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Recent Advances in Materials for 3D Bioprinting

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Abstract: 3D bioprinting has revolutionized manufacturing and design. Recent advances have enabled 3D printing of biocompatible materials, cells and supporting components into complex 3D functional living tissues. Inkjet printing, stereolithography (SLA), fused deposition modeling (FDM), selective laser sintering (SLS), and digital light processing (DLP) are prominent methods, each offering unique advantages. Materials used in 3D printing span plastics are PLA and ABS, metals, resins, ceramics, and other biocompatible substances. Scaffolds, crucial for supporting intricate structures during printing can be removable or water-soluble. In tissue engineering, the exploration of materials, nanoscale properties and the advancements in tissue decellularization methods offer opportunities for generating precise extracellular matrix (ECM) scaffolds with applications in regenerative medicine and bioprinting. Understanding natural compositions, striking a balance between removing cells and preserving structural integrity, and handling possible toxicity in cell culture on decellularized substrates are some of the factors to take into account. This review article explores the diverse materials used in 3D bioprinting and the role of scaffolds in the process.

Keywords: 3D printing, 3D printing materials, Nanoscale properties, Scaffolds.

I. Introduction

In the realm of modern manufacturing and design, 3D printing has emerged as a transformative technology, offering unparalleled flexibility and precision. This article delves into the intricacies of the 3D printing process, with a specific focus on the diverse materials utilized and the critical role played by scaffolds in achieving complex and structurally sound objects [1].

The advent of 3D printing, also known as additive manufacturing, has revolutionized traditional manufacturing methods. Unlike subtractive processes that involve cutting or molding materials, 3D printing builds objects layer by layer from digital models. This not only allows for unparalleled design freedom but also opens up a myriad of possibilities in various industries, including aerospace, healthcare, automotive, and consumer goods [2].

One of the key aspects explored in this is the spectrum of materials employed in 3D printing. From the versatility of thermoplastics like PLA and ABS to the strength and resilience of metals such as titanium and stainless steel, the article delves into how material selection influences the properties and applications of the final printed objects. Furthermore, it also investigates the use of resins, ceramics, and biocompatible materials, showcasing the diverse range of industries that benefit from these cutting-edge materials.

A pivotal component of the 3D printing process is the strategic use of scaffolds. Scaffolds are temporary structures that provide essential support during the printing of complex geometries or overhanging structures. The article delves into the types of scaffolds, including removable supports and water-soluble structures, and explores their significance in ensuring the integrity and success of the printing process [3].

II. Methods of Bioprinting

Although all bioprinting techniques result in aresult that are comparable, they can be divided into groups according to how the printing is applied. The majority of bioprinting techniques can be divided into four groups:

1. Inkjet-Droplet based bioprinting
2. Extrusion based on bioprinting
3. Laser-based bioprinting
4. Bioprinting using stereolithography (SLA)

Commercial 3D bioprinter manufacturers have assessed the characteristics needed by the optimum bio-printing technique as the sector has grown. The ideal bio-printer, according to industry experts, should have the following qualities:

- A significant amount of freedom of motion,
- Outstanding accuracy and resolution,
- Fast motions,
- The dispersion of several bio-inks at once,
- In reference to accessibility,
- Suitable dimensions,
- Ease of sterilization,
- The capacity for complete independence,
- Versatility and affordable price.

This entire list of qualities is yet to be achieved through several modern bioprinter technologies, and many of them still adhere to the same standard process [4]. CAD offers a toolpath layout to control dispensing and moving systems. The picture makes it clearer that, irrespective of modality, motion directs the dosage device via an x, y, and z contact plane. The general bioprinting procedure is broken down into two parts and into five broad actions. An engineer first creates the print's geometry and then manually confirms the print's viability. The scientists next choose the suitable biomaterials, such as cells and bioactive, before inserting the printer with bioinks. As indicated earlier, computer automation technology uses a variety of languages, including AutoCAD, G-Code, and LabVIEW, to control the printing process. The last actions are building a structure using bioinks deposited on top of it and using imaging methods like microscopy to verify and validate the tissues. The intended use of any bioprinter determines how post-production processing, such as sterilization, is carried out. We go into detail about each bioprinting technique in the following subsections:

A. Inkjet-Droplet Based Bioprinting

Due to the remarkable similarities between 3D and 2D inkjet printing, inkjet-based bioprinting was the first bioprinting method. As a result, this technique is thought to be the most well-known of the three primary printing techniques. Hydrogel pre-polymer solution-encapsulated cells are utilized to make bio inks for inkjet printing. Then, a standard ink cartridge has been filled with this bio ink. After that, the printer head is connected to the bioink-filled cartridge, and a scaffold with a high cell density is printed via electronic coding made with CAD software. Bio ink viscosity usually ranges from a limit of 0.1 Pa/s; higher viscosity can cause clogging of the printer nozzles. The use of bio-ink cartridges is because they are easy to adjust; nevertheless, the once-homogenous mixture of bioink eventually separates and the cells inside tend to sink to the bottom of the cartridge. This subsequently raises the viscosity ofthe bioink and leads to clogging problems [5].

B. Bioprinting-Based Extrusion

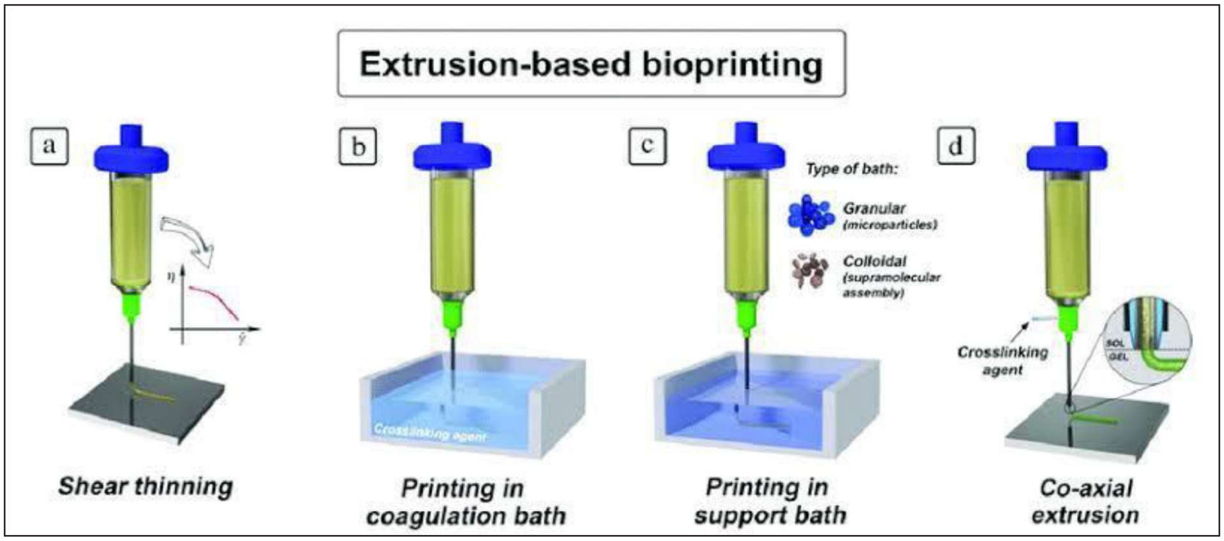

Fig. 1: Different Extrusion-Based Approaches for Bioprinting Structures

Although it was not the first bioprinting method produced, extrusion-based bioprinting is currently the most widely used due to the ease of use. These bioprinters can also quickly produce 3D structures on a larger scale than any other technologies. Inkjet printing is the main source of ideas for extrusion-based bioprinting. It originally emerged because of the more viscous bio inks that clog inkjet print heads. Unfortunately, almost any hydrogel pre-polymer solution, irrespective of viscosity, can be printed with extrusion bioprinters, together with the bioinks with many cells and a thin coating of a laser-absorbing materials, consisting of gold or titanium, and a laser-transparent foundation, like glass or quartz. The bioink layer or film is applied to the metal absorbent material of the ribbon when the cells have been suspended in a liquid or gel. Considering it only serves by collecting the bioink as it falls from the ribbon, the backing material is the component with the most specialization. Receiving surfaces usually represent biopolymers or other substances intended to encourage bonding and growth of the cells. Whenever pulses of laser light emit the ribbon across a small area, condensing the donor tissue layer there, a very high-pressure bubble appears at the bioink connection. This bubble pushes the biological ink droplet as it drops from the ribbon onto the receptive material. At that, the substrate starts cross-linking [6].

The most cutting-edge technique for bioprinting is laser-based, and as a result, it has several benefits. The absence of nozzles and the use of a non-contact printing technique that lowers the danger of contamination are the main advantages. Similarly, to extrusion-based bioprinting, and printing with laser light can produce massive structures that resemble a great similarity to structures found in life. To produce prints with a very high degree of resolution, one can adjust the thickness of the biologic layer, the viscosity of the bioinks, the amount of energy of the light from the laser pulse, the water-holding capacity of the material being printed, the printing rate, and the organization. The cell viability of laser-based approaches is often more than 95%, which is the best of the three main methods because there is no mechanical stress placed on the cells due to the lack of contact. Furthermore, laser printers not only produce more viable cells but also resolve the viscosity of bioinks problems experienced with inkjet and extrusion-based printers. Finally, laser-based technologies offer substantial manufacturing machine learning, and reproducible outcomes while maintaining quality and control over the creation of several different structures that have large cell counts counts [7].

However, compared to other processes, laser-based printers are more expensive, and their extensive control systems demand a high degree of understanding and expertise, which limits their popularity and availability in business and education. The high cost of production limits both the size of laser-based bioprinters currently in use as well as further study into the process. A few of the many basic issues that need to be solved include how laser exposure affects living cells, the effects of the droplet dimensions, excellence, frequency, difficulty, and pulsed time on pattern value, and how results are affected by the physical stabilization of cells in a solution. There are still numerous basic knowledge gaps due to a lack of study.

C. Laser-Based Bioprinting

Biomaterials are deposited onto surfaces using lasers in laser-based bioprinters. They were developed using laser direct-write and forward the induced laser transfer (LIFT). Although LIFT was first created for direct writing of metals, its impact enables the precision printing of living cells and other biological entities at an exceptionally fine scale, reaching down to the pico-micro level with remarkable accuracy and resolution. The substrate, coating through ribbon deposition and the use of pulsed laser technology are employed in the process.source make up the three primary components of the printer composition. The printer's energy comes from ultraviolet (UV) or wavelengths near UV, which are used in the nanosecond laser pulses. Additionally, ribbons are put together in two sections. To get over these restrictions, Wang et al created and tested a STA system that used visible light. Parts that were sold commercially were used to make the system. In the end, the researchers discovered that the printing of light-curable hydrogels was aided by the system's incorporation of an infrared ray water filter. As a result, more materials could be printed with a high degree of resolution (50 um) and about 85% cell viability [8].

D. Bioprinting using Stereolithography (SLA)

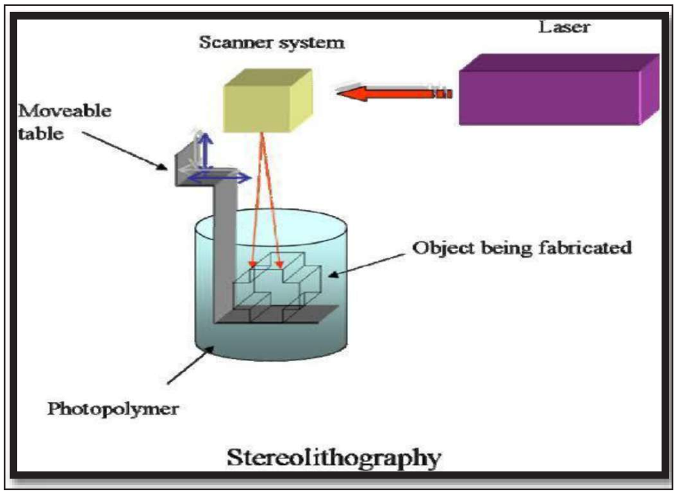

Fig. 2: Schematic Representation of STL

Stereolithography (SLA) is another well-known process that changed from a 2D direct writing printing process. However, like other bioprinting processes, it is considerably more advanced. The process of stereolithography is nozzle-less and freeform. Its main components are liquid photosensitive materials that dry when heated by laser light. Initially, UV light played a role in technological development. However, this method was abandoned due to its harmful effects on DNA. Digital Light Processing (DLP), a technique related to stereolithography and utilized in bioprinting, employs a digital micromirror array to control the brightness of projected light, inducing polymerization and cross-linking. Furthermore, the printing occurs in a layer-by-layer fashion. Each layer's printing time is distinct and remains independent of complexity or size, relying solely on thickness. This characteristic renders it a time-efficient approach for various desired prints. The entire process is overseen through top-down digital projection.

Stereolithography emerges as a highly practical approach for 3D bioprinting, particularly as our comprehension of biomaterials expands, leading to the availability of a growing number of photo cross-linkable polymers. With a precision rate exceeding 90%, SLA stands out for its superior precision in fabrication and maintenance of cell viability. Despite these advantages, certain limitations persist, primarily stemming from the scarcity of biocompatible and biodegradable polymers. Photo-curing agents, for example, may pose toxicity risks to cells, potentially leaving behind a residue harmful to tissue viability [9].

It is crucial to assess the bioprinter's ability to replicate the physiological conditions of in vivo cells effectively, ensuring the preservation or development of in vivo functionality within microenvironments. Successful emulation of native tissue requires the simultaneous printing of multiple cell types, necessitating the development of various bioinks. Consequently, the challenge lies in the formulation of multiple bioinks to accommodate the diverse needs of different tissue types and create constructs that closely mimic native tissues.

In different scenarios, a preference is given to cells with a faster doubling time, primarily because they need to attain a satisfactory density in vitro initially. The inclusion of growth factors or signaling molecules in a bioink rich with stem cells proves effective in reducing the quantity of bioinks and cell types required, promoting differentiation. However, the precise distribution of these growth factors and signaling molecules is crucial. Stem cells emerge as the most viable candidates for live cell printing due to the factors mentioned above. There are instances where an abundance of growth factors is unnecessary for stem cells in a bioink.

In contrast, when considering mechanical stresses generated by extrusion and inkjet bioprinters, particularly evident in mesenchymal stem cells, laser-based printing stands out by preserving the multipotency of stem cells. The three primary types of stem cells utilized in 3D bioprinting for targeted differentiation are mesenchymal stem cells (MSC), embryonic stem cells (ESC), and adult stem cells (ASC) [10].

III. Materials and Scaffolds

The 3D printing processes were originally developed for non-biological uses and were mainly targeted for use in manufacturing procedures that involved organic solvents. Applications in this context often involve the deposition of metals, ceramics, and thermo-plastic polymers. However, these materials typically necessitate elevated temperatures or involve crosslinking agents that are incompatible with biological materials and living cells. Consequently, a significant challenge in the domain of 3D bioprinting has been the identification of materials that not only interact effectively with biological substances and the printing process but also confer the necessary mechanical and functional properties to tissue structures. Currently, materials used for repair and regeneration in regenerative medicine predominantly originate from either naturally occurring polymers like alginate, gelatin, collagen, and chitosan, often sourced from human or animal tissues, or synthetic compounds such as polyethylene glycol (PEG112-115), fibrin, and hyaluronic acid.

Although polymers made from natural materials have characteristics comparable to the natural sources bioactivity of the human extracellular matrix (ECM), they give opportunities for biomedical procedures like 3D bioprinting. The advantage of artificial polymers is that the way they behave may be changed according to uses. The usage of synthetic polymers is hampered by their low biocompatibility, hazardous breakdown products, and mechanical property loss during degradation. Nevertheless, the hydrophilic and absorbent properties of synthetic hydrogels make them appealing for use in 3D bioprinting and regenerative medicine applications. Natural polymers offer benefits for applications in tissue engineering, including 3D bioprinting, due to their similarity to the organic bioactivity of the human extracellular matrix (ECM). The advantage of synthetic polymers lies in their capacity to be tailored to specific uses by adjusting their physical characteristics. The usage of synthetic polymers is hampered by their low biocompatibility, hazardous breakdown products, and mechanical property loss during degradation. Nevertheless, the ease of manipulating the physical attributes of synthetic hydrogels during synthesis adds to their appeal for utilization in 3D bioprinting and regenerative medicine applications. Synthetic hydrogels, being hydrophilic and absorbent, present an attractive option for 3D bioprinting and regenerative medicine due to the straightforward manipulation of their physical properties during synthesis. As the spectrum of biological materials for medical applications continues to expand, the criteria for desirable characteristics in printable materials have become more intricate and precise. Crucial attributes include appropriate crosslinking mechanisms to facilitate bioprinter deposition, favorable swelling characteristics, short-term stability, and long-term biocompatibility for transplantation. Ensuring short-term stability is imperative to preserve the initial mechanical properties, preventing the breakdown of tissue structures such as pores, channels, and networks. bio printed tissues need to be malleable during in vivo development, enabling the formation of structures guided by cellular and physiological needs [11].

The materials must especially facilitate cellular connection, growth, and function. some of the key features are printing ability, bio compatibility, loss of kinetics and waste products, structural and fundamental qualities, and material biological mimicry are in details below.

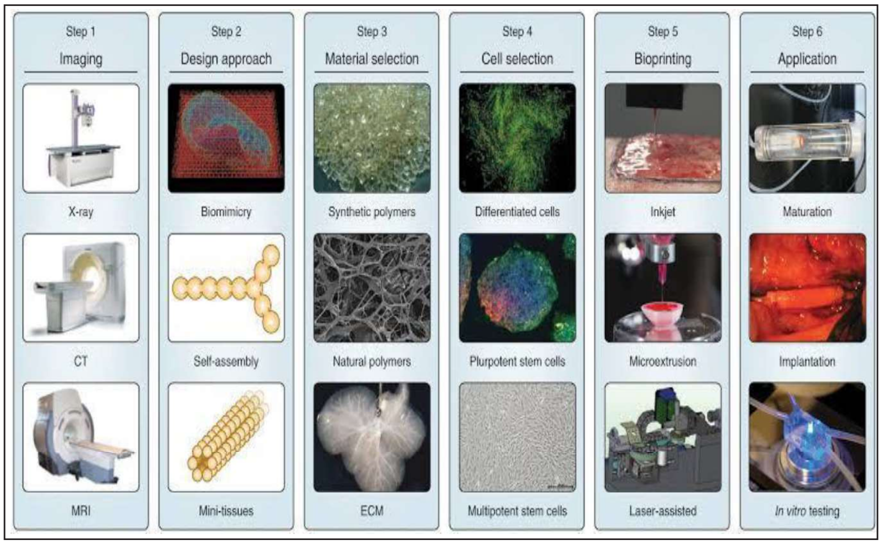

Fig. 3: Materials and Scaffolds

A. Printing Capability

The ability of a suitable material to be precisely and accurately deposited with the required spatial and temporal control is a crucial feature. Certain bioprinting technologies, like inkjet, have restrictions on the viscosity of the material, while others, like micro extrusion might require the presence of crosslinking or shear-thinning characteristics in the material. Processing parameters play a crucial role in defining the shear stress experienced by cells and the duration required for material deposition to construct a 3D structure, often assessed using tools like nozzle gauges. In the case of inkjet printing, it is crucial to have materials that exhibit swift crosslinking times to enable the formation of complex 3D structures through layering. On the other hand, micro extrusion can efficiently integrate highly viscous materials to preserve a three-dimensional shape after deposition, with the ultimate crosslinking taking place after the fabrication process.

The preservation of cell viability during the printing process can also influence the choice of material. Both LAB and thermal inkjet printing require the material to be heated locally to deposit cells. Materials that can protect the cells during delivery or have low thermal conductivity after printing, strengthen cell viability and function. Inkjet bioprinting can be achieved, but the post-printing cell viability may significantly differ based on printer specifications, material characteristics, resolution, and cell types. Research typically states that cell viabilities exceed 85%, that viability ranges between 40 and 80% are reported by micro extrusion printing research, and that viability exceeds of 90% 42, 54, 80, 104 in LAB research [12].

B. Biocompatibility

The requirement for biocompatibility has shifted from requiring an implanted material with the development of tissue engineering implanted materials being able to coexist with the endogenous tissue without causing any undesirable local or systemic effects in the host anticipated to either actively or passively cause the host118 to experience desired effects. When it comes to bioprinting, biocompatibility involves anticipating an active and manageable input to the construct's biological and functional elements. This might involve communicating with endogenous supporting healthy cellular activity and facilitating mechanical or molecular signaling systems in tissues and/or the immune system, all of which are necessary for a successful transplant and its proper operation, which are all necessary for a transplant to be successful and to properly function [13].

C. Loss of Kinetics and Waste Products

When a material scaffold degrades, the enclosed cells release proteases and subsequently generate extracellular matrix (ECM) proteins, contributing to the definition of the emerging tissue. Understanding and controlling the kinetics of materials is crucial for maintaining and enhancing their quality. Degradation has multiple facets that need to be considered initially, it is imperative to control the degradation rates, ideally aligning them with the cells' ability to substitute the materials with their own proteins, forming the extracellular matrix. This is difficult because, when a material breaks down, cellular components may be able to replace it more easily than a material with appropriate mechanical and functional characteristics for that tissue. The fact that degradation waste products often indicate a material's bio compatibility makes them essential as well [14].

The breakdown products need to be safe, easily absorbed by the body, and eliminated quickly. Small proteins and molecules, as well as nonphysiologically pH, temperature, and other variables that may be harmful to the survival of cells and function, can all be considered hazardous substances. For instance, certain initially inert large-molecular-weight polymers may break down into molecules or polymers which human cells can recognize, causing inflammation and other adverse effects. Especially in the development of products through tissue engineering methods, it is crucial to consider the swelling and contraction attributes of these materials. Excessive swelling in materials may lead to the absorption of fluid from neighboring tissues, while contraction might result in the closure of pores or vessels essential for nutrient delivery and cell movement.

Furthermore, it's essential to understand what occurs when applying different materials with different contraction or swelling properties because doing so may cause the result in creating to change shape or lose layer honesty [15].

D. Structural and Fundamental Qualities

If a substance is necessary as an anchoring point for mechanical leverage, to resist or produce specific forces, or to maintain a three-dimensional structure, then the construct must be maintained for it to continue to function. The selection of materials must be meticulous, aligning with the specific structural and mechanical prerequisites essential for diverse tissue types, encompassing bone, skin, and liver. One workaround for this constraint involves employing sacrificial materials that can provide the required structural and mechanical attributes for a predetermined duration.

When printing, this sacrificial material can be used to enable enough crosslinking to take place in the construct or conversely, could integrated into the construct, providing this purpose until the materials produced internally are able to do it adequately. When using this method, it's important to create a material with precise structural and degradation characteristics while preventing harmful degradation of waste products or possible foreign body reactions in the structure [16].

E. Material Biological Mimicry

Only recently has the significance of biomimicry for biocompatibility been investigated. The capacity to include biomimetic elements in a bio printed structure can have an active impact on both natural and external tissues' bonding, emigration, development, and its function. It has some solid reputation materials that significantly affect cell attachment. These principles, encompassing cell size and shape, can play a role in governing the growth and differentiation of cells within a scaffold. Surface ligands facilitate cell attachment and proliferation on a material substrate. Furthermore, the presence of minute structures such as ridges, steps, and grooves impact cytoskeletal assembly, cell attachment, and proliferation. The 3D environment within a tissue-engineered construct can also influence cell shape and the differentiation process. The nanoscale properties of materials can impact various cellular processes, including cell adhesion, cytoskeletal condensation, introductions, cell movement, presentation of antibodies, and regulation of intracellular signaling pathways. These pathways, in turn, govern gene expression and transcriptional activity. To produce materials with physiological functions using biomimicry, a fundamental understanding of the naturally occurring composition and spatial arrangement of extracellular matrix (ECM) elements in the tissue of interest is imperative [17].

Advancements in recent tissue decellularization techniques offer the potential for generating thorough extracellular matrix (ECM) scaffolds, facilitating in-depth examination of ECM compositions, spatial distribution, and biological functions. The method entails breaking down and removing the cellular components of a tissue, usually through perfusion with distilled water or mild detergents, while maintaining the unique extracellular matrix (ECM) characteristic of that tissue. The promise of employing bioprinting to create precise extracellular matrix (ECM) scaffolds is evident in potential applications within tissue engineering and regenerative medicine. However, achieving a balance between removing cellular components and preserving complex vascular and other tissue structures poses a challenge in the tissue decellularization process. Furthermore, cultivating cells on decellularized substrates has shown potential toxicity in tissue scaffolds, possibly attributable to the retention of decellularization detergents within the ECM. Mammalian systems are rich in ECM diversity, comprising over 300 ECM proteins, numerous ECM-modifying enzymes, growth factors binding to ECM, and various other ECM-associated proteins. Growth factors that bind to extracellular matrix and other proteins linked to extracellular matrix, collagen, pro-teoglycans, and glycoproteins are the most common and well-understood proteins. These proteins offer durability and space-filling activities, bind growth factors, control protein complexes, encourage adhesion of cells take part in signaling within cells, and might serve additional purposes. Taking a "scaffold-free" approach to bioprinting could be a fascinating way to approach the idea of material by biomimicry. Bio printed cells create and deposit the extracellular matrix cellular spheroids that self-assemble could create an ECM environment that is ideal for their own purpose. constructing these dynamics. Adding ECM mechanisms to materials allows for even more control over the behavior of cells. The challenge is to find out how to combine these materials into bio printed constructs and still preserve suitable degradation times and waste products, as well as a knowledge and managed the biological impact on the construct's structure and function [18].

IV. Conclusion

3D printing, a transformative technology, encompasses various methods and types for creating three-dimensional objects. Inkjet printing, stereolithography (SLA), fused deposition modeling (FDM), selective laser sintering (SLS), and digital light processing (DLP) are prominent methods, each offering unique advantages. Materials used in 3D printing span plastics like PLA and ABS, metals, resins, ceramics, and biocompatible substances. Scaffolds, crucial for supporting intricate structures during printing, can be removable or water-soluble. In tissue engineering, the exploration of materials' nanoscale properties and the advancements in tissue decellularization methods offer opportunities for generating precise extracellular matrix (ECM) scaffolds with applications in regenerative medicine and bioprinting. Considerations include understanding natural compositions, achieving a balance between cellular removal and structural preservation, and addressing potential toxicity in cell cultivation on decellularized substrates.

V. Conflict of Interest

Authors declare no conflict of interest.

VI. Animal Ethical Approval

Not Applicable.

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