International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Innovative 3D Bioprinting Techniques: An In - Depth Review of Fabrication Methods and Emerging Tissue Engineering Applications

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Abstract: ***Three - dimensional (3D) bioprinting has evolved as a revolutionary technology in tissue engineering and regenerative medicine. Bioinks, composed of living cells and biomaterials, enable the construction of layer - by - layer complex tissue structures that closely mimic human biological environments. The ability to create such biomimetic constructs holds immense potential for developing transplantable tissues like skin, cartilage, and bone. While current bioprinting techniques are restricted due to limitations with cell deposition and vascularization, numerous innovative approaches are emerging, each offering unique capabilities to address the critical shortage of transplantable organs. This review explores the various printing techniques such as extrusion based, inkjet, and laser assisted methods highlighting their advantages, limitations, and the innovative approaches being developed to address current challenges. Additionally, it delves into the bioink compositions that optimize cell viability and functionality, paving the way for future advancements in organ transplantation and personalized medicine.***

Keywords: 3D bioprinting, tissue engineering, bioinks, regenerative medicine, biomaterials

1. Introduction

Bioprinting is an emerging sector of engineering that contains regenerative applications in making functional tissues. Specifically, bioprinting is the use of a computer - aided transfer process for patterning and assembly of living and non - living materials with a prescribed 2D or 3D organization to produce bio - engineered structures according to Moroni L. et. al., 2018 [1]. Bioprinting involves the deposition of layers of living cells, crosslinkers, growth elements, and other biomaterials to form a living vascular structure [2].3 - dimensional (3D) printing, otherwise known as additive manufacturing (AM) [2] is a typical form of bioprinting used for creating a wide range of in vivo organoids that behave like cells [3]. In addition, 3D bioprinting falls under the category of fabrication methods where the design of the fabricated bio - construct contains living cells within programmed geometries [4]. The living cells and biomaterials used in the construct are termed bioinks. These bio - ink cells serve as the mandatory foundation for the printing formulation of single cells or cell aggregates (several cell types) and are typically formed within hydrogels. Additionally, these inks are not restricted to specific biomaterials and the cell - seeding process occurs post - fabrication [5]. As bio - inks are ideal for 3D bioprinting, there has been a great development in formulating the optimal bio - ink that is printable, biodegradable, and affordable. These regulatory guidelines have also led to the development of high - quality hybrid bio - inks [6].

The human body's limited regenerative capabilities significantly challenge organ/tissue damage, post - injury complications, chronic disease, and aging. To address these setbacks, procedures like organ transplantations are performed but are immediately faced with issues such as donor shortages, organ rejection, and complications during surgery. These concerns, cumulatively, are high - magnitude issues for the human body, which calls for novel resolutions [7]. Biofabrication of 3D structures that mimic human tissue can serve as a safer alternative. Utilizing living cells and other biomolecules allows these engineered structures to maintain cellular interactions and a high level of reciprocity in given biological environments.3D bioprinting addresses the major issues of organ shortages and high rejection rates by providing a significantly advanced form of regenerative medicine [7]. Bioprinting also displays impeccable potential in clinical organ modeling. Within bioprinting, there is in - situ bioprinting and in - vitro bio - printing. In - vitro bioprinting is a conventional approach that depends on a large bioprinter to print 3D constructs before being introduced into a human body. In - situ bioprinting, on the other hand, is when the construct is fabricated within the living body [8] [1]. In addition to the diverse options, 3D bioprinting is acclaimed for its ability to blend multiple cells within printed scaffolds and other complex 3D structures. This high - quality technique has also been accredited for fabricating more biomimetic scaffolds that require cell proliferation and differentiation [6]. As mentioned above, bioprinting has strong applications through in vitro modeling which has fostered opportunities to create personalized implants and innovative medicine. The rapid progression and attention that 3D bioprinting has attained demonstrates that it could serve as a novel technology in the biomedical domain [9] [10]. In this review, we discuss the various bioprinting technologies and offer an in - depth overview of their integrated functions. Additionally, we discuss the different types of bio - inks and biomaterial combinations that work simultaneously with the printing technologies to produce customized tissue scaffolds. This review is significant as it sheds light on the rapidly evolving field of bioprinting, providing insights into the future of organ transplantation, regenerative medicine, and the development of personalized therapies, which are crucial in addressing the global shortage of transplantable tissues.

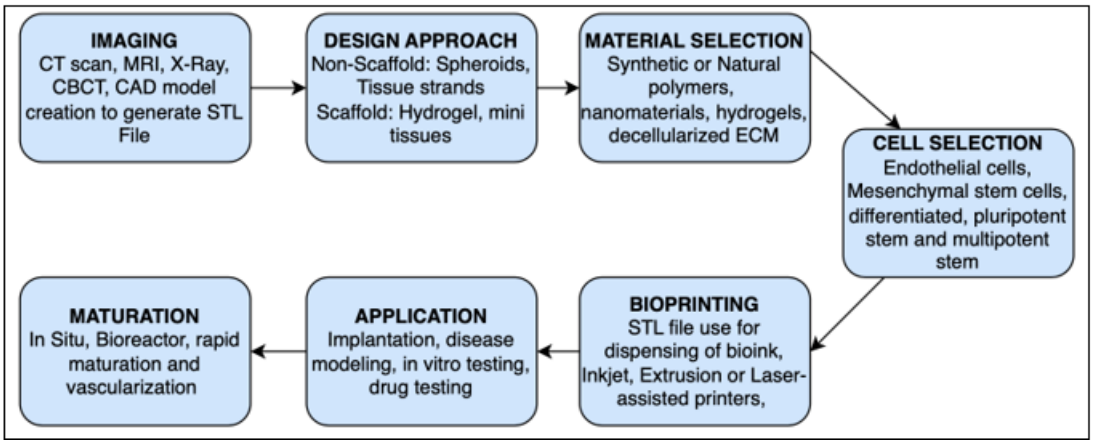

Figure 1: Flowchart going over the process of bioprinting

2. Bioink Materials

2.1 Alginate

Alginate is a biodegradable and biocompatible polysaccharide that is typically obtained from seaweed [11]. Due to their low toxicity, alginates can be fabricated into hydrogels, microspheres, and sponges [12]. Alginates are used widely in wound healing and tissue engineering [13] [14]. The alginate - based hydrogels that are prepared via cross - linking methods pose similarities to extracellular matrices of living tissues. This facilitates the delivery of bioactive agents and cell transplantation. Specifically, in cell transplantation, hydrogels serve as a carrier for cells to reach their desired site, provide space for tissue formation, and influence the function of the engineered tissues [15]. Alginate allows ease in printing and extruding which in turn provides protection for encapsulated cells [16]. Recently hybrid hydrogels have been used to address common mechanical issues associated with 3D bioprinting such as hierarchical structure, topological morphology, and mechanical strength [14]. Hybrid hydrogels of sodium alginates combined with carboxymethyl cellulose (CMC) have shown enhanced improvements. After a set of quantitative characterization tests, it was found that there was a composition of 4% Alg - 4% CMC that maintained good printability and shape fidelity [17]. Most bioprinting methods currently use alginate or hybrid forms of alginate as bio - inks. For instance, Yu Y. et al., 2013 created a process for printing vascular - like channels via encapsulation of cartilage progenitor cells in alginate and eventually obtained a hollow tubular construct that presents great biological and mechanical qualities. In addition, this group created a nozzle system where alginate solution was extruded through a case with a calcium dichloride ($CaCl^2$) to create a tubular construct [18] [19].

2.2 Chitosan

Chitosan (CHT) is a biopolymer derived from chitin which is found in invertebrate exoskeletons. Chitosan is insoluble in neutral solutions but can be obtained in acidic aqueous solutions. A slightly basic aqueous solution of chitosan at a pH greater than 6.2 allows for the precipitation of hydrogels [20]. Hydrogels based on chitosan have found application in wound dressings, drug delivery systems, cartilage tissue engineering, and cell encapsulation [21]. Chitosan - based bio - inks are notable due to their low cost and high biocompatibility. According to Luca, M. D. et al., 2022, There have been reports of CHT ink being used to deliver therapeutics within printed scaffolds in breast cancer treatment. Although chitosan is not suitable directly for 3D printing due to poor mechanical strength, it still is very well adapted for drug delivery applications. Poly ε - caprolactone (PCL) is suitable for 3D bioprinting due to its in - vivo biodegradability. Chitosan in addition to PCL can overcome some of the limitations of its individual characterization. For example, a 3D - printed scaffold could contain one layer of PCL and another layer of chitosan to act as a suitable implant for a human body [22]. Another reason chitosan has gained immense attention is due to its Food and Drug Administration (FDA) approval making it utilizable and safe for biomedical applications [23]. Chitosan bio - ink has been utilized in bioprinting cartilage tissue, bone tissue, liver/heart valves, and neural connections [24] [25].

2.3 Hyaluronic Acid

Hyaluronic Acid (HA) is a promising bio - ink candidate due to its histocompatibility, biodegradability, anti - inflammatory properties, and non - immunogenicity. HA is an acidic polysaccharide that is found commonly in the extracellular matrix (ECM) of human connective tissue. Its functions such as stem cell differentiation and wound healing have made it favorable to use in 3D bioprinting [26]. In the chemical structure of HA, both hydroxyl and carboxyl functional groups have been found to enhance its biological properties [27]. The chemical composition can be modified through the use of cross - linking mechanisms to further improve bioactivity. HA can be chemically modified with the addition of functional groups which results in HA derivatives with high molecular weights to retain physicochemical properties [26]. In addition, there is a built - up resistance to HA degradation [28]. Another chemical modification can be done through HA crosslinking. Crosslinked HA hydrogels have macromolecules that are highly aggregated and folded which makes them less susceptible to degradation [29]. In terms of bioprinting technologies that use HA - based bio - inks, there are droplet - based, extrusion - based, and laser - assisted. Unmodified HA bio - inks are not compatible with droplet - based bioprinting due to the low viscous modulus of certain aqueous HA solutions. However, HA can be mixed with synthetic/natural polymers to produce suitable bioinks for droplet - base bioprinting [30] [26]. Wang L. et al., 2022 created an extrudable hydrogel bioink with photocrosslinked

gelatin methacryloyl (GelMA) and dynamic hydrogel - crosslinked HA for 3D bioprinting. HA can be produced through microbial fermentation, artificial enzymatic synthesis methods, and tissue extraction [26]. With HA being highly accessible and the scope for its applications growing, HA - based bioinks are an emerging sector in the biomedicine field. One major advantage that HA provides is that its chemical modification results in gel - based derivatives maintaining shape over prolonged periods of time, degrading at slower rates than regular HA [26].

2.4 Cellulose

Cellulose is a very abundant biopolymer found naturally in algae, fungi, and bacteria. It is known for its high purity, mechanical strength, and water retention capacity which makes cellulose an applicable material [31]. In addition, cellulose is very sustainable and eco - friendly, making it a viable option for 3D bioprinting. Bacterial cellulose is a chemically modified material that has a high potential for mesoscale alignment of colloidal scale nanofibrils [32]. In addition, the hydroxyl groups present in the cellulose matrix maintain the surface hydrophobicity and thermodynamic stability [33]. Pitton M. et al., 2021 developed multi - component bio - inks based on 2, 2, 6, 6 - tetramethylpiperidine - 1 - oxyl radical (TEMPO) - oxidized cellulose nanofibers to improve printability and ensure stable biocompatibility for the printed scaffold in future uses. At the end of their process, they finalized a bio - ink and performed in - vitro tests for cytocompatibility to show the potential use of the developed materials in tissue engineering applications [34].

Agarose

Agarose is a polysaccharide derived from agar - agar which is extracted from red seaweed (class *Rhodophyceae*). When discussing agarose hydrogel formation, two major influencing factors are temperature and concentration. Agarose chains at high temperatures result in a coil structure. When the temperature is lowered, the chains form into single or double helical structures which form into a gel later. Since agarose is prone to form hydrogels without toxic crosslinking agents, its biocompatibility is further enhanced [20]. Between temperatures of 26 and 30 (degrees Celsius), agarose is rapidly gelled which allows it to be printed. Agarose, however, is limited in cell growth as it degrades over time. Therefore, agarose is used to create cell aggregates and is sometimes mixed with collagen to increase the effectiveness of cellular functions [35]. In addition, agarose fibers are printed from predefined patterns and serve as a base that is covered with other functional biomaterials. A great advantage of agarose is the temperature control which allows agarose fibers to be manipulated easily through melting and extraction [35].

Table 1: Bio - ink Materials for 3D Bioprinting: Correlating Properties with Target Tissues, Advantages, and Challenges in Tissue Engineering Applications.

Reference [temp]	Bio - ink Material	Key Properties	Target Tissues/Organs	Advantages	Challenges
[36]	Chitosan	Antimicrobial properties, Molecular weight, derivatives, pH, biodegradable	Cartilage, bone tissue, and skin tissues	Good biocompatibility biodegradability, nontoxicity, immunogenicity, antimicrobial activity	Poor mechanical strength, presence of endotoxins,
[37]	dECM	Cytocompatibility, shape memory polymers	Cartilage, Muscle	Excellent mechanical properties, great biomimetic elastomeric mechanical properties, Promising printability	Weak immunogenicity
[38]	HA	Shrinkage properties, viscosity, printability, cell proliferation, high water content	Cartilage, wound healing	Improved cellular functionality, good biocompatibility	Structural Stability, poor mechanical strength, rapid degradation
[39]	Alginate	Gelation properties, pH,	Human mesenchymal stem cells	Low toxicity, low cost, high genetic selection capacity	High degradation rates, enzyme epimerase hinder procedures
[40]	Collagen	Hydrophilicity, cell adhesion	Skin	Good biocompatibility, increased support for sustained drug release	Unstable mechanical properties, slow gelation rate
[41]	Fibrin	Biodegradable	Wound repair, tissue repair	Enhanced cell migration	Rapid degradation, poor printability
[42]	Matrigel	Basement membrane extract	Tumor tissue	Good biocompatibility, good cell growth, and differentiation	High cost
[43]	Tannic Acid	Antioxidant	Various tissue applications	Good biocompatibility, great antioxidant properties	Poor printability
[44]	Hydroxyapatite	Osteoconductive	Bone tissue	Good biocompatibility	Brittle
[45]	Polyvinyl Alcohol (PVA)	Water - soluble, biodegradable	Various tissue applications	High water content, good biocompatibility	Lacks crosslinking, poor cell adhesion
[46]	HAMA	Young Modulus, Hydrogel stiffness, Matrix composition	Cartilage, tissue engineering application	Good biocompatibility, custom matrix	Fibrocartilage formation, Phase separation, cell behavior variability

[47]	GelMA	Electrical conduction, low viscosity, viscoelastic properties, Osteogenic differentiation,	3D functional cardiac tissue, cell - instructive nanocomposite, hepatocyte cultivation, neurons, soft tissue, bone tissue	Good biocompatibility	Hydrophobicity of carbon - based nanomaterials, High cost, potential cytotoxicity
[48]	Thermoplastic material	PCL (Polycaprolactone), Porosity, crosslinking	Bone tissue, Cartilage	Reduced shear stress	Long - term stability, Volume limitation for bio - ink
[49]	Resin	Photopolymerization compatible	Cardiac tissue, Liver tissue	Low biotoxicity	Process optimization
[50]	PDMS	Decellularized Liver Matric (DLM),	Liver tissue	Liver - specific ECM, high - throughput capability	Spheroid size effects, limited cell types, hydrophobicity

3. Bioprinting Techniques

3.1 Droplet – based Bioprinting

Droplet - based bioprinting (DBB) is associated more with excreting distinct droplets as base units which eventually results in high resolution. Through the physical manipulation of a given bio - ink, this technique is deemed to be simple, yet capable of achieving accurate control of growth factors, biomaterials, and cells. Droplet - based bioprinting has a wide set of applications ranging from tissue engineering, regenerative medicine, drug delivery, clinical approaches, and cancer research [51x]. Within the domain of droplet principles, the latter can be divided into inkjet bioprinting and electrohydrodynamic jetting (EHDJ). Furthermore, inkjet bioprinting can be sectioned into thermal, piezoelectric, and electrostatic.

Table 2: Integration of Bioprinting Techniques with Microfluidics: Cellular Components, Scaffold Materials, and Their Applications in Tissue Engineering

Reference	Integration of Bioprinting and Microfluidics	Cellular Components	Scaffold Composition	Intended Tissue or Organ
[52]	Extrusion - based with microfluidic nozzle	Human umbilical vein endothelial cells (HUVECs)	Gelatin methacrylate (GelMA)	Vascularized tissues
[53]	Extrusion - based 3D bioprinting	HepG2 and NIH/3T3	Alginate, GelMa, cellulose nanocrystal	Liver tissues
[54]	Fixed deposition modeling	Structural purposes	Nylon, polycarbonate	Cartilage, bone tissues; prosthetics
[54]	Vat Polymerization (SLA)	Human Mesenchymal Stem Cells (hMSCs), Fibroblasts	Photopolymers, curable resins	Medical models/prototypes
[55]	DBB ~ Inkjet	Human Adipose - Derived Stem Cells (hADSCs), Neural Cells	PEG hydrogel, beta - TCP	Biomolecules; protein, nucleic acids
[56]	Extrusion - based printing	Cartilage progenitor cells	Alginate	Vascularized tissues
[4]	Laser - assisted bioprinting	Human keratinocyte cell line (HaCaT)	Collagen	Skin
[4]	Extrusion - based printing	Chondrocyte	Hyaluronic acid	Osteochondral
[57]	Pneumatic extrusion printing	Human nasal chondrocytes	Type I collagen	Nasal cartilage
[58] [59]	Pneumatic extrusion printing	Human bone marrow - mesenchymal stem cells and human nasal chondrocytes	Nanofibrillated cellulose/alginate	Nasal cartilage
[59]	Digital light processing (DLP)	Rabbit auricular chondrocyte	Silk fibroin methacrylate	Trachea
[60]	Pneumatic extrusion printing	Human nasal chondrocytes	PCL/atelocollagen	Trachea

3.1.1 Inkjet Bioprinting

Inkjet bioprinters deposit a precise amount of bio - ink on the printing surface through either a smooth flow or individual drops from a nozzle. The desired cell - laden biological material is stored within the ink cartridge of the printer and is then digitally controlled [61]. The overall process for inkjet printing can be done in two steps: 1) the development of individual droplets to be deposited on a specific location of the substrate; and 2) the interactivity between the deposited droplet and substrate. As mentioned above, the smooth flow of bio - ink correlates with the Rayleigh - plateau instability occurrence in Continuous InkJet (CIJ), which describes the tendency for a liquid to follow through as a stream of discrete drops. The ink applied in this way is typically conductive thus allowing it to be manipulated with a magnetic field. Drop - on - demand (DOD) inkjet, on the contrary, follows the concept of individual drops from a nozzle where the droplets are deposited over a specific location. CIJ inkjet contains high drop - rate frequencies, while DOD contains rates at lower frequencies, which tends to result in higher printing resolutions [62x]. DOD printing has shown immense potential in addressing the challenges of spatially carrying compositions and properties. Specifically, DOD printing has allowed for more precise 3D constructs through multiple depositions of polymer inks at interval - based timings. This displays the high - quality spatial controllability of DOD printing [63]. An advantage of CIJ printing is its ability to print at faster speeds. For instance, CIJ printers are commonly used for printing traceability information onto pharmaceuticals and beverages [64].

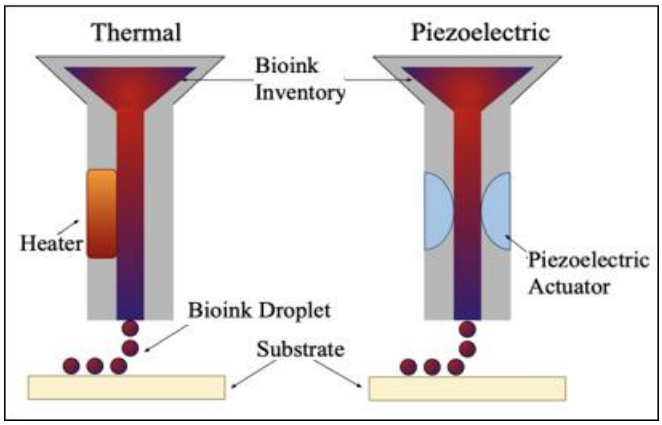

Figure 2: Inkjet bioprinting.

3.1.2 Electro - hydrodynamic Jetting

Electro - hydrodynamic (EHD) printing is superior for its low cost, high resolution, and DOD printing. EHD has been utilized in sensors, microfluidics chips, and mobile electronics [65] [66]. In a Microlens Fabrication study conducted by Fang F. et al., 2020, they explored the DOD EHD printing process of printing glycerol solution in fabricating molds for polymer - based microlenses. They exploited the advantages of EHD printing to finally master their polydimethylsiloxane (PDMS) mold [67].

3.1.3 Fused Deposition Modeling Bioprinting

Fused deposition modeling (FDM) is a technique in which 3D structures are deposited with thermoplastic material onto a substrate in layers. The printhead of an FDM printer melts the plastic filaments to allow for more consistent polymer layers. FDM has become widely used in scaffold fabrication and has its advantages in low energy consumption, great durability, good affordability, and low temperature [68]. The FDM process is mainly based on the foundation of melting a raw substance to form it into new shapes. The filaments from this are fed through a liquefier where the material becomes heated. Then, the partially liquefied material excretes through a nozzle in a layer - by - layer motion. To produce a discrete part of a construct, all the feeding material is used, thus ensuring less material waste for each dispenser. With FDM, the software allows for faster printing speeds, a proper melt flow rate, and great infill density [69].

3.1.4 Extrusion Based Bioprinting

Extrusion bioprinters contain multiple printheads for printing various materials into a single construct. Additionally, they also function at higher cell densities [70]. With two or more printing heads, extrusion bioprinters disperse material with continuous pressure through a tiny - sized nozzle. During this dispersion, the direction of the deposited layers varies, but usually, the cartridge is fixed to an arm that moves accordingly in a z - y direction, thus creating said 3D patterns. The low speed and pressure of extrusion printers aid in avoiding the typical harsh conditions encountered via bioprinting (shock, heat, tears, etc.) [70]. Extrusion Based Bioprintig (EBB) is utilized for creating live cell - incorporated structures. Therefore, hydrogels are primarily used for this printing because of the high water contents and crosslinking ability [71]. EBB deals with printing continuous filaments with constant diameters to form a final 3D scaffold. An important physical characteristic of EBB is surface tension. The attractive forces between aqueous particles affect the contouring on the surface. However, surface tension is usually neglected in EBB because it plays a more pivotal role in inkjet - based printing [72].

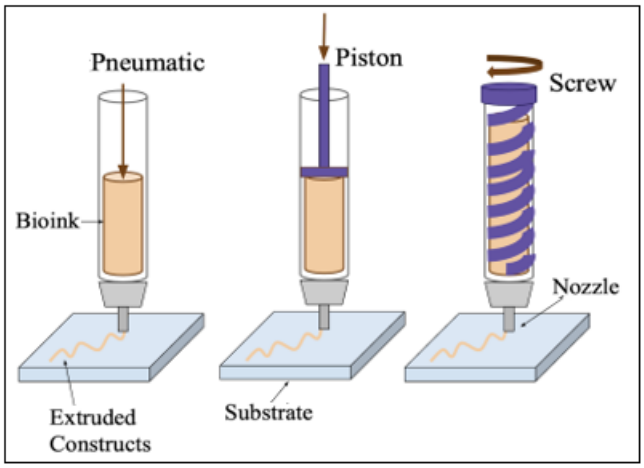

Figure 3: Extrusion bioprinting

3.1.5 Vat Polymerization printing

Vat polymerization (VP) is a 3D printing method that makes use of photopolymerization to cure a specific liquid bio - ink through a layer - by - layer format. VP has made a presence in biological applications such as biomedicine, medical devices, human tissue regeneration, and human - based scaffolds [73] [74]. Continuous VP printing has allowed for higher fabrication velocities which in turn have resulted in printed objects with smooth surfaces. VP printings can be subdivided into stereolithography (SLA), digital light processing (DLP), and continuous liquid interface production (CLIP). These few fast - printing approaches have immense potential to expand the biomedical application of VP [75].

3.1.6 Vat Polymerization Stereolithography

Stereolithography (SLA) depends on spatially controlled illumination to crosslink with specific hydrogel features. The layer - by - layer printing technique provides a seamless domain of multi - material integration. Vat polymerization is predominantly employed in fabricating both cellular and acellular structures. The contactless feature of using SLA enables it for higher spatial resolution than other bioprinters. SLA uses a more streamlined approach to photocrosslink liquid bioninks into desired solid features which contribute to the final construct [76]. As mentioned, SLA relies on a computer - controlled laser to cure a photocurable bio - ink. Once the liquid bio - ink is exposed to the raster - scanning laser, it is cured directionally (z - direction) into a layer. The platform on which the layer is deposited slides aside to allow for a refill on the fresh bio - ink of the second layer. Once this process is iterated through, the 3D bio - printed construct is finished and typically goes through an additional polymerization processing (eg. heating, photocuring) [77]. SLA - based 3D bioprinting is a commonly used AM process in Dentistry because of the high resolution and accuracy offering smooth finishings on the constructs. The consecutive deposited layers of photocurable material call attention to the influence of thickness and positioning of printed layers in the dimensional accuracy of constructs. In addition, other factors such as sturdiness, surface anatomy, and bacterial observations [78].

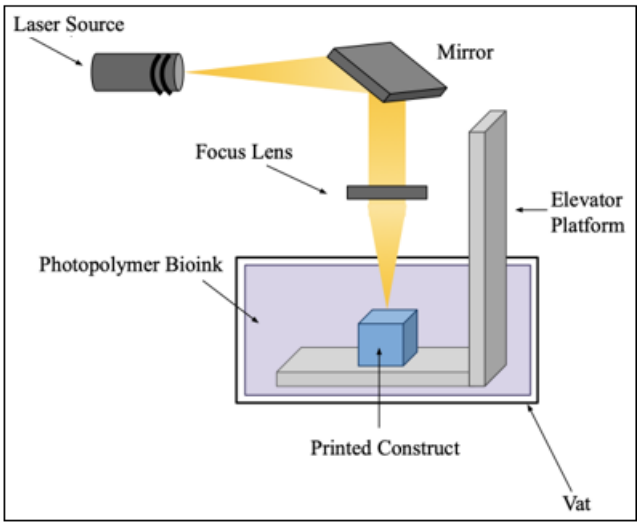

Figure 4: Vat polymerization stereolithography bioprinting.

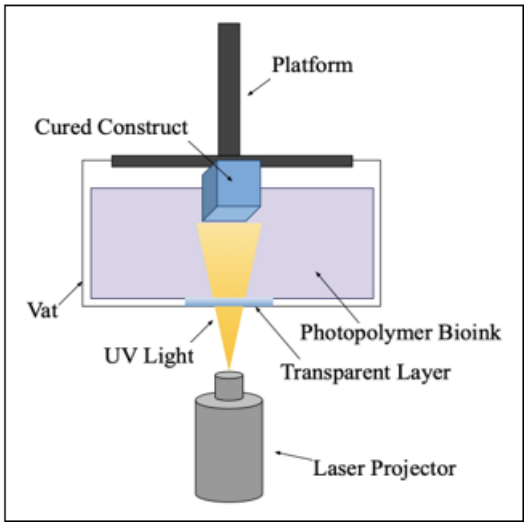

Figure 5: Vat polymerization digital light processing bioprinting

3.1.6 Vat Polymerization Digital Light Processing

Digital light processing (DLP) systems use digital photomask to project the light on a platform. This mask is usually a liquid crystal display (LCD). DLP usually reflects light to a surface rather than passing through and DLP projectors are more mobile and reliable for delivering high - intensity light reflections. DLP development lies in enhancing light properties for higher resolution, toxicity levels, and sample sizing. For accessing better control over light penetration, it is optimal to apply small amounts of absorbing agents. This ensures that the light penetration depth is at the ideal wavelength length thus preventing unwanted over - cured waste of the bio - resin [79]. Between DLP and SLA mechanisms, DLP printers tend to be cheaper and offer faster printing speed with high resolution (25 - 50 um). Specifically, DLP has been explored in bone tissue engineering. The interconnected micropores of a human bone can be printed at high resolution with DLP. However, there has been difficulty in finding the appropriate photopolymer that works with DLP and is biocompatible. Most industrial photopolymers are toxic to the human body and fail to serve as material for biological implants. Fortunately, isosorbide is non - toxic and low - cost thus making it an optimal monomer for 3D printing [80]. DLP has given rise to many functionalities in 3D printing. New DLP - based techniques have allowed for more tailored constructs and have portrayed immense complexity in 2D/3D lattices. Another favorable aspect of DLP is the stiffness displayed in constructed organ models which has proved this technique to be an influential tool in the biomimetic domain [81].

3.1.7 Vat Polymerization Continuous Liquid Interface Production

Continuous Liquid Interface Production (CLIP) is an improved version of the DLP techniques such that the speed has been enhanced by a factor of 1000. Tumbleton J. R. et al., 2015 took advantage of the challenges displayed with oxygen in photopolymerization by replacing the regular vat with an oxygen - permeable. This modification circumvents the visible layer lines and reduces printing time drastically [82]. The CLIP method diminishes the need to use a layer - by - layer printing process with the mentioned oxygen - permeable window. With improved speeds, CLIP has achieved time ranges between 50 and 150 ms resulting in an overall increase by 100 ms [83]. Resin in the CLIP techniques can freely flow into the “dead zone” which is located at the window surface. Johnson A. R. et al., 2016 have created a CLIP - based prototype microneedle with modular control. CLIP techniques have expanded further than just 3D bioprinting. Microneedles through CLIP have displayed chemical properties essential for penetrating skin and performing drug delivery. The rapid methodology of CLIP has accelerated the transition of microneedle technology into a clinical setting [84].

3.1.8 Laser - Assisted Bioprinting

Laser - assisted bioprinting (LAB) has been performed in in - vitro tissue while still displaying high resolution and speeds. A very well - known method of LAB is laser - induced forward transfer (LIFT). The LIFT technique uses an upper (donor) side and a lower (collector) side which are placed in parallel. Underneath the donor side, a laser - absorbing metal (eg. gold, or silver) is covered with a desired biomaterial that is to be deposited. A single laser is pulsed at the metal which causes the metal to absorb the pulse and thus force the biomaterial to fall on the collector side [85]. LIFT has been used to print micron - scale precise constructs with wide viscosity ranges. These factors contribute to improving cell viability and make it capable of spheroid bioprinting. More specifically, these spheroid structures (microbeads) are more resistant to applied stress than an actual spheroid therefore making it optimal for creating tissues and organs [86].

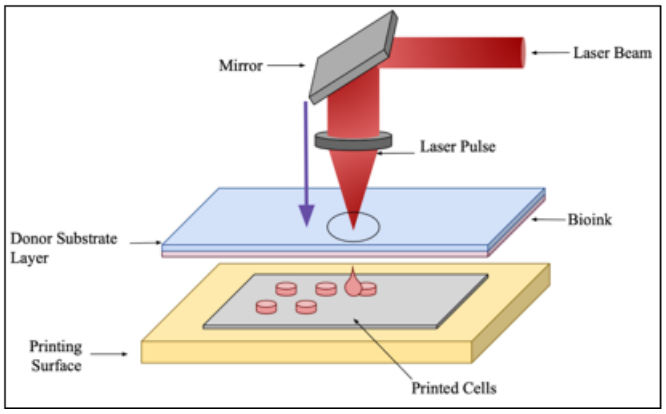

Figure 6: Laser assisted bioprinting

4. Applications of 3D Bioprinting:

4.1 Infectious Disease Studies

Typically, infectious diseases have been treated through conventional mediums such as antibiotics, antifungals, and antivirals when the immune system of the host becomes unable to fight against the infection.2D cultures have been studied to understand host - pathogen interactions, however, the cells in the cultures are not placed in similar conditions as the organism. Therefore, 2D cultures don't fully represent the biological processes that take place in native tissues in vivo [87].3D models, on the other hand, serve as a better replacement for developing desired drugs and vaccines that can mimic the microenvironments of the organism much more effectively [88] [87]. A great example of a bioprinting application in infectious diseases is the reconstruction of a human respiratory tissue that was used in the SARS - Cov - 2 infection study to test prospective antiviral drugs [89]. These accurate models aided in the verification of many present drugs used in reducing the SARS - CoV - 2 infection and were even used to develop the first approved treatment for COVID - 19 [90]. Most infectious diseases are the products of microorganisms such as bacteria, viruses, and fungi. Specifically, bacteria is a notable cause of diseases such as the Haitian cholera outbreak and the *Escherichia coli* outbreak in Germany which has led to damage in the human gastrointestinal tract [91]. Bioprinting applications such as in - vitro modeling and vaccine development have created the potential to positively impact future pandemics [92] [93].

4.2 Tissue Grafts

Tissue engineering is a specific field within bioengineering that is highly focused on fabricating artificial tissues and organs to replace damaged tissues. Tissue - engineered vascular grafts (TEVG) can provide low immune rejection rates and mass reducibility of prosthetic grafts [94]. In order to ensure that TEVGs are successful in the human body, they must mimic the in - vivo properties of blood vessels (tear strength and burst strength) [95]. In addition, TEVGs must also be hemocompatible with the host to ensure stability and prevent thrombosis [94]. On the other hand, some patients require invasive surgical intervention to aid in healing their infection from bone defects. This is usually done in the form of bone grafts, substitute materials, or vascular grafts to aid in bone regeneration. Bone grafts can usually be taken from another part of the patient's body but are usually limited by their restricted size and the higher associated risk of infection that may follow. Therefore research has been done in bone tissue engineering (BTE) to create alternatives to traditional bone grafts. In addition, there have also been porous 3D scaffolds that have had directed effects on bone regeneration [96].

4.2 Hollow Organs

End - stage lung disease is a major influencer in global mortality rates with an estimated 1.8 million deaths [97]. From tobacco smoking to exposure to harsh air pollutants, patients are obtaining chronic obstructive pulmonary disease (COPD) rapidly which requires an immediate solution [98]. However, the known solutions have certain limitations. There is a high scarcity of donor organs and the current therapeutic methods are not addressing issues at an immune system level. To solve these setbacks, development in tissue bioengineering has made it possible to circumvent the lack of organs and immune suppression [98].3D bioprinting has the capability of creating functional tissues to mimic the native tissue of the host through the right usage of materials and a proper geometrical scaffold design. Printed scaffolds for lungs have made biocompatible, non - toxic, and chemically stable which should ensure reduced adverse immune responses after implantation [99].

5. Limitations and Challenges

The field of fabrication covers various independent topics such as regenerative medicine, engineering, and embryology. These areas have recently intersected with the conventional method of biomedical engineering [100]. Specifically, bioprinting has many modalities and printing methods: laser, light, inkjet, and extrusion printing [101]. Though each bioprinting technique has evolved rapidly, significant limitations remain in generating true biological substitutes for various tissue types. Replicating tissue microstructures and maintaining complex cellular organization still remains a crucial challenge in the bioprinting field [100]. One specific example of a complex cellular architecture is liver tissue [102]. The liver is susceptible to irreversible damage and is faced with transplantation difficulties. In addition, the hepatic lobule, the standard building block of a liver, is complicated. In a lobular unit, there are 3D layers of cells that are connected through a palisade of hepatocytes. This palisade hepatocyte is bordered by the region of Disse (a place - rich region). Neighboring that, endothelial sinusoidal cells coat the lumen of the sinusoids [100]. Despite the current structural organization, this description only represents a basic view of a single liver lobule. From a design perspective, the liver proves to be a very complicated tissue to be recreated through engineering mediums [103]. Along with the liver, there are other tissue types that display intricate architectural organization [100].

In addition to these, there are also engineering challenges within individual bioprinting techniques. As a very common technique, extrusion - based bioprinting is known for its ease with syringe print heads and its positioning systems. However, a serious limitation of this printing technique is that heterogeneity of some complex tissues occurs at a scale that is orders of magnitude below what current extrusion printers

are capable of achieving. Usually, a theoretical size for a dispensed droplet is the width of a single cell, however, extrusion printers dispense bioinks as fibers which creates complications for micropattern tissue regions [104] [101]. Another set of techniques with their own limitations are laser and inkjet systems. They have a narrower window of printability and a limited amount of bio - inks that they are compatible with. In addition, there is a trend of structural weakness present in bioprinting constructs due to the limited viscosity of bio - inks which restricts the maximum dimensions of the structures. A major limitation is found with light - based bioprinters and their difficulties in fabricating heterotypic complexity for tissue constructs. Most of the light - based bioprinters, including DLP and two - photon polymerization, function through the use of a vat filled with a photo - curable biomaterial in which every additional layer requires a fresh coating of material to be layered on the already printed construct. A common strategy that is used to deposit the material is through a coordinated motion in which a robotic arm lowers and raises a build platform in an X - Y dimensional plane. Even though this strategy does work, it has a few issues that hinder the final product quality. The issue lies in the fact that the coverage of the printed material is a more direct way of providing diverse compositions of tissues through either different biomaterial inks or cell types. With that, the method creates a challenge as the body of the construct is not built in even layers making it difficult to achieve true tissue heterogeneity [105] [101].

Connecting hydrogels of different compositions through a microdevice, under the exposure of light, allows for different shapes and structures to be built within a device to create the desired heterotypic constructs with higher levels of microscopic intricacy [105]. This solution has opened paths to other alternatives that can be used. For example, tissues could be built in the Z - plane for the purpose of increasing volume and circumventing the setbacks faced with thin footprints [106]. Another avenue open for improvement in the field of bioprinting is microgels. The development of microgel assembly has created simpler strategies for fabrication. Bertassoni L. E. et al., 2021 have developed a method for high - throughput bioprinting of injectable pre - vascularized microgels so that they can be easily injected into 3D - printed micro - cages. When the pre - vascularized microgels were implanted and differentiated with cell - laden hydrogels containing the same polymer materials, it was found that the formation of the vascularized tissue had significantly improved with the pre - vascularized matrix [101] [107].

6. Conclusion

Although bioprinting is relatively new and has posed a continuous set of questions, the progress it has achieved in the last 20 years is still outstanding. What started as a mere robot dispensing cell droplets on a plate has transformed into a machine piecing individual layers, cell - by - cell, into entire functional organs.3D - printed livers [102], flexible cartilage, and cellularized vascular channels fall short [108] to describe the full extent to which 3D bioprinting has influenced the biomedical field. While questions and challenges are formed each day in response to bio fabrication and printing, it does not serve as a setback to the overall progress made. Building functional and biocompatible organs, tissues, and body parts from scratch is not a simple task, but it is certainly not an unattainable goal. In order to develop and progress further into the field of bioprinting, future work should primarily focus on addressing and outlining the critical challenges still present. Rather than focusing on superficial issues such as speed, precision, and cost, it would be preferable to develop more enhanced engineering tools that are concentrated on fixing biological problems. A good example of this would be spending more time analyzing the barriers regarding biocompatibility and vascularization of tissues than solely focusing on dispensing biomaterial with higher resolutions and faster printing speeds. Therefore, a balanced system that incorporates both the ideals of engineering and biology ensures a successful path to the fabricating organs that can potentially function within humans. The rapid advancements in 3D bioprinting have reshaped the field of tissue engineering, with exciting potential to revolutionize regenerative medicine and organ transplantation. While current techniques offer remarkable possibilities, critical challenges, such as cell viability, vascularization, and structural fidelity, persist. Addressing these issues will be key to unlocking the full potential of 3D bioprinting in creating viable, functional organs. Future research must focus on refining bioink formulations, enhancing bioprinting resolution, and developing new techniques that mimic the complexity of native tissues.

Supplementary Material
Not applicable

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