rapid manufacturing are now a highly evolving domain. Modifications are regularly made to old techniques, while new devices and methods are continuously developed. The Rapid Manufacturing (RM) sector is today being shaped by both commercial makers and research institutions of software and hardware. However, the rapid and ever-changing nature of industrial progress poses challenges in organizing current methodologies.

Several manufacturers typically make comparable gadgets using various names for the same manufacturing procedure, many of which are registered trademarks. These names became prevalent simultaneously, leading to significant misunderstanding. It is important to understand that words like 3DP, additive manufacturing (AM), Solid Free-Form Fabrication (SFF), and rapid prototyping are practically interchangeable. For the rest of this document, we have opted to use the phrase 3D. This is a recently developed technique for creating TE scaffolds with precise architectural control. Although there are numerous 3DP techniques available, such as Selective Laser Sintering (SLS), stereolithography, Digital Laser Printing (DLP), bioprinting, inkjet printing, Electron Beam Melting (EBM), Fused Deposition Modeling (FDM), laser beam melting, Precision Extruding Deposition (PED), and polyjet, they all share the fundamental principle of depositing material layer by layer to create the final product.

The 3D Tissue Engineering (TE) scaffold is created by adding continuous 2D layers of a material in succession. AM offers many benefits, including the capacity to fabricate intricate structures and the potential for using CAD (Computer-Aided Design) techniques. It facilitates the use of diverse biomaterials. The utilization of live cells with biodegradable polymers enables the advancement of techniques and innovative approaches to fabricate intricate tissues and even, in the future, whole organs. A three-dimensional (3D) printed tissue engineering (TE) scaffold may be created based on individualized patient data. The CAD approach enables accurate design of the its missing component or 3D organ. The CAD 3D model may include specific characteristics of biological organs, like vasculature or porosity. 3DP is becoming more popular in the fields of RM (regenerative medicine) and TE due to its notable benefits.

3DP procedures may be classified into two states: direct 3D and binder 3DPs. The former method is sometimes referred to as the “drop on powder technique” (see Fig 1). Objects are fabricated by depositing a binder solution onto a powder substrate using inkjet technology. The procedure starts with the even distribution of the powdered layer onto the construction platform. The positioning program utilizes droplet deposition on the powdered layer to create the desired pattern. Subsequently, the construction platform, powder, and component are descended, allowing for the subsequent layer to be applied. Subsequently, the powder is eliminated, allowing for the visual examination of the printed component. The approach has drawbacks such as a comparatively poor resolution and issues about the dependability of the printhead. A diminutive nozzle might exhibit superior quality; however, it is more susceptible to obstruction. One benefit is that it is possible to fabricate complex scaffolds with internal passages since the bordering powder provides support for the items.

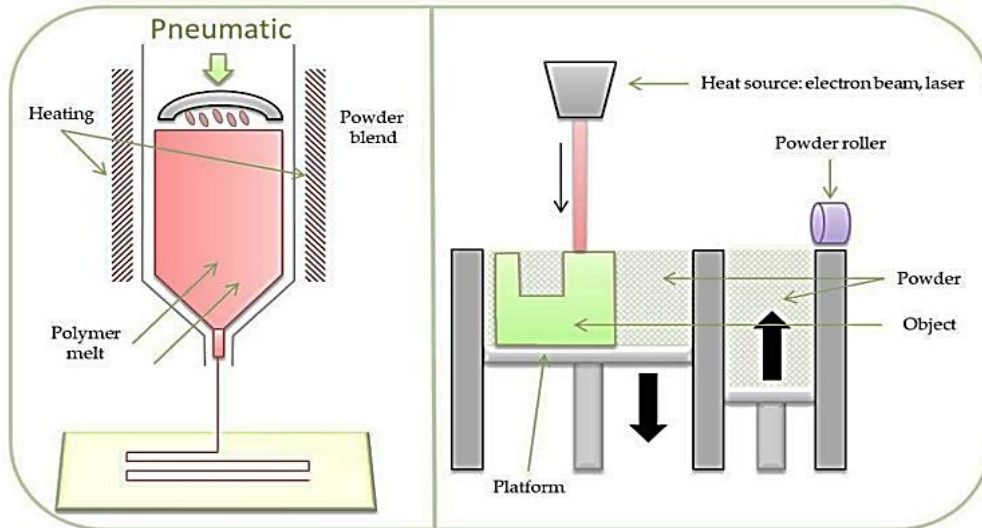


Fig 1. Schematic Representation of the Direct 3DP Approach on the Left and the “Drop on Powder Technique” on the Right.

The main purpose of this paper is to discuss different approaches of AM aimed at designing frameworks and scaffolds in the domain of tissue engineering and regenerative medicine. The approaches may design customized and complex models with high accuracy and precision. They enable the integration of biological agents, temperature-sensitive compounds, and live cells. To effectively optimize the process of manufacturing and develop custom frameworks for particular requirements of tissue engineering, scholar may gain more insights of the merits and demerits of each approach. The remainder of the article has been arranged as follows: Section II focusses on 3DP technology for tissue engineering frameworks or scaffolds. Section III reviews the most present developments in FDM, 3DP, SLS, and stereolithography. Section IV presents a summary of the findings and recommends directions for future studies.

II. 3D PRINTING OF TISSUE ENGINEERING SCAFFOLDS

The 3DP technology, as defined by Mostafaei et al. [2], is a process that creates 3D structures by using an inkjet printer to apply a solution of liquid binder onto a powder bed. Printing has made use of a diverse array of materials due to the fact that the biomaterials majorly exist in either a liquid or solid form. The conventional process for 3DP is shown in Fig 2.

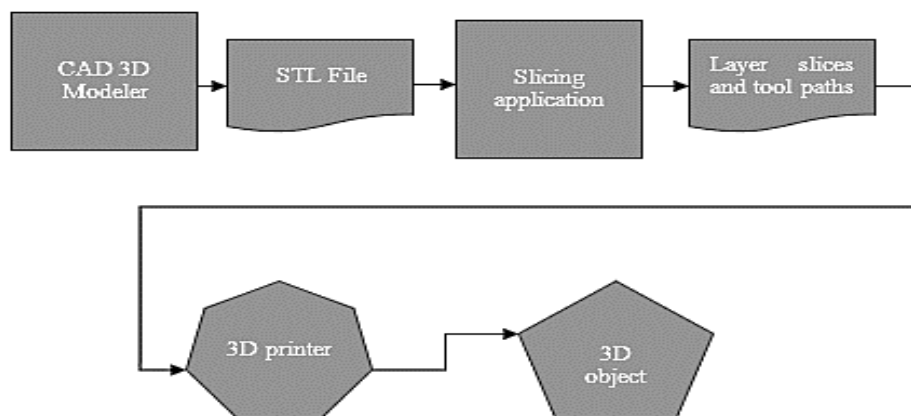


Fig 2. 3DP Process Typical Workflow.

To begin with, a framework is created by use of a 3D modeling software. Subsequently, it is saved to a file using a widely used 3D information interchange standard. The most widely used format in the 3DP business is STL (Stereolithography), which will be further elaborated upon. Subsequently, in most 3DP processes, the stored information is analyzed to break down the framework into individual layers. This results to the design of a 2D contour lines collection, which are then analyzed to offer control instructions for positioning the laser beams or printing head. The approach allows for the creation of intricate scaffolds, including internal channels and dangling elements, by using limitless powders to provide support for the items.

Cox et al. [3] used 3DP to build scaffolds with a high level of porosity. They also employed particle leaching methods to enhance the scaffold's structure. Furthermore, they successfully proved that cells were able to grow into these scaffolds. Moreover, using room temperature manufacturing factors enables the integration of substances that are temperature-sensitive, like biological and pharmacological agents, into scaffolds. The viability of utilizing biological substances and live cells in manufacturing processes was discussed in [4], to produce clean water to generate frameworks using starch. The capability of this method, which entails “multi-color” printing, where every ink can be effectively integrated in a particular location, is an added advantage in the field of tissue engineering. This ability provides significant potentials to disperse wide-range extra-cellular matrix materials, arrange various types of cells in a concurrent manner, and control live cells tissue cell production.

The technology of 3DP provides more options for printing materials than other SFF approaches. The technology has effectively designed various biological substances like proteins, peptides, live cells, and DNA plasmids. The integrations of these substances require the transformation of industrial 3DP tools. Cells need an appropriate habitat that provides oxygenation, maintains an ideal temperature, and supplies food.

III. RECENT MATERIAL AND TECHNOLOGY ADVANCES

Fused Deposition Modeling

Fused deposition modeling (FDM), also known as material extrusion additive manufacturing (AM), utilizes polymers in the form of filaments as the main material [5]. The filament is intensely heated until the point it attains a molten state before being expelled via the 3D printer nozzle [6]. The nozzle head has the ability to move in three DoF (degrees of freedom) in order to place the extruded polymer onto the build plate according to the directions given in the G-code [7]. The process of FDM is shown in a diagrammatic diagram in Fig 3, illustrating its concept. As shown, the filament is consistently supplied to the nozzle and extruder of the device by means of the two rollers rolling in opposing ways. The substance is sequentially placed on the layer by layer, build plate, until the anticipated form and dimensions of the result are attained.

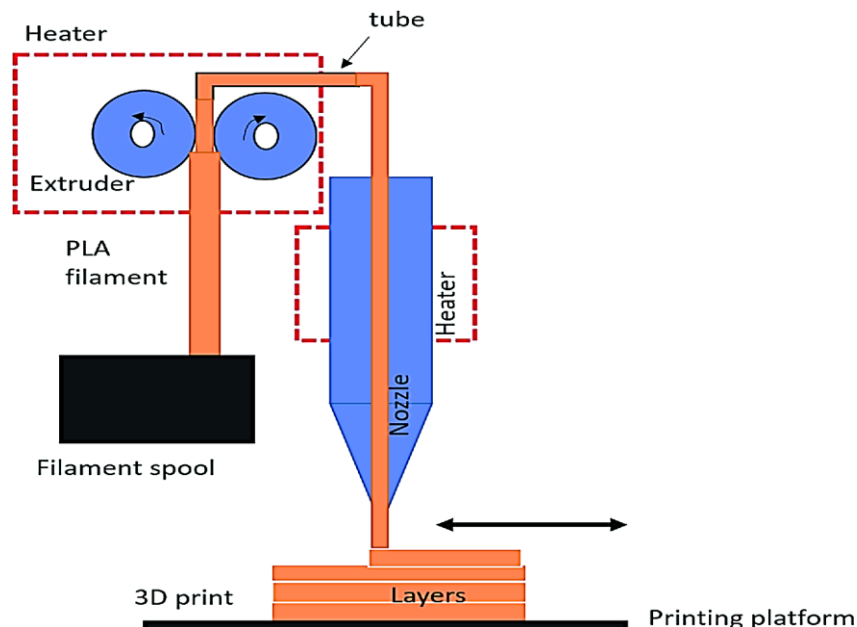


Fig 3. FDM Principle.

Throughout the process of layering, the printer nozzle moves in a back-and-forth motion according to the original CAD model spatial integrates in the files of G-code. This continues until the element reaches the needed form and size. In some systems of FDM, numerous explosion nozzles may be used to apply the polymer ingredients, particularly when compositional gradients are needed for the components. The efficacy and resolution of the extrusion process mostly rely on the characteristics of the thermoplastic filament. Consequently, several 3D printers are specifically engineered to accommodate different filament substances. Typically, inexpensive FDM 3D printers are restricted to handling a single kind

of thermoplastic, with polylactic acid (PLA) being the prevailing choice [8]. The elements are typically applied in layers onto the plate of construction (platform), and may be detached by either snapping them off or immersing them in a detergent, basing on the specific thermoplastic used. Subsequently, the printed components might undergo surface cleaning, sanding, painting, or milling to improve both their visual appeal and functioning.

Fused deposition modeling (FDM) involves the precise displacement of melted substances of thermoplastic using two heated extrusion heads with a tiny opening, following a predetermined scheme. The extrusion process starts with the expulsion of molten polymer via the nozzle system (extrusion die) and its subsequent deposition onto a structure. FDM commences with a software procedure that swiftly handles STL files, systematically slicing and aligning the model for the building process. Support structures may be produced automatically if necessary. The machine distributes two substances—one for the prototype and one for a temporary supporting framework. FDM operates on the principle of additive manufacturing, whereby materials are progressively accumulated in layers. A metal wire or plastic filament is extracted from a fed or coil into an extrusion nozzle, which has the ability to control the material flow. The nozzle is heated to induce material melting and may be maneuvered in both horizontal and vertical directions by a numerically controlled system. The materials are arranged in successive layers with a thickness as small as 0.330 mm. The component is constructed from the bottom to the top, adding one layer at a time (see Fig 4).

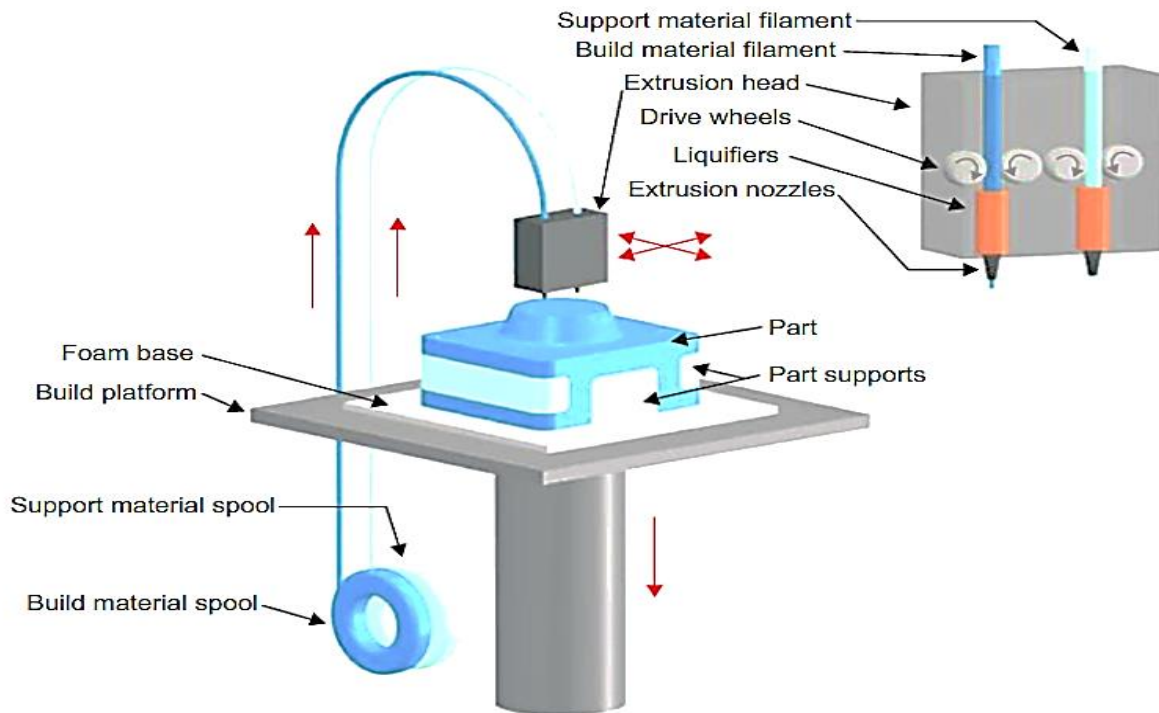


Fig 4. Fused Deposition Modeling (FDM) Technique.

Fused deposition modeling (FDM) utilizes frequently used polymers that are biocompatible characterized by low melting points. The utilized materials in FDM for scaffold fabrication include composites of bioactive glass and PCL, ϵ -caprolactone/L-lactide, PLA, Collagen infiltration PLGA, PMMA, Gentamicin PCL-TCP, PLGA-TCP coated with HA, PCL, PCL-TCP, gelatin-coated PCL, PLGA-PCL, and PCL-PLGA-TCP. Experiments have been conducted using bone marrow-derived mesenchymal, and swine chondrocytes stem cells. Animal models, including mice for wound healing, humans with craniofacial defects, and rabbits with bone defects, were used in in vivo research. Applications include the fields of antibiotic delivery systems, cartilage tissue engineering, treatment of osseous craniofacial abnormalities in bone tissue engineering, and humans.

Stereolithography

Stereolithography (SLA) is largely acknowledged as the pioneering method for fast prototyping, which first emerged in the 1980s [9]. The first SLA employed a HeCd-laser beam to regulator the polymerization process of the photocurable resins in a multifaceted manner. Following the curing of each layer, the platform, together with the solidified structure, descends in a bottom-up manner, while a fresh layer of liquid resin is applied on top. The uppermost layer is now prepared for patterning. In the top-down technique, light is directed onto a plate that is transparent that is first placed near to the bottom of the container with liquid resin. Once a layer has been printed on the transparent plate, the solidified substance is separated from it. The elevated cured structure facilitates the infusion of uncured liquid resin into the gap between the clear plate and the structure. The subsequent layer is now prepared to undergo the process of patterning. Due of the potential slowness of rastering a laser beam, particularly for larger components, the masked lamp approach was devised to simultaneously cure a

complete photopolymers layer. Once the model is constructed, the unreacted liquid resin is eliminated by drainage. UV oven's post-curing transforms any remaining unreactive functional classes and enhances the structural integrity of the component.

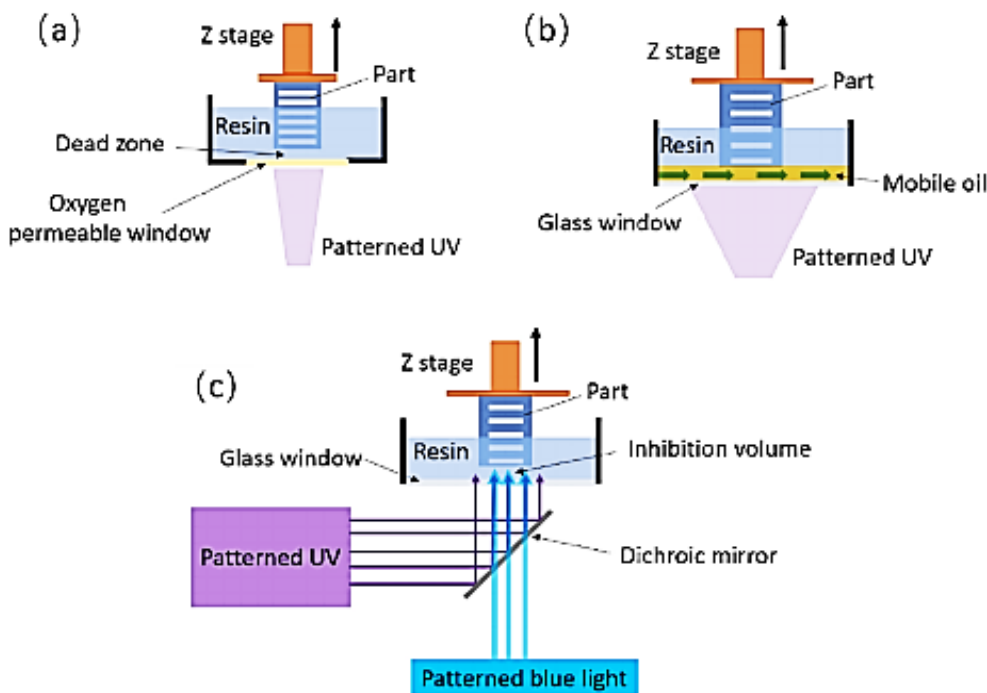


Fig 5. Depicts The Ongoing Steps of Stereolithography. (A) Diagram Illustrating the CLIP (Continuous Liquid Interface Production) Method. (B) Schematic Representation of the HARP (High-Area Rapid Printing). The SLA Is Achieved by the Use of Dual-Network Irradiation.

Recent advancements in SLA technology include the expansion of the use of multi-resin systems and the library of photo cross linkable polymers for a single build. Several scholars have documented their own continuous stereolithographic methodologies. In his study, Alamdari et al. [10] (**Fig 5b**) described a temperature controlled continuous stereolithographic method that employs a movable liquid interface to minimize the adhesive forces between the curing and the window objects. The use of an interface of mobile liquid, namely a flowing immiscible fluorinated oil, serves to reduce adhesion at the build zone and facilitate heat dissipation. As a result, the HARP technique may achieve quick print speeds without being constrained by thermal restrictions. Li et al. [11] (**Fig 5c**) presented a dual-wavelength continuous stereolithography system. In this system, one specific wavelength of light is used to prevent the resin from solidifying, while another specific wavelength of light is utilized to start the solidification process of the resin. By manipulating the intensity of two light sources, it is possible to generate an inhibition area between the window and the curing sections. This region prevents adhesion and allows for uninterrupted printing.

There has been a significant rise in the production of polymers including aliphatic polyesters, which allows for biodegradation [12]. Subsequently, the macromer is acrylated to provide the capacity for photo cross linking. Arcaute, Mann, and Wicker [13] demonstrated the use of several resins in a single construction by combining PEG-DA and PEG-DMA with bioactive PEG, labeled bioactive PEG, fluorescently labeled dextran, in the scaffold distinct sections. When substituting the material, the scaffold will be extracted from resin reservoir, cleansed with pure water, and fresh resin will be introduced into the container. A fixture was used to preserve the scaffold registration of X-Y, so guaranteeing the layers arrangement. The dynamic mask projection SLA technique has attained a vertical resolution of approximately 1 μm and a lateral resolution of about 2 μm for PPF resin. The microstructures achievable with this method exhibit exceptional intricacy, while there are still obstacles to overcome in terms of fabricating horizontal channels and mitigating structural shrinking.

Selective Laser Sintering/Melting

Selective Laser Sintering (SLS) is a manufacturing technique that has similarities with 3DP. Both models involve the consolidation of powder particles into thin layers. However, in SLS, a CO2 laser beam is used for this purpose. Powder bed fusion (PBF) is a process in additive manufacturing where specific areas of a PBF are selectively fused together using heat energy. The most often used PBF printing technologies are SLS, selective heat sintering (SHS), SLM, DMLS, and FBM.

Currently, the primary emphasis of SLS research is on optimizing processes, developing materials, and extending applications. The PBF technique employs an electron laser or beam to liquefy materials powdered. The PBF method involves the application of powder material onto the previous layer, using various devices such as rollers or blades. A hopper, located

beneath or adjacent to the bed, offers a continuous source of material (see **Fig 6**). EBM necessitates a vacuum environment and is applicable for the components' production using alloys and metals. DMLS is a process that is similar to SLS, except it use metal powder to create objects by fusing the powder layer by layer. SHS distinguishes itself from other methods by using a heated thermal print head to simultaneously fuse powder materials and use rollers to introduce layers between each fusion. Additionally, the platform is lowered to accommodate the model.

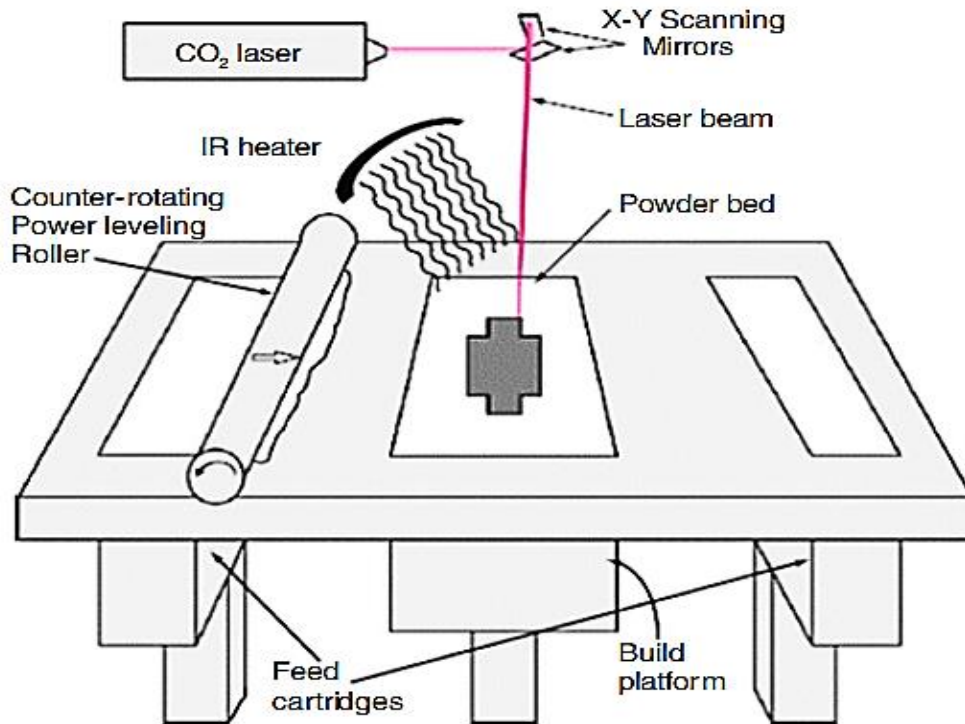


Fig 6. Schematic of the SLS Process.

Some benefits of this technology include: (i) relatively low cost; (ii) suitability for creating visual models and prototypes; (iii) integration into small-scale office-sized machines; (iv) support system specified by the powder; and (v) a broad variety of material alternatives. However, this technique also has some drawbacks, including: (i) a relatively sluggish velocity; (ii) materials that lack systematic qualities; (iii) limits in size; (iv) high power use, making it costly; and (v) the final result being determined by the size of the powder grains.

The laser employs a precise 2D pattern to heat the powdered polymer particles over the glass transition temperature, so causing them to sinter. During the process of sintering, the movement of molecules on the outer surface of the particle causes the creation of connections (known as necks) between adjacent particles. After the formation of a single layer, the piston, which secures the component, is dropped and a fresh coating of powdered substance is added to the top surface. A succeeding layer is created and tightly connected to the prior layer. The unconfined, free-flowing particle is eliminated after the component is finished and undergoes heat treatment to get maximum density. Temporary support systems are unnecessary in this case, as opposed to SLA, since untie solid crystals provide support for cantilever materials. Due to the incomplete melting of powder particles during sintering, the original porosity between the particles may be maintained, allowing for the processing of various pure and mixed materials.

While solid state sintering may be accomplished for most structures at temperatures ranging from 0.5 to 1 time the melting temperature, EBM and SLM use high-energy sources to heat the fine particles above melting point. This process ensures the entire fusion of particles, resulting in a consolidated and fully-dense structure. Within In practical terms, the process of melting is more readily achievable when all the powder has a uniform MP (melting point). Consequently, melting is easier to achieve with pure metals compared to alloys, mostly because of the differences in the behavior of surface tension, liquid metal flow, and interactions between the laser and the material. Resultantly, the wide-range of materials that may be used for Selective Laser Melting (SLM) is more limited in comparison to Selective Laser Sintering (SLS). The resolution of the features is dependent on the size of the powder particles, the measurement of the diameter of the laser beam used for focusing, and the efficiency of heat transmission inside the powder bed. The particle size must not exceed 10 μm due to insufficient quick sintering and propagating, which might lead to inaccuracies at the edges. The utilized substances are PCL and a composite of hydroxyapatite and polyethylene glycol ether ketones. Biomaterials are frequently employed in the fabrication of thin, solid disks, generally with diameters ranging from around 400 to 500 μm .

The latest developments in SLS technology have enabled the production of scaffolds with reduced stiffness and improved features resolution. Scaffolds of PCL have been manufactured with a lower hardness of 300-400 kPa, which is lower than

the previously reported range of 14.9 to 113.4 MPa. The reduced stiffness enables the use of soft TE in areas such as heart tissue. Efforts have been made to optimize the CAM/CAD process for creating FGS with varying hardness inside a component. This has been achieved by using polyhedrals collection to regulate the permeability. The permeability of the scaffold is determined by its hardness and is exemplified using PCL in SLS. Furthermore, Finite Element Analysis (FEA) has been used to assist in the development of microarchitecture and forecast the mechanical characteristics for SLS.

3D Plotting/Direct-Write Bioprinting

Bioprinting is the process of creating structures of hydrogel by directly including cells in the creation process (see Fig 7). Several methods of cell printing are used to introduce cells during processing: extrusion of alginate-cell solution from a syringe; bovine vascular endothelial cells inkjet printing in culture medium by means of electrostatic forces; embryonic chick spinal cord cells direct writing guided by means of a laser; and cells transfer suspended in alginate by means of laser-induced forward transfer cells. This method enables precise and regulated placement of cells or growth factors, together with the supporting scaffold structures.

Nevertheless, this manufacturing method is often restricted to hydrogel substances like fibrin and alginate, which may not be optimal for inculcation in biological settings that need robust automatic characteristics. An instance of practical use is the use of collagen droplets (650 μm in diameter) containing rat smooth muscle cells to generate precise spatial arrangements of cells in a three-dimensional environment. The SFF technique is particularly effective for materials with low viscosity, since the buoyancy resulting from the extruded materials' density matching with the liquid media avoids any deformation of the shape [14]. The thickness of the strand may be adjusted by factors such as the viscosity of the material, the speed at which it is deposited, the diameter of the extrusion tip, and the amount of pressure applied.

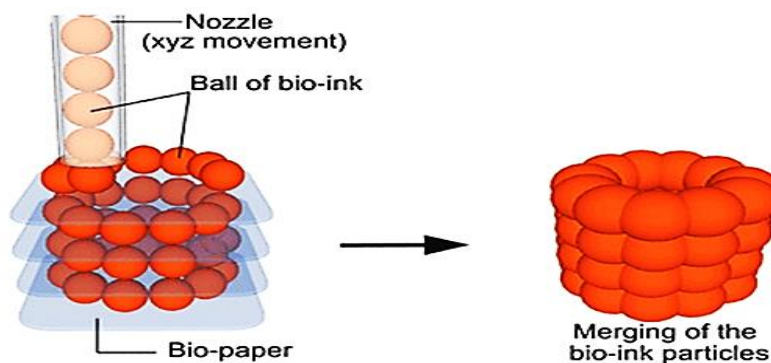


Fig 7. The Process of Bioprinting.

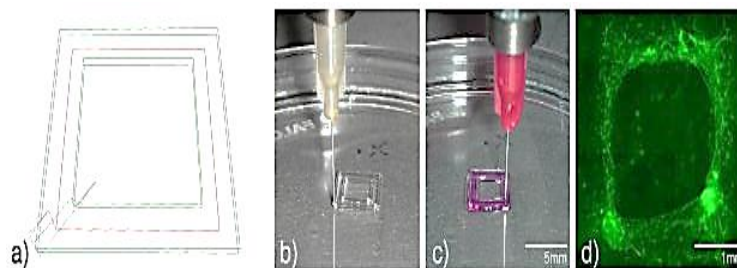


Fig 8. A Simple Pattern Created Using Direct Writing, Using Just Two Materials. A) Line Trajectory Depicting the Flow of Low Viscosity Material (Red) And High Viscosity Material (Green) During Printing. (B) Photographs Depicting Bio Printed Structures with a High Viscosity Of 40wt%. (C) Pluronic F127 Is Used, And Then Low Viscosity Collagen Is Combined with Micro Vesicle Fragments and Microvascular Cells. (D) Following A 7-Day Incubation Period, Fragments and Cells (Green) Develop Networks of Neovascular Inside the Structured Collagen.

Bioprinting involves the printing of tiny bioink spheres made up of hydrogel and cells substances, such as decellularized or alginate extracellular matrix, in a specific form [15]. Every direct-write bioprinting technology, whether it is custom-made or commercially available, is specifically engineered to release a substance onto a surface. Usually, substances are released from either a syringe or a reservoir. Some systems have the capability to accurately dispense several materials in a sequential manner without requiring any modifications. These methods include numerous printing units inside the bioprinter's software and hardware. Every unit of printing operates autonomously and may be filled with either comparable or different substances.

By using this approach, it is possible to use distinctive assortments of components and compositions. In previous research, we encountered challenges in preserving pattern resolution while working with low viscosity materials. The width of the lines was significantly influenced by the kind of substrate material used and the ambient humidity level of 40. Consequently, the breadth of the lines varied greatly and neighboring lines often came into touch with one other. The

challenges escalated while trying to print numerous layers. Consequently, our current pattern designs have prioritized a two-fill method and material mold. This involves printing a mold made of a high thickness structural substance, followed by filling the empty areas with a low viscosity biological substance. **Fig 8** illustrates a simple mold and fill design.

The primary benefits of bioprinting are the ability to treat materials at 20° C (if applicable), the cells direct integration, and the uniform dispersion of cells. The primary drawbacks are restricted automatic rigidity, precise timing requirements for gelation, the need for precise liquid medium densities and matching of material to maintain desired forms, and resolutions' poor level. Advancing the materials design suitable for biofactor printing, along with the development of advanced printheads capable of depositing multiple biofactors and materials separately on a single platform, has the potential to create constructs that meet the intricate biological needs of tissue engineering scaffolds.

Jang, Koo, and Kim [16] describe the capability to use bioprinting technology to create individual cells and structures made of hydrogel-PCL scaffolds that contain cells. The technique known as "Block-Cell-Printing" has shown the ability to print single-cell arrays at a high rate. Book-shaped traps in microfluidic arrays are used for the purpose of capturing individual cells. Cells that are confined may be connected and then separated by a 5 µm gap to study cellular communication. The study included cultivating primary rat neurons in the cortex that were immobilized, and the cells exhibited neuronal morphology. High cell viability was shown for human osteoblast-like cells and human mesenchymal stem cells after the procedure of processing. Finally, a sequential method has been used to deposit hydrogel droplets containing chondrocytes (either decellularized or alginate extracellular matrix bioink) and PCL in alternating layers, resulting in the formation of a three-dimensional structure.

IV. CONCLUSION AND FUTURE SCOPE

This paper has reviewed the approaches of Additive Manufacturing (AM), and their applications in the manufacturing field and tissue engineering. These approaches are beneficial to selection of materials, which establish complex scaffolds with upmost accuracy. 3DP has been employed to establish scaffolds with higher porosity, highlighting its capabilities to promote the development of cells. Moreover, it allows for the inclusion of thermosensitive substances into frameworks, thereby promoting the merging of biological agents and live cells. FDM can only process a single kind of thermoplastic material and uses thermoplastic filaments. Nonetheless, post-processing methods may be used to enhance printed items' practical and aesthetic qualities.

Both the development of photo cross linkable polymers and the use of multi-resin systems in the SLA field have made significant strides. In order to overcome the limitations of heat, new stereolithography techniques have been created that enable faster printing. The SLS process, along with its variants like SLM and EBM, provides a broad choice of materials and the ability to build thick, fully solid structures. Technological advancements have led to the development of scaffolds that exhibit reduced stiffness and improved accuracy in representing features. Bioprinting enables the direct integration of cells into the printing process, enabling precise placement of cells and growth factors. However, its use is often limited to hydrogel materials and may not have the most advantageous mechanical characteristics for usage in biological settings.

The future of 3DP technology has immense promise for advancements and practical uses, notably in the fields of SLS, 3DP, SLA, FDM, and Bioprinting. Within the realm of 3DP, more study might focus on enhancing the structure and porosity of scaffolds, as well as exploring the integration of thermosensitive substances like pharmacological and biological agents. The integration of biological materials into industrial 3DP technology shows great potential for progress. Possible advancements in FDM include improving the precision and effectiveness of the extrusion process, along with expanding the range of printable materials beyond polylactic acid (PLA). In order to improve the appearance and functionality of printed parts, more improvements may be made in cleaning the surface, scrubbing, the painting process, and milling methods.

In relation to SLA, future research might focus on expanding the range of photo cross linkable polymers and developing multi-resin systems for a unified structure. Advancements in stereolithographic methods, such as temperature-controlled processes and dual-wavelength systems, may increase printing speed and guarantee continuous printing. Advancements in material choices, velocity, and size constraints have the potential to increase the advantages of SLS. Ongoing research is dedicated on improving the structural characteristics of materials and reducing power consumption. Bioprinting has great potential for use in tissue engineering. Subsequent examination should give priority to the advancement of materials with long-lasting mechanical characteristics suitable for implantation in biological settings. The advancements in bioprinting technology, such as the ability to manipulate materials at room temperature, the requirement for accurate timing in gelation, and the development of advanced printheads for depositing different biofactors and materials, have the potential to greatly enhance the capabilities of bioprinting.

CRedit Author Statement

The author reviewed the results and approved the final version of the manuscript.

Data Availability

No data was used to support this study.

Conflicts of Interests

The authors declare no conflict of interest.

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