



## **Survey Paper on 3-D Bio-Printing for Hard Tissue**

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### **ABSTRACT**

Three-dimensional bioprinting is basically for creating or formation of the natural developing which includes allocating cells into the biocompatible stage by applying a liberal layer-by-layer for dealing with the tissue-like three-dimensional(3D) structures. As we know each muscle in the body is made out from various types of cells, various advances for the printing of these headsets will differ in their size to maintain assurance on steadiness and reasonability of headsets which exists within the Assembling process. There is a huge exploration going on bioprinting innovation and discovering its possibility as a primary upcoming hotspot for injecting and complete transplantation. Manufacturing organs like instance liver and kidneys which are made by the bioprinter have arisen to meet the need crucial components that result in the human body, such as veins, tubules and for the development of billions and billions of cells required for these organs. This survey paper outlines the current most noteworthy advancement in bioprinting innovation, depicting the expansive scope of bioprinters and bio-ink utilized as a part of preclinical investigations. Refinements between the types of laser-based bioprinting, expulsion-based bioprinting, and inkjet-based bioprinting innovations again proper and prescribed bio-inks are talked about. Also, the current most astounding improvement in bioprinter innovation is looked into with a direct the business perspective.

**Keywords:** 3-D Bio Printing, Hard Tissue

### **1. INTRODUCTION**

The bone tissue is made up of two different assemblies cancellous and cortical bone is called Osseous. Cancellous which is also called the inner

portion of the bone which is soft in nature and it consist of 50–90 volume absorbency. Though, cortical bone is the solid outer layer of bone with less than 10 volume absorbencies. Both of them listed above will experience dynamic remodelling, maturation, a variation that is controlled via interactions among the three types of cells they are osteoblast, osteocyte and osteoclast [1]. Osteoblasts are mainly in charge for formation of new bone and osteoclasts are in charge for the reabsorption of old bone. This type of active process which relating osteoclasts and osteoblasts cells is acknowledged as bone remodelling. This does maintaining a healthy bone. Bones are very important to some living creatures and it is famous for its self-healing capabilities. large-scale bone defects are major in number and they cannot be healed completely by the body itself [3], and in majority of the cases, external interface is necessary and much needed to restore the normal operations. There are different treatments, for instance like autografts (Where bone is taken from the same person's body) and allografts (Where bone tissue is taken from the infected donor), the bone tissue engineering focuses on the approaches to create and restore bone to maintain its purpose is becoming popular.

Effective utilization of this i.e, engineering on bone tissue can maintain a strategic distance from challenges identified with other treatment alternatives including distinctive materials, for example, allografts, autografts. Aside from this material issues, a reasonable comprehension of science including cells, EMC-Extracellular Matrices and development factors are urgent in bone tissue designing [7]. Platforms are a fundamental all bone tissue designing. Platforms are 3 dimensional biocompatible structures which are capable to imitate the Extracellular

Matrices properties, (for example, helping mechanical, cell movement and protein creation from biological and mechanical establishments) give a layout to cell connection and stimulate bone tissue arrangement [5]. For the ceaseless in growth of bone tissue, combined absorptive is critical.

Pores which are Open and interconnected enable supplements, particles to pass to all internal portions of a platform to encourage cell growth, vascularization, in like manner squander material evacuation [4]. Pore volume which panels the penetrability supplements to the framework and their existing powered properties. Porousness in PCL (poly-e-caprolactone) expanded with huge pore size and realize improved bone recovery, vein penetration, and compressive quality while other parameters were kept in the similar manner [13]. Away from organic execution, the underlying mechanical characteristics and quality of debasement frequency must coordinate to the host tissue for the ideal bone mending [14]. Since higher porosity expands surface territory independently volume, the energy of biodegradation for platforms may affected by divergent pore constraints. Biodegradation concluded a cell intervened process compound disintegration are together vital to learn settled patch-up and platform supplanting with a new bone with no remainder [8]. Pore estimate additionally assumes a vital part of ECM creation and association.

PDLLA (PolyD, L-lactic Corrosive) frameworks with pore measure 325 and 420 mm propel efficient collagen I arrange; while, the little pore size of 275 mm keep the human osteosarcoma-inferred osteoblasts to deliver, multiply and separate useful ECM [12].

Other than science, pore estimation, pore capacity, mechanical quality are essential bounds which characterize platform's execution. At a beginning period, ingrowth of the bone occurs at the fringe of frameworks having negative slope in mineralization toward the inward parts [4]. A base pore estimate in the vicinity of 100 and 150 mm is required for bone development [4]; be that as it may, upgraded bone arrangement and vascularization are accounted for frameworks with pore measure past 300 mm [9]. The quality debasement energy of permeable platforms is very influenced by pore size, geometry, and swagger introduction as for the stacking course [15]. At long last, surface properties, for example, science, surface charge, and geography additionally impact

hydrophilicity and as a decision cell– material associations for bone tissue ingrowth.

## 2. PROBLEM STATEMENT

The Organs and Hard tissues present in the human body incorporate teeth, bones, and ligament, comprising of firm interesting kinds of cell and generous natural and inorganic extracellular frameworks (ECMs). For instance, the bone which is made from osteoblasts and calcified ECMs, in which the larger part inorganic ECM. The tooth is another type of exceptionally hardened hard tissue. It includes cementum, dentin, lacquer, and endodontium. creation of the hard tissues and organ alternatives (called as inserts, joins, prostheses, biomaterials, forerunners, and analogs) is defined as a vital piece of the regenerative solution. Among which the manufacture of tissue repairing resources has begun before and medical applications are more and more effective [11]. An uncommon need and necessary of hard tissue and tissue replacements is that they need a high substance of mineral ECMs having solid mechanical properties. Especially, biomaterials, which have been utilized much of the time as organ and hard tissue inserts, have experienced a few advancement stages, for example, latent business items, cell-loaded hydrogels, no bioactive frameworks, and pre-outlined activity composites. Also, some inflexible organs, for example, ears, and nose have complex bent sides which need precise handling advancements to produce. In this way, the improvement of new organ substitutes and hard tissue and appropriate physical and natural capacities in view of the bionic standards is a vital territory of hard tissue and organ building.

The primarily preferred standpoint of 3D bioprinting innovations in organ designing and huge hard tissue is their ability to create composite 3D questions quickly from PC demonstrate with changing inside and outer constructions, for example, obey to procedures. These mind-boggling 3D items can be either tissue designing permeable platforms, biomaterial or cell complexes, homogeneous materials, or different muscle contained organs. In the wake of printing, A permeable 3D platforms may embed or can be planted with autologous tissues fill in as osteoconductive formats expansive tissue engineering. In a perfect world, new tissue frames along the adhere to procedures amid the platforms debase gradually to the human body [2]. The cell compounds may utilize as a part of the vivo or vitro for huge rigid tissue developmental study. homogeneous materials i.e,

tissues of same kind may utilize for substantial hard tissue imperfection restoration. While different tissue containing organs will be utilized to tweaked organ designing and replacement. As of now, here is an extensive variety of materials can be utilized for 3D printing forms. At present, there is an extensive variety of resources which have utilized for 3D bioprinting forms. For instance, metallic joints which were made by the 3D printed are impressively lighter in weight when compared to the ones created by regular strategies. With the adhere to procedures, the inserts can stay longer time in the body than customary embeds because of the blend of the inserts with host tissue. Hard tissue can develop effortlessly to the adhere to procedures and upgrade the restoration impacts. Along these lines, engineered polymer created platforms comparative substantial possessions as normal genuine bones has broadly inquired about. Benefits of the manufactured platforms is that distinct to metallic inserts carry on impartially in X-beam gear [4]. It is presently conceivable to remake a framework of a jaw or an ear that precisely mirrors patients vast muscle, organ shapes in light of the pictures procured by attractive MRI reverberation imaging or CT modernized tomography filters specifically from the patients. The predefined use procedures directly affect the results of the hard tissue and organ repairs.

### 3. BACKGROUND WORK

#### 3.1. Bioprinting Materials / Bio-inks

Mirroring the local tissue engineering and structure utilizing 3-D bioprinting approaches is very testing, since adjusting the physical and compound prompts of the phone facilitating biomaterials need comprehension of cell ecology and cell ECM 189 cooperation [2]. A Built 3-D micro-environment can be accomplished by using normal (e.g., hyaluronic corrosive, hydroxyapatite, alginate, collagen and fibrin) and manufactured (e.g., polycaprolactone (PCL), PLA polylactide, PGA polyglycolide), PLGA (poly lactic-co-glycolic corrosive) and polyethylene glycol (PEG)) polymers half and half biomaterials that union regular and engineered materials.

##### 3.1.1. Natural Materials

Physical and synthetic creations of the common hydrogels may adapt by the target tissues and cell types [7]. The Physical properties of biopolymers, for example, solidness, consistency, and porosity assume

a basic part of the continuation and usefulness of produced construct [2]. Most of the cells composed with hydrolyze characteristic and hydrogels and can discharge their own respective particular cell grid, also permitting the space for cell development and relocation. In addition, these frameworks are tend be intended for contain tissue particular development elements for example, changing development like factor-beta(TGF- $\beta$ ), non-sensitive layer of the skin called epidermal development factor (EGF), insulin development factor (IGF) and also grid metalloproteinase (MMP), where these help to re-establish the substance signs of the microenvironment. Mainly one of the confinements of regular hydrogelly is the clump to-bunch changeability, which might influence the approval of the designed microenvironment as various clusters might have marginally unique composition [2]. The Collagen-and fibrinogen-based hydrogels are normally determined grids are generally utilized as a part of tissue engineering Collagen write I am the most bounteous segment of the local ECM and give an ideal 3-D condition for cell grip and proliferation[11]. Photocrosslinkable gelatin methacrylate (GelMA)- based joins natural highlights allowing proteolytic degradation and integrin-interceded cell attachment. GEIMA which is comprehensively utilized as a part of designing fake tissues because of its simplicity of control and cross photo linking properties. Alginate is an exceptionally biocompatible characteristic polymer that can be effectively crosslinked in the calcium arrangements; in this manner, frequently utilized as bio-ink in 3-D bioprinting applications.

Age of 3-D manufactured tissues can likewise be accomplished by using reconstituted storm cellar films from the mouse tumors like Cultrex and Matrigel into the bioprinting process [10]. Storm cellar films are made out of ECM maintaining proteins like laminin, fibronectin, that assume critical parts in cell attachment and spatial organization [8]. These characteristic films can be connected in implanted and overlay culture for advance 3-D cell associations. Presenting cellar layer in a bioprinting polymer arrangement has appeared to support the geometrical design and upgrade function of the cell [6].

##### 3.1.2. Synthetic and Semi-Synthetic Materials

For defeating downsides of naturally determined bio materials, for example, group tobunch changeability,



a study on semi-manufactured and engineered bio materials has been built. The particular kind of grids bargain a significant option as they have local ECM parts composed with tuneable substantial properties, bringing about better reproducibility and likeness between various investigations. In this way, biocompatible engineered polymers, for example, PLA, PCL, PGA, PLGA, and PEG, are every now and again utilized as a part of bioprinting applications[1] Biomimetic PEG hydrogels can be blended by means of the fuse of an assortment of attachments from oligopeptides to entire development elements re-establish grid inferred biochemical flagging. For instance, the consideration of arginine–corrosive glycine–corrosive aspartic–corrosive peptides empower integrin-intervened tissue attachment advances movement. Notwithstanding, engineered networks are not as organically applicable as normally determined 3-D lattices, and don't contain the flagging particles gave by local ECM [2]. self-amassed peptide hydrogels are likewise generally used to create 3-D microenvironments required for the cell philosophy. Peptide frameworks made up of peptide arrangements it can enable platform to self-gather under firm physical circumstances, allowing tissue embodiment in hydrogel [8] for example, BDPuraMatrix is type of crossbreed peptide hydrogel [3]. The structure of BDPuraMatrix is same as like additional semi-manufactured of hydrogels which holds 99% of water and just 1% of amino acids in it. Such designed framework permits switch over the piece of development factors, ECM proteins, cytokines and tissues. Among many ways which is the latest ways deal with building a local like 3-D microenvironment to utilize tissues that can create common ECM [4]. system depends on the introductory planting method of permeable unite with death customized conciliatory tissues can discharge ECM and prompted death of cells[4]. The deaden unite can be additionally put away as an off the rack item till planted with autologous cells

#### 4. TECHNIQUES

3-D bioprinting is encountering a fast change from essential research in scholastic labs to a developing industry because of its potential business esteem in wide fields including pharmaceutical revelation, customized solution, and tissue transplantation. The market size of 3-D printing in 2012 was around \$2.2. billion, now it is relied upon to reach the worth of \$10.8 billion by 2021. In the year 2014 itself there are

excess of 30 organizations global taking occupational specifically identified with 3-D bio printing and bio printed items. Items and administrations gave by these organizations are centered around 3-D bioprinters and bioprinted platforms. More than seven sorts of 3-D bioprinters are accessible in the market, which targets R&D clients in both scholarly establishments and biotech/Biomed organizations.

- Rapid prototyping – essential for the one by one impeachment or removal of absurd number of cells required for the formation of thick tissues
- High precision –precision is the utmost familiar feature from the bioprinting scheme. The skilled accuracy is majorly used in tissue engineering

High resolution –Printing system which is having the determination is decent sufficient to be utilised for this persistence.

- Computer control – it is the most and mainly required component for controlling a number of processes. Without any computational regulation it might be terrible to switch the number of processes to be completed in this procedure.

The consequence of bioprinting is at last to build prepared supplant for the harmed muscle in patient's body. build should be possible in a perfect world it ought to be get done, in an entire 3D modelled piece in a solitary print, however, because of a few impediments the vast majority of the exploration centre the development in coating under coating mode. After these deposits are printed they can be well organized to make hard or denser tissues. bio printing should be possible fundamentally with 3 unique strategies listed below:

- Inkjet-based – this type of printers are mostly known method because of the marketable bioprinters which we use in our day-to-day life.
- Laser-based – In today's world all the inkjet printers are being replaced by this technology. This is more complex and it produces a well laser pulsations venture bio-ink drops on to the desired material.
- Extrusion-based – these are the technology which is cell responsive technique. The advantage of this method is that it condensed shear strain triggered by the cells.

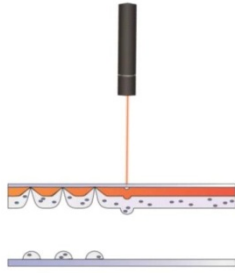
**Table -4.1: Comparison of different types of bio printers**

Characteristics VS Printer types	Laser-based printers	Inkjet-based printers	Extrusion-based Printers
Resolution level	High	Medium	Medium-low
Droplet size (in mm)	>20lm	50–300lm	100lm–1mm
Accuracy Level	High	Medium	Medium-low
Materials Used	Cells in media	Liquids, Hydrogels	Hydrogels, cell aggregates
Commercial availability	No	Yes	Yes
Multicellular feasibility	Yes	Yes	Yes
Mechanical/structural integrity	Low	Low	High
Fabrication time	Long	Long-medium	Short
Cell viability	Medium	High	Medium-high
Processing modes	Optical	Thermal and Mechanical	Mechanical, Thermal and Chemical
Throughput Level	Low	High	Medium
Control of single-cell printing	High	Low	Medium
Hydrogel viscosity	Medium	Low	High
Gelation speed	High	High	Medium
Advantages	High accuracy can be achieved, single cell manipulation, a high-viscosity material	Cost-efficient and versatile	Multiple compositions, good mechanical properties
Disadvantages	Unfriendly Cells, low scalability and viscosity prevents to build-up in 3D	Low viscosity and strength. prevents build-up in 3D	Shear stress on nozzle tip wall, limited biomaterial used, low accuracy

- **Laser-based:** One of the most popular and promising printers are Laser-based. printing methods used in laser based printers will use laser-assisted technology. This technology will scheme the bio-ink drops on to substrate. This particular technique is difficult to implement because of the

price and technical requirements. This method reunites the finest features of supplementary methods i.e., inkjet and Extrusion. As shown in figure 4.1 the laser pulses generate a reply at the time it contacts the laser engraving film under it and the zone where laser contact vanishes and

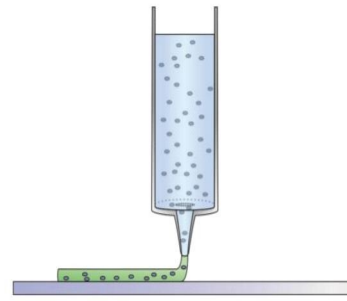
large amount of gas pressure is generated to push biomaterial on to substrate.



**Figure 4.1.** Representation of Laser-based bioprinting process.

In a recent time, the work done by L. Koch and his team presented a 100% tissue feasibility can be accomplished from various kinds of cells using laser assisted bio printing. They proved a direct relationship present among droplet size and laser pulse energy, by doing it which gave the key to control the droplet size. Later printing this they had analysed the cell viability which was having 100% where stated earlier and importantly they have studied phenotype of the cells which are printed using laser based printer thus proving that there is no loss of cell kinds. Sylvain Catros and rest of the others issued a publication in 2012 which determine layer by layer hybrid method for producing cell constructs, they have combined the laser bioprinting and electrospinning scaffold interleaved. This technique gained well outcomes compared by the standard technique of cell seeded scaffolds.

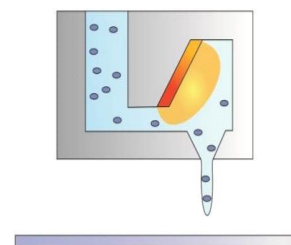
- Extrusion-based:** This method reduced amounts of shear stress and it is treated as the most inoffensive method in bioprinting. The bio-ink which is present at the cylindrical deposits and waiting for the mechanical pressure, a continues plus from a piston which sends the biomaterial from the cylindrical tube over a nozzle on to the surface compared with supplementary approaches this methods is having the following disadvantage like, there is a determination loss because of the larger spigot is vital where there is precision loss will be the method limitations. The illustration of method is shown in Fig:4.2



**Figure - 4.2:** Representation of Extrusion-based bioprinting process.

To overcome the precision and resolution loss. few people have invented technique where the cells are compressed in spheroids. spheroid consist of cell collections which is made up of biomaterials. Few people call as structure [17]. tissue spheroid are considered as voxels where voxels represent slightest element in the 3D environments, same as the pixel in a 2D atmosphere, and truth be paradigm a physical structure. In this kind method spheroids may have to maintain a even structure and size. They have demonstrated and described plan of micro patterned chamber used to fabricate cell spheroids having continuous size and constant cell figure [16]. To attempt the use spheroid method which provides a feasible construct, Cyrille Norotte published a paper work [11] in which they reported vascular tissue construction without the use of a scaffold in 2009.

**Inkjet-based:** This method is commonly the most rich and use for the marketable so it is the inexpensive technology used in bio printing. high precision, resolution make them perfect for bioprinting Besides the cost. New methodologies proven decent outcomes with this method which have ability to overwhelmed its faults, limits and time. In Inkjet method bio-ink is deposited in the containers and it is associated to sequence of firing compartments. compartments are minor in size and consist controlled actuator (heating element or piezoelectric) which ventures bio ink on to the surface Fig. 4.3.



**Figure -4.3:** Representation of Inkjet-based bioprinting process.

Since cells are sensitive to temperature changes for the increase in temperature (actuation) can be injurious to them. However, heating component can increase upto 300°C the bio material usual temperature doesn't rise more than a limited degree. Therefore, more studies show better results. [14]. Apart from temperature deviations, in this method there is another disadvantage that burden made by the rapid pressure can transform can origin harm the cells when they squash through the tiny little spigot. Despite the negative report feasibilities are up to 92% .in 2005 T. Xu published their work with mammalian cells [15]. In a subsequent work, T.Xu demonstrated about electrophysiology, feasibility of printing neural-cells with thermal inkjet method [16] . keeping up expectation for this method in upcoming because neural cells are very delicate and delicate to environmental changes and stress.

## 5. CONCLUSION

Current Three-Dimensional printing innovation had empowered the creation of organ 3D models and hard tissues, analogs and platforms specifically from CAD information. This 3D printing innovation can be classified into different classifications as per distinctive systems.

Particularly in the surgical innovation field, these advancements were utilized for the generation of patient-specific inserts, for example, permeable frameworks, bioartificial tissues, cell-loaded builds, and organs, for organ designing and hard tissue. The incorporation of CT strategies. Organ regenerative models, visual hard tissue and natural useful models have accomplished incredible achievements in vast tissue and organ deformity recuperating and improvement the course of the most recent decades, the expulsion based 3D printing advancements had grown rapidly and outstood exceptionally among all the accessible conventions.

The laser-based and ink-jet 3D bioprinting advancements are in second or third area because of impediments of programming and equipment of the inkjet based hardware and tedious, harmful impacts the cells of laser based gadgets. Acknowledgment ought to be given for low-temperature twofold spout expulsion based 3D printing advancements, which will particularly helpful for persistent hard tissue and organ designing in specific. A conspicuous achievement is unidirectional for expanded vascular framework joined in the vast organs and tissues.

Numerous real-time issues for vascular organs building were overwhelmed by Wang Group Centre for Tissue Manufacturing, Department of Mechanical Engineering, Tsinghua University. Contrasted and conventional tissue designing methodologies, where the tissue should be planted towards permeable frameworks into shape tissues, 3D printing innovation has numerous focal points making bio artificial organs and tissues. The gelatine based regular hydrogels, engineered-polymers has given the tissue an agreeable convenience to develop, multiply and separate. The controlled adhere to procedures and stretched vascular frameworks are fundamental for blood going infiltration of new tissue in the development. The technical progresses expulsion based joined multi spout type of 3D bio printing innovation, novel PU biomaterials, foundational microorganism commitment conventions, bioactive specialist (e.g., development cryoprotectant, and separation factor) fuse procedures, and spatial full scale and small scale condition governing methodologies offer ascent to open doors for assembling bio artificial organs and hard tissues with the entire range of their local partners

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