International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Avenues and Future Prospects Of 3D Bioprinting

Pradyun Iyer[1], Vanessander De Matos Muinge[2], Shivaay Chhillar[3], Adesayo Elumaro[4], Rajkanwar Singh[5]

[1,2,3,4,5]Co-author, Incognito Blueprints

Abstract

3D bioprinting has opened numerous avenues in the medical field, particularly advancing biomedical techniques for administration of drugs, fabrication of prostheses, surgical operations and restoration of damaged body parts. In this review we discuss the principles of 3D bioprinting, its applications, advantages over traditional methods, recent advancements and the future prospects of automation within the field.. The review also delves into the creation of more physiologically relevant tissue models for drug screening and toxicity testing. Finally, we also explore more recent advancements showcasing the impact of printing in space, integration of artificial intelligence (AI), and development of 4D bioprinting. Expressing current limitations and challenges, the review underscores the potential of 3D bioprinting in personalized medicine.

Keywords: 3D Bioprinting, Qualitative Research, Applications of 3D Bioprinting, Future prospects of 3D Bioprinting, Artificial Intelligence, Machine learning, Medicine, Automation

1. Introduction

For years, physicians have relied on traditional manufacturing methods and tools when solving problems in the medical field. That was, until the emergence of the transformational process known as three-dimensional (3D) printing and 3D bioprinting which is changing the whole arena of manufacturing methods being used in the medical field [1].

3D printing, formerly known as ‘Rapid Prototyping’ or ‘Additive manufacturing’, was initially a method used for rapid prototyping, and was first patented by Charles Hull in 1984. Hull described his invention as: “A system for generating three-dimensional objects by creating a cross-sectional pattern of the object to be formed at a selected surface of a fluid medium capable of altering its physical state in response to appropriate synergistic stimulation” [2] [3]. Hull is considered the inventor of the stereolithography (SLA) method, which works on the principles of solidification of layers comprising photopolymer resin. This transformational process (3D printing) is a mechanical process whereby solid objects are created by ‘printing’ successive layers of materials in order to replicate a shape modeled by a computer [4].In this technology objects are created by a controlled addition of materials, rather than traditional subtractive methods.

3D printing related to the direct biomedical field is regarded as 3D bioprinting [5]. In the field of medicine, 3D printing has a market size of around $2 billion- an estimate which is increasing at a rapid rate with increasing research being done in the field [6]. There are various applications to this technology with the leading ones being tissue and organ regeneration, drug testing, and personalized medicine at 30.79% [7]. 3D bioprinting can be defined as- a process that enables the precise layer-by-layer deposition of bioink, a specialized material consisting of living cells, biomaterials and bioactive molecules [8] [9]. A computer-guided pipette is used to layer bioinks on top of one another, to create artificially living tissues. The medium used to 3D print the product is organic, rather than a medium such as plastic or metal which the world is currently fighting against due to climate changes. Therefore this technique allows for the fabrication of complex and functional tissues and organoids while being environmentally friendly and contributing to the reduction of global warming at the same time.

Bioprinting offers both fast and precise solutions for cases such as grafts and tissue/organ depending upon the urgency. Laser-assisted bioprinted objects range from 20 to 100 μm printing at a rate of 10-20k droplets per second [[10] [11]] , while inkjet bioprinters work at a faster rate of around 30k droplets per second with a fineness of 100-500 μm.

90% of proposed drugs fail in clinical trials which results in wastage of time and resources, this can be prevented through the usage of cell cultures that mimic clinical trials and in vivo environments [12] [13]. 3D bioprinting can be used to create models for use in surgical preparation, where surgeons use copies of patient-specific organs to use for practice before performing complicated surgery operations, it can be used as a way to train future surgeons and it is also used as a way to educate patients and their families about upcoming surgeries [14] [15] [16]. 3D bioprinting plays an integral role in the creation of surgical instruments. It allows for the fabrication of personalized surgical tools tailored to each patient's unique anatomy and by fabricating surgical and exclusive tools for sui generis operations, thus, improving a surgeon's level of comfort. This highlights how 3D bioprinting has the potential to shape the landscape of surgical instrument's manufacturing [17] [18] [19]. 3D Bioprinting also has the potential to drastically reduce the huge need for organ donors around the world. For example, the need for organ donors in the United States remains critical. As of June 2024, over 103,000 men, women and children are on the national transplant waiting list, and tragically, everyday around 17 people die waiting for a transplant, highlighting the urgent demand for more organ donors [20] [21]. These statistics clearly show the potential 3D bioprinting has in the printing of organs and tissues and the impact it can really have if its application and deployment is successful.

Although Bioprinting offers numerous benefits to physicians and surgeons, it has certain limitations that need to be addressed. The quality and the diversity of materials of bioinks need to improve in order to match the highly demanding and diverse complexity of human biological structures, which are extremely complex and intricate! Therefore the development of bioinks is a key point for bioprinting [22].

The reproducibility, scalability and standardization of 3D bioprinting processes are areas that also need improvement, especially for production at large-scale, which is usually needed in the pharmaceutical field for standardized products targeted to the broader public. Regulatory and ethical processes should also be worked on and improved to ensure a safer and more controlled environment for all parties involved.

Recent interesting developments and applications of 3D bioprinting include, bioprinting use for space, bioprinting in microgravity, integrations with AI and machine learning and the introduction of four-dimensional (4D) bioprinting .

In this review, we explore the avenues of bioprinting in the medical field. Firstly by delving deep into the underlying principles of bioprinting processes and techniques, secondly by exploring its applications in surgical training, subsequently an exploration at the transformational applications of 3D bioprinting in drug discovery was presented, a detailed and very clear comparative analysis between the traditional methods of manufacturing vs the 3D bioprinting process was crafted and ultimately an exploration of many of the most impactful new advancements of 3D bioprinting in the medical field and how it is going to look like in the future was performed. The paper provides an overview of 3D bioprinting in healthcare, with a focus on how it can be used in surgical preparation to help physicians understand complex patient anatomies, and it discusses its advantages and limitations while providing a future outlook of how bioprinting can be effectively used in 3D printed medicine.

2. Discussion

2.1 Principles of 3D Bioprinting Techniques.

2.1 Bioink Selection

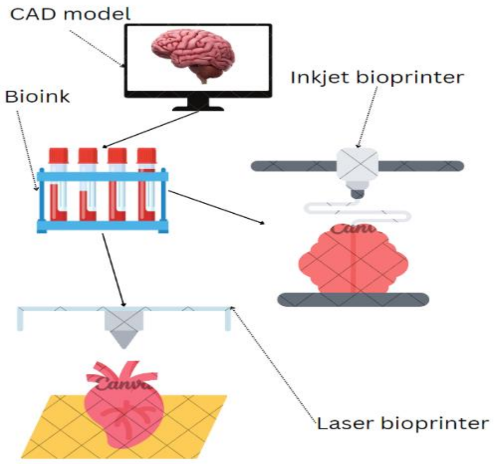
3D Bioprinting is highly used in tissue engineering and regenerative medicine for development of complex tissue. 3D bioprinting can mimic native organs and tissues. Bioinks contain living cells and biomaterials that mimic the extracellular matrix environment, supporting cell adhesion, proliferation, and differentiation after printing [23]. In contrast to traditional 3D printing materials, bioinks must have:

1. Print temperatures that do not exceed physiological temperatures
2. Mild cross-linking or gelation conditions
3. Bioactive components that are non-toxic and able to be modified by the cells after printing

2.1.2 Properties of Bioinks

Based on the requirements of the specific tissues and organs, bioinks are modified and created to regenerate the right tissue or organ. A bioink is essentially a biomaterial which is used to construct organic bodies through the process of 3D bioprinting. Polymers obtained through the cultivation of natural resources are known as natural biomaterials in the biomedical fields. Naturally obtained bioinks have various advantages over synthetic materials; being able to biomimic the ECM composition/structure, having the ability to self-assemble, higher levels of biocompatibility as well as being biodegradable [24]. However, various advantageous properties are attributed to synthetic polymers as well such as being more controllable in terms of mechanical stability, photo crosslinking ability, pH and temperature responses.

Fig 1: The figure elucidates the existing methods and techniques which are used in order to bioprint organic structures. The Agarose-based bioninks can be used to develop either synthetic or natural bioinks which can be further bioprinted using either the inkjet or the laser approach of bioprinting. However it is much more efficient using a laser bioprinter as it eliminates the use of nozzles thus increasing adaptability to the viscosity of the bioink as well as minimizing the damage to the cell cultures allowing for bioprinting of much complex organic structures rather than organoids.



2.1.3 Types of bioinks

2.1.3.1 Synthetic bioinks

Synthetic bioinks are essentially engineered materials, designed to have precise control over their chemical and physical properties. Few synthetic bioinks which are widely in use are polyethylene glycol, polylactic acid, and polycaprolactone [24].

2.1.3.2 Natural bioinks

Natural bioinks are derived from biological sources. Examples of such bioinks are collagen, gelatin, alginate, fibrin, and hyaluronic acid. These bioinks are inherently biocompatible and often have built-in biological cues to promote cellular interactions [24].

An integral part of 3D bioprinting regards with the careful consideration of bioinks. Bioinks are selected with the purpose of the project in mind. Thus the bioinks possess a certain array of properties which would be essential for a successful outcome. Combining different methods and altering certain properties are necessary in order to develop more successful bioinks for the 3D bioprinting of organic structures.

2.1.3.3 Agarose-based bioinks

Agarose, a marine polysaccharide obtained from seaweed, is a primarily used biopolymer in the biomedical field as it has diverse applications due to its remarkable gel formation [24].Agarose is a linear polymer chain with an agarobiose repeating unit. This agarobiose backbone chain consists of disaccharides namely D-galactose and 3,6-anhydro-L-galactopyranose. Though the gelation, mechanical and biocompatibility properties of agarose are commendable, its ability to support cell growth is limited. Hence, blends of functional biomaterials along with the agarose gel were used. Kreimendahl et al. (2017) reported the use of agarose-based bioink consisting of collagen and fibrinogen, separately. They demonstrated the ability of these agarose-based blend biomaterials to form stable 3D structures and support endothelial and fibroblast cell growth. In a similar work, Yang et al. (2017) used agarose/collagen along with sodium alginate as bioink for cartilage tissue engineering application. The bioink was incorporated with chondrocytes and a cartilage-like tissue was printed and evaluated in vitro. The printed biomaterial showed enhanced mechanical properties without affecting the gelling behavior considerably. Such high preference to choose agarose in bioprinting by scientists around the world is mainly owing to its excellent gelation properties, biocompatibility and rheological properties which are highly desired in 3D bioprinting. Gu et al. (2016) used agarose along with alginate, carboxymethyl-chitosan to produce 3D printed structures with induced pluripotent stem cells or human-derived neural cells for developing functional neurons. Thus the successful printing and formation of stable 3D structures with encapsulated cells in it was demonstrated [24]. Chemically modified agarose such as carboxylated agarose was used as a bioink to develop mechanically tunable 3D tissue constructs. In the study, hMSCs were used for the evaluation, and the constructs yielded very high cell viability up to 95% than the native agarose gel.

2.1.4 Approaches to three-dimensional bioprinting

2.1.4.1 Inkjet approach to 3D bioprinting

Inkjet, pressure-assisted, and laser-assisted are among the three standard methods of 3D bioprinting. Each has its own set of benefits. Scientists have made great progress using inkjet bioprinters. Various printing technologies have been tested, practice of printing various types of tissue, including vasculature, heart, bone, cartilage, skin and liver have been successful. Additive manufacturing technology is increasingly recognized as a potential solution for constructing complex interfacial tissue engineering scaffolds. AM forms complex 3D biocompatible structures via automated deposition of biological substances on a substrate using computer-aided design or computer-aided manufacturing technology [25]

Bioprinting technology has drawn increasing attention as fabrication methodology for producing scaffolds, cells, tissues and organs develop. It has advantages in precise control, repeatability and individual design, yet many challenges remain for building complex tissues including multiple cell types in a spatial structure. More importantly, bioink materials development, resolution enhancement and vascularization are necessary to apply bioprinting technology clinically.

2.1.4.2 Laser-based approach to 3D bioprinting

Laser based bioprinting consists of three parts: a pulsed laser source, a ribbon and a receiving substrate. The lasers irradiate the ribbon, causing the liquid biological materials to evaporate and reach the receiving substrate in droplet form. Its physical mechanism makes it possible to print cells and liquid materials with a cell-level resolution [26]. By giving tissue engineers control over cell density and organization of 3D tissue constructs, laser assisted bioprinting holds much promise for fabricating living tissues with physiological functionality. Laser-assisted bioprinting employs a laser to precisely deposit bioinks containing living cells onto a substrate, forming intricate three-dimensional tissue structures layer by layer. This technique comprises several key components: a pulsed laser source, a donor slide coated with a bioink, a receiving substrate, and a ribbon-like absorbent material.

2.1.4.3 Applications in Tissue Engineering

Laser-assisted bioprinting has high potential through various biomedical applications:

1. **Organ Transplantation:** Laser assisted bioprinting lets the fabrication of patient-specific tissues and organs, mentioning the critical shortage of donor organs for transplantation.
2. **Disease Modeling:** Laser assisted bioprinting facilitates the construction of 3D tissue models which mimic the complexity of native tissues- a valuable platform for studying disease progression and drug efficacy.
3. **Regenerative Medicine:** Laser assisted bioprinting allows the fabrication of scaffolds embedded with cells and bioactive molecules, promoting tissue regeneration and repair in damaged or diseased organs.

2.2 Applications in the Surgical Domain

3D bioprinting, a sophisticated form of additive production, has revolutionized the clinical subject by fabricating products of incredible accuracy, personalized models and implants. This generation involves the layer-by-using-layer deposition of biomaterials, cells, and growth factors to create complicated organic systems. In surgical practice, 3D bioprinting performs a crucial position in improving the precision and outcomes of numerous procedures. This paper delves into the applications of 3D bioprinting in understanding individualized anatomies and developing surgical publications, which include anatomical models, printed phantoms, and biologically active implants.

2.2.2 Personalized Aids and Medical Emergencies

One of the most critical applications of 3D bioprinting in surgical guidance is the arrival of the affected person’s anatomical map. The models are derived from imaging statistics, which includes CT scans or MRI, which is probably then transformed into digital 3D fashions. The subsequent physical replicas permit surgeons to test the ideal anatomical functions of every patient earlier than the actual surgical operation. This customized approach permits deeper data of the patient's specific situations, which include the exact size and area of a tumor or the complex vascular systems in a specific place. Few applications of 3D bioprinting in this field are listed below.

1. **Detailed Visualization:** Surgeons are able to visualize complicated anatomies better by fabricating a tangible layout, enhancing their spatial recognition and making plans.
2. **Pre-Surgical rehearsal:** Surgeons exercise personalized models in order to refine their strategies and decrease the risk of intraoperative mistakes.
3. **Affected individual training:** Patients better recognize their situation and the proposed surgical procedures via one's tangible fashion.

2.2.3 Treating Complications

For complicated surgeries, involving congenital anomalies or reconstructive surgical techniques, individualized anatomical models are crucial. They enable multi-disciplinary agencies to collaborate more efficiently by offering a not-unusual reference point. This collaboration is critical for developing major surgical techniques, selecting suitable tools, and looking forward to capability complications.

2.2.4 Publication of Surgical Auxiliaries

3D models of published anatomical maps serve as treasured surgical guides. Those fashions replicate the patient's anatomy with immoderate fidelity, supplying an in-depth map that courses the medical professional through tough techniques. For example, in craniofacial surgical treatment, 3D published fashions can define the precise contours and systems, bearing in mind prices decreasing and reconstruction.

2.2.5 Applications in medicine

3D Bioprinting has various applications in different fields of medicine such as the orthopedic practice of medicine where custom models of bones and joints can be developed, this would ease and fasten the pre-surgical planning and intraoperative navigation. In the discipline of neuroscience, bioprinted models of the spinal cord would aid in identifying and mapping out critically affected areas, plausibly allowing for minimally invasive processes. 3D Bioprinting has implications in surgical treatment regarding cardial maladies with models of the heart assisting in the creation of corrective surgical procedures for complex congenital defects.

2.2.6 Bioprinted Phantom Replicates

Bioprinted phantoms, which replicate the mechanical and biological homes of human tissues, are increasingly utilized in surgical training. These phantoms provide a sensible platform for training surgical techniques, trying out medical gadgets, and schooling clinical experts. The advantages of this technology are listed below.

1. **Realistic schooling:** trainees can work out phantoms that carefully resemble real human tissues, improving their abilities and self-perception.
2. **Tool finding:** scientific devices and devices may be examined on these phantoms to ensure their efficacy and safety earlier than scientific use.
3. **Device Optimization:** surgeons can refine and optimize new surgical strategies on phantoms earlier than utilizing them for patients.

2.2.7 Biologically active Implants

Biologically active 3D revealed implants have developed surgical training and effects significantly. Such implants are designed to combine seamlessly with the affected person’s body, promoting herbal healing and lowering the threat of rejection. Few applications of 3D Bioprinting in this field are listed below.

1. **Scaffolds for Tissue Regeneration:** printed scaffolds offer a framework for the boom of the latest tissues, helping in the repair of broken organs and tissues.
2. **Customized implants:** personalized implants, which include prosthetic limbs or dental implants, are

designed to match the patient's unique anatomy, making sure of higher capability and luxury.

3. **Drug-Eluting Implants:** These implants launch recuperation sellers over time, presenting localized remedies and lowering the want for systemic medicines.

There are various advantages to biologically active implants such as offering herbal tissue regeneration which is essential to quicker and further effective restoration. Such implants have a decreased rate of rejection as well. This is because these types of implants are customized and biocompatible to the individual, which lessens the hazard of implant rejection and headaches. Additionally, biologically active implants have the capability to be 3 times stronger than traditional implants, enhancing the overall functionality and first-rate life for patients.

2.2.8 Personalized surgical instruments

3D bioprinting lets in the appearance of custom surgical units to precise strategies and patient anatomies. The devices can be designed to match unique surgical conditions, improving precision and decreasing the hazard of mistakes. Examples of such devices are listed below.

1. Custom-reducing publications: These devices are tailored to the patient’s anatomy, those guides ensure correct and steady cuts in the route of surgical procedures.
2. Personalized Retractors: Designed to shape precisely round anatomical structures, those retractors offer better publicity and access at some point of strategies.
3. Device Handles: Ergonomically designed handles decorate the healthcare expert's comfort and management, reducing fatigue for the duration of lengthy techniques.

2.2.9 Preoperative Planning and Simulation

3D bioprinting helps in making plans based on simulations. Such simulations make use of published 3D models, allowing surgeons to explore surgical procedures and potentially demanding conditions. Combining 3D bioprinting with digital fact (VR) technology complements preoperative making plans. Surgeons could interplay with VR models of the affected character's anatomy, manage systems, and exercise techniques in virtual surroundings as well.

2.2.10 Regenerative medication and Transplantation

3D bioprinting holds a big capacity in regenerative remedy and transplantation. 3D bioprinting is used to create engineered tissues for repairing or converting broken tissues. programs embody pores and skin grafts for burns, cartilage for joint restoration, and neural tissues for spinal cord injuries. Researchers are developing techniques to print complete organs and complicated tissue configurations that might eventually deal with the shortage of donor organs and revolutionize transplantation. However, this idea is currently in experimental tiers, the bioprinting of organs together with kidneys, livers, and hearts is progressing unexpectedly. These bio-printed organs may additionally need to someday offer a sustainable answer for patients in need of transplantation.Guidelines. Challenges, and Advancements.

2.2.11 Improvements and Advancements made to this technology

The destiny of 3D bioprinting in surgical guidance will be shaped by the usage of advancements in printing technology, biomaterials, and bioinks. Persisted studies and development are crucial for enhancing the precision, capability, and scalability of bioprinting tactics. Few advancements to this technology are listed below.

1. **Multi-cloth Bioprinting**: growing printers able to concurrently deposit a couple of substances will decorate the complexity and functionality of printed structures.
2. **Superior Bioinks**: growing bio-inks that carefully mimic the homes of nearby tissues will enhance the biocompatibility and average overall performance of printed implants and models.

3. **Excessive-decision printing**: improving the selection of 3D printers will permit the creation of finer and more focused structures, crucial for replicating tricky anatomical functions.

2.2.12 Legal, Regulatory and ethical limitations

As 3D bioprinting becomes more included in surgical exercises, regulatory and ethical concerns will play a crucial characteristic. ensuring the protection, efficacy, and moral use of printed products is paramount. Few regulatory frameworks by which 3D Bioprinting is bound by, are listed below.

1. **Requirements and suggestions:** developing entire necessities and guidelines for the production and use of printed clinical merchandise should ensure consistency and safety.
2. **Approval strategies:** Streamlining regulatory approval methods for printed products should facilitate their clinical adoption without compromising patient safety.
3. **Ethical concerns:** Addressing moral troubles associated with the use of bio-printed tissues and organs, collectively with consent, ownership, and accessibility, may be vital for accountable advancement.

2.3 Drug Discovery and Fabrication

In recent years, the cost of drug discovery and development has been progressively increasing faster than the number of drugs approved for treatment. This is because existing in vitro models for drug development do not sufficiently ensure safety and efficacy, owing to their lack of physiological relevance. Additionally, preclinical animal models are extremely costly and present problems of inaccuracy due to species differences. 3D bioprinting serves as a solution to the major hindrance in drug discovery and development. 3D bioprinting can be used to create highly accurate models of human tissues and organs. These models can be used to study disease mechanisms in a controlled environment, allowing researchers to observe the progression of diseases and the effects of various treatments in a way that closely mimics human physiology.

3D bioprinting offers numerous advantages in drug discovery by providing more accurate and human-relevant models for testing and research. This leads to a better understanding of diseases, more efficient drug development processes, and ultimately, more effective and safer drugs for patients. This effectively provides broader drug development pathways in drug testing and screening, toxicology studies, regenerative medicine, and many other biomedical endeavors.

2.3.1 Tissue models and applications in drug-screening

Tissue models are critical tools in the drug discovery process, providing more accurate and physiologically relevant systems for screening potential drug candidates. Traditional drug development methods involve analyzing the effects of drugs in 2-D laboratory-grown cells or animal models. However, growing evidence makes it clear that 2D models often fail to accurately predict drug efficacy *in vivo*. The main reason for this is that 2D models fail to replicate the intricate microenvironment found *in vivo* [27]. 3D tissue printing provides a more relevant and human-like platform for drug testing [28]. Given below are the most majorly used types of tissue models used in drug screening.

2.3.1.1 Applications of Cell cultures in drug screening

Cell cultures refer to laboratory methods that enable the growth of eukaryotic or prokaryotic cells in physiological conditions [29]. This imitator is used to investigate the biology, biochemistry, physiology (e.g., aging), and metabolism of wild-type cells and diseased cells. The 2 major cell culture techniques used in drug discovery are the use of monolayer cell cultures and spheroids and organoids. Monolayer cell cultures are anchorage-dependent cultures of usually one cell in thickness with a continuous layer of cells at the bottom of the culture vessel [30].

In monolayer cultures, cells are grown as a single layer on a flat surface (e.g., petri dishes or multiwell plates). These cultures are easy to maintain and allow high-throughput screening. However, cell cultures lack the complexity of three-dimensional (3D) tissues.

On the other hand, spheroids and organoids are self-organized functional cell clusters located in a 3D protein matrix [29]. They can better represent *in vivo* conditions and interactions between different cell types. Organoids are derived from stem cells and can self-organize into miniaturized organs. However, they are typically met by the same constraints as monolayer cultures.

2.3.1.2 Applications of Tissue Explants in drug screening

Tissue explants are fresh human tissues dissected into small blocks or biopsies that are cultured at the liquid-air interface on collagen rafts [31]. These tissue blocks retain their cytoarchitecture and support productive infection of various pathogens without exogenous stimulation. Ross Harrington first implemented the technique in the United States [32] [33]. They are commonly used for drug penetration studies and evaluating drug distribution within tissues. However, since they are cultured *ex vivo* the tissue explant method is met with several drawbacks: Tissues start to deteriorate after 3 weeks in culture, there exists difficulty in monitoring cells beyond the depth of confocal microscopy (unless cells are isolated for analysis), and the system does not reflect the effects of *in vivo* systemic factors [32].

2.3.2 Perfused Organ Systems (POS) & Bioengineered Tissues

These are the 2 major types of tissue models which employ 3D bioprinting in the process of fabrication. The term “organ perfusion” when applied to preservation, inherently emphasizes the utilization of non-static preservation methods rather than counting merely on the current standard of care, SCS. *In vitro* studies benefit from perfusion because it positively impacts cell survival mechanisms and simulates real-life growth conditions for research purposes. Types of POSs typically employed in drug discovery include Microfluidic systems and Bioreactors. A tissue chip or Organ-on-a-chip was coined by merging 3D organotypic system culture with microfluidics. They shall be explored further later in the research article. Bioreactors are devices that culture cells in a controlled environment. Maintaining relevant physiological conditions in cell cultures is of paramount importance to ensure the reproducibility of published findings and the translational relevance of experimental data to clinical applications [34]. Meanwhile, Bioengineered tissues involve employing biofabrication to create 3D tissue constructs using bioprinting.

2.3.2.1 Disease-exclusive and Personalized tissue models

3D bioprinting allows for the creation of patient-specific tissue models using cells derived from individual patients. This enables personalized drug testing, where treatments can be tested on bioprinted tissues made from a patient’s cells, leading to more tailored and effective therapies.

Organoids are self-organized, three-dimensional structures derived from stem cells that can mimic the structure and physiology of human organs. Patient-specific induced pluripotent stem cells (iPSCs) and 3D organoid model systems allow cells to be analyzed in a controlled environment to simulate the characteristics of a given disease by modeling the underlying pathophysiology. The recent development of 3D cell models has offered the scientific community an exceptionally valuable tool in the study of rare diseases [35]. Patient-specific 3D organoid modeling systems represent *in vitro* cell models that can overcome some of the limitations of traditional drug models and cell cultures. These models allow researchers to study cells in a controlled environment, simulating disease characteristics by modeling the underlying pathophysiology.

3D bioprinting has yet to successfully overcome the many challenges related to building 3D structures that closely resemble native organs and tissues, which are complex structures with defined microarchitecture and a variety of cell types in a confined area. Meeting this challenge is being made possible by directing the 3D bioprinting to manufacture biomimetic-shaped 3D structures, using organ/tissue images, obtained from magnetic resonance imaging and computerized tomography, and employing computer-aided design and manufacturing technologies. With the addition of these techniques, personalized/patient-specific tissue models can lead to more effective treatment and therapies.

2.3.3 Organ-on-chip (OOC) for drug metabolism

3D bioprinted tissues can be integrated into microfluidic devices to create organ-on-a-chip systems. These systems provide a more physiologically relevant environment for testing drug efficacy and toxicity compared to traditional 2D cell cultures.

1. **Cardiac models**
2. **Liver models**
3. **Skin models**
4. **Neural models**
5. **Intestinal models.**

2.4. Comparative Theoretical Analysis

The comparative theoretical analysis highlights the benefits and limitations of traditional approaches versus 3D bioprinting approaches. By having a focus on the contrast between traditional vs new approaches, scalability, reproducibility and cost. Therefore providing a thorough understanding of how 3D bioprinting can be introduced into healthcare practices and its potential.

2.4.1. Traditional vs Novel Approaches

Traditional methods in the medical field have long been the cornerstone of medical device production. Mainly in tissue engineering and regenerative medicine the traditional methods relied heavily on the use of two-dimensional (2D) cell cultures and animal models [29]. While these techniques are well understood and have proven reliable over decades, these methods have limitations in terms of laboratory safety as in a cell culture laboratory there is risk of potential hazards linked to infectious agents harbored by cultured cells (e.g., HBV or HIV), the methods require significant time and labor investment and have limitations in terms of their ability to accurately mimic human tissue and the complexity of the human body. In traditional prosthetics manufacturing, the manufacturing process involves creating molds and then casting the prosthetic device which requires large-scale production to cover the overhead costs of making the tools, also requiring expensive precision and skill to assemble intricate parts which often result in costly prosthetics that lack customization, and take a long time to manufacture leading to less comfortable fits for patients and long waiting times.

In contrast, 3D bioprinting offers new and revolutionary approaches. Specifically, 3D bioprinting offers a new approach to tissue engineering and regenerative medicine by allowing for the creation of three dimensional (3D) tissue models using a combination of bioinks and biomaterials that more accurately mimic human tissue and improves the precision of the final product while significantly reducing the time and cost required to produce customized medical solutions [36] [37]. 3D printed prosthetics are manufactured through a process of layer-by-layer creation of the prosthetic device directly from digital designs which eliminates the necessity of mold and large-scale production. 3D printing can create complex constructs with ease therefore eliminating the need for expensive precision and skill which results in more affordable, comfortable and customized prosthetics manufactured at shorter times [4].

Bioprinting use in surgical preparation is notably promising! By creating patient-specific personalized 3D models of patient anatomy, surgeons can better understand complex patient anatomies and plan surgeries much more effectively, resulting in improved patient outcomes and reduced complications during surgery [36] [37].

2.4.1.1 Key Distinctions compared to Traditional Methods

Traditional methods are generally designed for large-scale production in industry, making it difficult to tailor personalized patient-specific solutions. In contrast, 3D printing excels when it comes to production of highly customized solutions, allowing for the creation of personalized medicine, implants, customized surgical tools and prosthetics that perfectly fit with the patient's anatomy providing better patient outcomes while being affordable [4]. 3D bioprinting's precision clearly outperforms those of traditional methods. The improved precision is especially important for applications such as tissue engineering where slight deviations may hinder the treatment's effectiveness [38].

2.4.2. Scalability of 3D Bioprinting

Scalability is significantly important in the widespread adoption of manufacturing technologies in the medical field. Traditional methods, commonly optimized for mass production, can efficiently produce large quantities of standardized products. Traditional method's scalability is achieved through the use of automated machinery and assembly lines which can result in economies of scale (reduction of per-unit costs as production volumes increase).

In contrast, 3D bioprinting faces unique scalability challenges. While the technology is highly efficient for producing personalized, patient-specific solutions, scaling up production to meet large demands remains complex. Current speed of 3D printers and the intricacies involved in printing biological tissues can limit the volume of production [36] [37].

To tackle this issue, researchers around the world are currently investigating new bioinks and biomaterials that can be used in larger-scale bioprinting manufacturing. Furthermore, the recent advancements in 3D printing technologies, such as the development of faster and more precise 3D printing systems, are helping to improve the scalability of 3D bioprinting [36] [37].

2.4.2.1 Points of Consideration:

Traditional methods are currently much more efficient for mass production as there are established industrial processes and economies of scale. Even though 3D bioprinting is comparatively slower; it offers unparalleled benefits when it comes to mass producing customized medical devices. The scalability of 3D bioprinting is expected to grow significantly with the current rapid global technological improvements in 3D bioprinting, possibly revolutionizing the way medical devices are manufactured.

2.4.3. Reproducibility of 3D Bioprinting

The ability to consistently produce identical items is a key quality metric in medical manufacturing. Traditional methods benefit from decades of refinement which consequently result in current high reproducibility. Traditional methods such as injection molding and CNC machining involve standardized processes and materials, allowing for the consistent production of medical devices and implants. The use of mold and computer controlled machining in traditional methods ensure that each product meets the same specification, enabling reliable repeatable manufacturing [39].

In 3D bioprinting, reproducibility is a sector which is undergoing constant improvement and research, it is more complex. While 3D printing achieves a very high degree of customization and personalization, the existing variability in biological materials and the sensitivity of the printing process can make it more challenging to ensure consistent, reproducible results. Many factors such as the existing composition rheological properties of the bioink, the printing parameters, and the complexity of printed structures can all impact the reproducibility of 3D bioprinted products. Many efforts and ongoing research around the globe are underway, including the development of more standardized bioinks and printing processes, aiming to improve the reproducibility of 3D bioprinting [40].

2.4.4. Costs of 3D Bioprinting

Cost considerations play a key role in the adoption of new manufacturing technologies. Traditional methods have lower unit costs in mass production as a consequence of more established industries and processes. However, traditional methods such as injection molding and CNC machining generally have high upfront costs compared to 3D printing. The methods used require the creation of molds, tooling, and other specialized equipment which can be expensive, especially in the case of low-volume production. Traditional methods are capital intensive (the initial capital required is quite large) but they often benefit from economies of scale [5].

3D bioprinting has a lower upfront cost due to not needing expensive specialized tooling and equipment making it more suitable for low-volume or personalized medical applications. For example, when producing costly molds 3D bioprinting is better as traditional mass manufacturing is too expensive for low-volume manufacturing [36]. 3D bioprinting provides an economical and fast solution for developing personalized surgical equipment tailored to each surgeon's needs and operations [4]. However, costs of 3D bioprinting can be higher for individual units in the early stages of technology development.

The comparative theoretical analysis of traditional manufacturing methods in the medical field and 3D bioprinting presents us with unique benefits and limitations for each approach. Traditional methods provide efficiency in mass production and high reproducibility, while 3D printing offers a greater customization and precision, with promising advancements in scalability and overall cost-effectiveness.

2.5. Recent Advancements

2.5.1. Bioprinting in microgravity

Bioprinting in such conditions is now possible and tested in space stations such as the ISS and are funded by organizations such as NASA and the ESA [41]. Organizations such as the 3D BioFabrication Facility and Redwire Corporation are working on projects which utilize this technology to sustain and fabricate models [41] [42].

Bioprinting in microgravity is beneficial in the creation and fabrication of soft tissues as there are minimal external forces which may cause physical conformations or changes to the printed object. Bioprinting in space shuttles, where effects of gravity are not observed, allows for scaffold-free bioprinting and lower viscosity bioinks [43] [41]. Scaffold-free bioprinting doesn't utilize a medium or a lattice to print bioinks upon, rather the bioink is directly deposited on the substrate, making it a much more economical substitute to terrestrial 3D bioprinting [44]. Living filters can be designed and engineered with specifications that would prove to be much more economic as well as revivify dying ecosystems [45]. Mastering this technology would prove useful in the future in cases of emergencies on missions aiming to colonize or explore outer space [43].

However there are disadvantages to this method such as; it being a lengthy process spanning a few weeks due to the required strengthening of the 3D printed object by self-culturing [43].

2.5.2. Bioprinting with the medium of stem cells

The ability of stem cells to regenerate and its quality of self-renewal hints at the idea of an unlimited source of bioink. Implications of this attribute of stem cells would be in patient-personalized medicine and treatment, boosting and fastening recovery of trauma inflicted area. This technology has the potential to replace organ transplantation, thus increasing the availability of treatment for patients suffering from organ failure. It has applications in the bioprinting of cardiovascular, musculoskeletal, neural, hepatic, adipose and skin tissues [46].

2.5.2.1. Fabrication of Cardiovascular tissues

Due to the lack of resident stem cells in the heart, regeneration of specialized cells in the heart is not possible, thus by utilizing the differentiation of pluripotent stem cells into other cells, cardiac tissue can be fabricated [47].

2.5.2.2. Fabrication of Musculoskeletal tissues

Biomimetic skeletal tissues can be made through printing patterned layers of Ca2C12 myoblasts which have the ability to perpetually differentiate into specialized syncytia found in the myotubes comprising the bones [46].

2.5.2.3. Fabrication of Neural tissues

Bioprinting of neural tissues have seen various advancements in recent years. This includes the fabrication of functional neural tissues and the establishment of neural networks amongt other neural subtypes. This was achieved through bioprinting induced pluripotent stem cells and human embryonic stem cells through a traditional fashion. Due to the usage of scaffolds and molds, neural cells weren’t evenly distributed and there were no neural pathways formed amongst other layers [48].

2.5.2.4. Fabrication of Hepatic tissues

Recent advancements in bioprinting allow for the creation of organs. Deposition of hepatic tissue fabricated using induced pluripotent stem cells onto a bioprinted scaffold can make it biomimetic to the original body part. Allowing for transplantation of scaffolds undergone recellularization and autologous structures as well as future research on the characteristics, causes and specifications of the disease [49].

2.5.2.5. Fabrication of Skin tissues

Skin tissue differentitated from stem cells have the potential to fasten up skin grafting procedures, as well as blend with the microenvironment and surrounding skin tissues in nature. However, there are chances that development of diseases may occur due to the accumulation of bodily fluid. Additionally, due to its recency, this technology is in a premature state of clinical testing [50]. However due to the complexity of bodily tissues including the cellular network, varying cells and cell structures and the frailness of structural integrations, materializing such a 3D printed device proves to be difficult [46].

2.5.3. Integration With AI and machine learning

Artificial Intelligence (AI) proves to be useful while getting insights on the optimality of a virtual design for the proposed 3D printed model. AI can assist in optimizing the printing and manufacturing time taken for the creation of such models by directing the movement and the path of the nozzle such that the most efficient route is adopted. Running the output generated by Machine learning (ML) models, allows for the detection of plausible defects, better constituent materials as the bioink and simulating the proposed model [51] [52]. Additionally it allows for much refined and high-quality models [51].

ML when incorporated with 3D bioprinting helps fasten the analysis of various inputs due to the absence of factors such as overwhelming complexities and fatigue which a human would face. Comparing input such as the internal composition of organic structures and microenvironment of the organic structure to a database of preset procedures, specimens and examples allows for the creation of a virtual model - a proposal of a plausible substitute for the organic structure in theory. An advantage of incorporating ML with Bioprinting is that accuracy and precision increases as throughput increases as a much refined ‘guess’ is developed by investigating similarities [53]. This allows for a much personalized prosthesis rather than a generalized prosthesis offered by manual diagnosis and analysis of the organic structure.

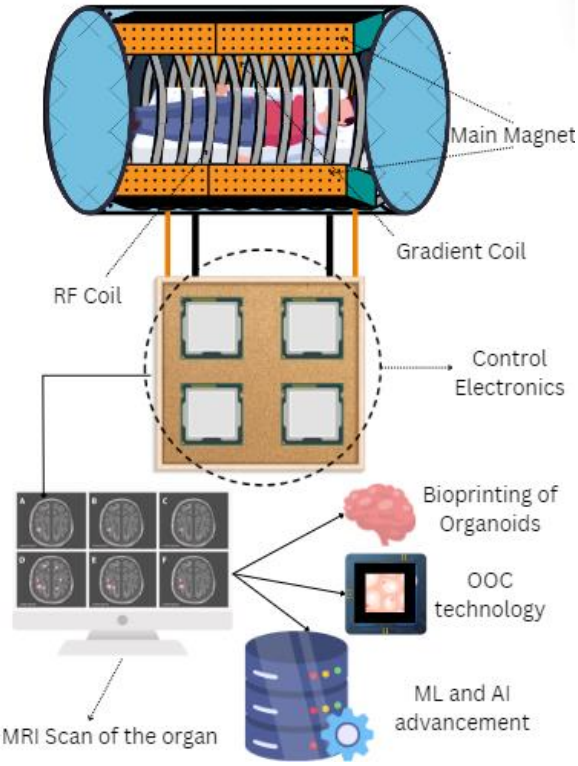


Fig 2: The rotating magnet in the MRI causes a strong magnetic field thus aligning the protons with the field of the MRI. The RF coil and the gradient coil communicate with the gradient amplifiers, RF electronics, pulse sensor computer and the image reconstruction computer to generate a complete scan of the organic body. This can be made into a virtual CAD model using softwares such as Instructable. There are countless applications of this technology such as bioprinting of organoids for research or healthcare, usage in OOC chips, creation of databases, ML programs and AI for advancing the diagnosis and treatment of diseases.

Current challenges with incorporating AI and ML models with bioprinting include the lack of a database consisting of viable solutions, lack of communal knowledge on the subject matter, cybersecurity and dependability of such models, legal and ethical beliefs /constructs, and financial issues for significant research [54].

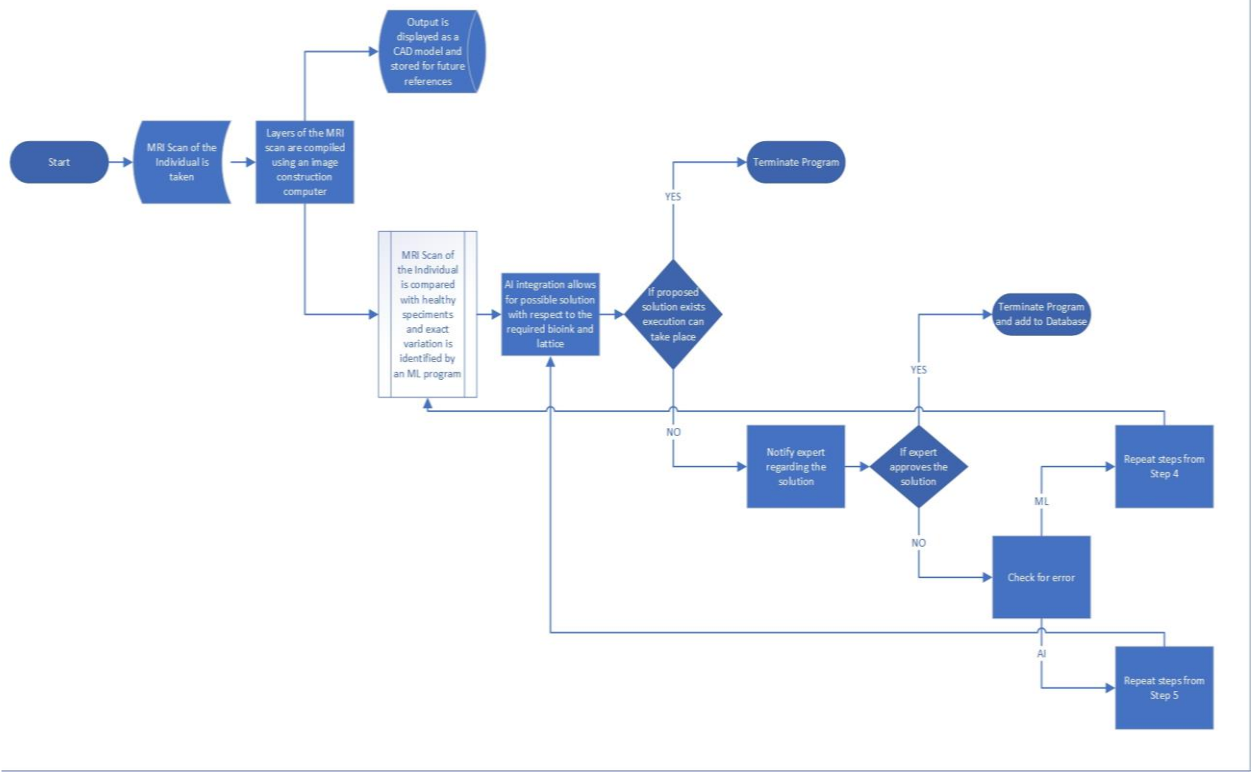
2.5.4. Four Dimensional (4D) Bioprinting

4 Dimensional bioprinting overcomes the primary fault of static 3D bioprinting: the inanimate stature with conditions specific and limited to the original printed object. Thus, enabling the development and creation of complex structures which mimic bodily or programmed functions, respond to stimuli and substitutes for prostheses and failing parts of the body. Bioinks with properties variable to stimuli, printed on certain lattices, allows for such yield of results. 3D bioprinting inhibits tissue-regeneration, an integral property of organic structures achieved through cellular mechanisms, dynamic activities of cellular components and self-initiated response to changes or disturbance of the microenvironment with homeostasis. 4D bioprinting offers a solution with the incorporation of biomimetic stimuli-responsive shape-recovery polymers as a lattice for bioinks, allowing for personalization of medicine and treatment [55].

Due to the premature state of 4D Bioprinting, there are only a few which do not degrade through the process of stimulation, and maintain stability of the structure and function throughout the process of stimulation, additionally the models require to be programmed and mathematically modeled in order to perform functions such as morphing [55] [56].

3. Discussion

3D bioprinting holds the potential to revolutionize the medical industry and the way surgeons operate in numerous ways, bringing about numerous positive transformations! [36] The research conducted a literature review exploring the potential of how bioprinting use in surgical preparation helps physicians (surgeons) better understand complex patient anatomies. The study conducted a thorough research on the principles of bioprinting, bioprinting use in drug discovery, a comparative theoretical analysis between 3D bioprinting vs traditional methods, the future of bioprinting through its integrations with AI and machine learning, bioprinting in microgravity and much more interesting avenues.



This flowchart represents the software/device which would effectively incorporate the entirety of this research article. It is a recursive machine which increases in precision and accuracy with increasing throughput. It operates using an AI and an ML program, which work synchronously in order to create a solution in the form of a 3D bioprinted model which would substitute and match the requirements. These solutions are produced using past case studies and specimens, additionally as it corrects itself, it tends to approach a self-sustainable status and reaches a level of optimity.

Improved preoperative planning and surgical outcomes are some of the positive results generated by 3D bioprinting [37]. Physicians (surgeons) gain a deeper understanding into the spatial relationships between different organs and tissues. With the use of patient-specific models surgeons are able to more carefully and accurately visualize and thoroughly understand complex anatomical structures in three dimensions, which facilitates better surgical navigation while reducing risks of complications during surgery. 3D bioprinting can also transform surgical instruments by allowing for the creation of personalized surgical instruments and implants which are made specifically for each individual patient anatomy, which significantly improves compatibility and efficacy of medical interventions, especially highly complex unusual ones! This results in reduced postoperative complications that may have otherwise occurred [4].

In the realm of drug discovery, 3D bioprinting enables researchers to conduct much more accurate and predictive drug screening assays through the 3D manufactured functional tissue models that closely mimic our human physiology, which can lead to the identification of novel therapeutic agents with higher efficacy and lower toxicity levels [38] [57]. Researchers are also able to replicate the intricate cellular microenvironments found within living organisms, providing a more physiologically relevant platform for drug testing and validation. An example of a successful 3D bioprinted drug is Spritam (levetiracetam), the first 3D bioprinted drug approved by the FDA which is used to treat seizures in patients with epilepsy [58] [59].

Despite the many advantages, 3D bioprinting also presents certain limitations and challenges that need to be addressed [40]. The scalability and reproducibility of 3D bioprinting use for mass production remains a considerable challenge. Further research and development into the reproducibility and the standardization of bioink formulations is required. Some ways to optimize 3D bioprinting's scalability is to improve the time taken for 3D bioprinting processes and introduce quality control systems to check the consistency of quality present in finished products (ensuring consistency and reliability of 3D bioprinted products is essential for their widespread adoption in clinical practice). The establishment of standardized tests and protocols to test performance/quality of bioprinted tissues and organs would bring significant help in terms of regulatory improvements by health organizations. Promotion of ethics around bioprinting practices should be cultivated, ethical considerations such as the patient consent, regulatory frameworks and oversight must be carefully addressed in order for a more ethical implementation of bioprinting technology around the world to take place [60].

Researchers have used many methods to develop agarose-based bioinks for bioprinting. One of the approaches involves the preparation of agarose solutions at selected concentrations, ranging from 1% to 4%, which are mixed with cells and other bioactive components. The bioink is often crosslinked using physical or chemical methods post-printing to maintain its structural integrity. Physical crosslinking methods include temperature-induced gelation, where the printed structure is cooled to room temperature for the formation of a stable gel. Chemical crosslinking uses crosslinking agents such as Geni pin or glutaraldehyde to covalently link agarose chains, increasing the mechanical properties and stability of the printed constructs.

Researchers also have various additives in agarose-based bio-inks to enhance their properties. Like, the addition of nitrocellulose has been shown to improve the printability and mechanical strength of agarose-based bio-inks, their reinforcing effects, and their ability to form strong hydrogen bonds with agarose molecules. The incorporation of growth factors, such as vascular endothelial growth factor or basic fibroblast growth factor, into agarose-based bio-inks has been looked into to promote cell proliferation and tissue regeneration.

Despite the numerous advantages of agarose-based bio-inks, several limitations still exist. One of them is the relatively low mechanical strength of agarose hydrogels, which may lower their ability to support complex tissue architectures. One more challenge is the limited control over the degradation kinetics of agarose hydrogels. Agarose undergoes enzymatic degradation by agarase enzymes produced by bacteria, which may cut its long-term stability in vivo. The diffusion of small molecules through agarose hydrogels leads to degradation in the structural integrity of the printed constructs. Ways to control the degradation rate of agarose-based bio-inks, like modifying the chemical structure of agarose or incorporating crosslinking moieties with tunable degradation kinetics, are needed to ensure the long-term stability and functionality of the printed tissues.

Despite the current limitations, agarose-based bioinks hold great potential for advancing the field of tissue engineering and regenerative medicine. Future research directions may focus on further enhancing the mechanical properties and stability of agarose-based bioinks through the development of novel crosslinking strategies and the incorporation of advanced biomaterials. For instance, the integration of nanocomposites or hybrid hydrogels composed of agarose and other biopolymers may offer synergistic effects, leading to bio-inks with superior mechanical strength and biocompatibility.

Moreover, the development of bioinks capable of recapitulating the complex microenvironment of native tissues holds immense potential for applications such as organoid fabrication and disease modeling. By incorporating bioactive molecules, such as growth factors or extracellular matrix components, into agarose-based bioinks, researchers can create biomimetic scaffolds that promote cell differentiation and tissue morphogenesis. Additionally, advances in bioprinting technologies, such as multi-material and multi-photon printing, will enable the precise patterning of multiple cell types within agarose-based constructs, facilitating the engineering of complex tissues with spatially controlled functionalities.

4. Conclusion

3D bioprinting offers certain leverages over traditional methods, in ways such as: being time and cost efficient, less labor intensive, mimicking the cellular and structural arrangement much more accurately, being personalized to the requirement of the individual, as well as being a transformational tool for surgeons in presurgical preparation and in the making of personalized surgical instruments. Agarose-based biomaterials are chiefly used as bioinks for 3D bioprinters. The quality and the time taken for the end-product depends upon the type of bioprinter being used. Laser bioprinters are regarded for their fineness, whereas inkjet bioprinters are used to save time.

3D bioprinting is used to fabricate drugs with precise dosages and OOC chips in order to gauge their effectiveness [61]. MRI scans can be processed through an image processing computer in order to create personalized tissue models. The 3D CAD model can be further used in surgery, where custom surgical instruments could be fabricated to increase the ease and efficiency [62]. With future advancements, techniques which integrate 4D bioprinting could help simulate the CAD model. This would result in a better prenotion regarding the surgery and a reference to treat other cases with similar nature. Effects of natural phenomena can be leveraged to enhance print quality as well.

The research article approaches 3D bioprinting influence across the medical field in a clear and extensive manner, and provides insights over the plausible future advancements in these fields. It discusses the applications and increasing integration of 3D bioprinting in these fields, the extensiveness of the field with regards to 3D printing and highlights the use of Artificial Intelligence and Machine Learning algorithms in accordance with 3D Bioprinting which is an emerging field [63]. However, this research paper is bound to theoretical suggestions and ideas based on existing research.

Acknowledgements

This research was supported by Incognito Blueprints Pvt. Ltd.

Author contributions

V.D.M.M. Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. P.I. Visualization, Methodology, Investigation, Writing - original draft, Conceptualization. S.B. Methodology, Writing - original draft. R.S. Methodology, Writing - original draft. A.E. Methodology, Writing - original draft.

Competing financial interests

The authors declare no competing financial interests.

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