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THE FUTURE OF 3-D BIO-PRINTING

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***Abstract*:** Bio printing is a new regenerative medical field in which the In-vivo bio-manufacturing of tissues or organs takes place which is driven by two needs one is organ transplantation and the other is accurate tissue models. Bioprinting was first demonstrated in the year 1988 by Klebe. He used standard Hewlett-Packard (HP) inkjet printer to deposit the cells using cytoscribing technology. The ability to create a cell-laden, 3 D structures, that mimic bodily tissues not only in tissue engineering but also in drug delivery and cancer research. For the fabrication of tissue engineering scaffolds, bioprinting can provide patient-specific spatial geometry, controlled microstructures, and the positioning of various cell types. Over the last few decades, three-dimensional bioprinting has been widely used in the construction of many tissues/organs such as skin, vessels, hearts, and so on, which cannot only lay the groundwork for the grand goal of organ replacement, but can also be used as In-vitro models for pharmacokinetics, drug screening and so on. As conventional techniques are in capable of fabricating constructs with the required structural, mechanical, and biological complexity, there is an increasing demand for alternative fabrication approaches to develop tissues and organs. 3D bioprinting is an additive manufacturing technique that uses a "bioink" to build devices and scaffolds layer by layer. Because organs are so complex, many bioprinting methods are used to overcome the challenges of various applications. Nozzle based techniques such as inkjet and extrusion printing, as well as laser-based techniques such as stereo lithography and laser-assisted bioprinting can yield on cell viability, resolution, and print fidelity. The different fabrication techniques, laser-based, extrusion-based, stereo lithography and inkjet-based bio-printing are defined in this article. The benefits, current research status of each technique in relation to different tissue types, challenges and prospects are discussed.

***Key words*: 3D scaffolds, Extrusion, Stereo lithography, Inkjet, Organ printing, Bioink, Polymers.**

INTRODUCTION:

Bioprinting is rapidly evolving industry with the potential to reshape regenerative medicine. It is a subject of additive manufacturing (AM) also referred to as three-dimensional (3D) bioprinting. 3D bioprinters create 3D tissues using bioinks made of living cells and bio materials (1). This procedure follows a work flow that includes computational modeling, bioink preparation, bioink deposition and subsequent maturation of printed products. Bioprinting is versatile tool capable of producing a wide range of tissues and organs. Access to bioprinted organs could aid in the resolution of current human organs shortage crisis. These technologies, when combined with advances in tissue engineering, could aid in the treatment of variety of veterinary conditions, including equine bone fractures, articular cartilage repair and generation of more accurate disease models (3). Bioprinting is also defined as the use of viable cells, biomaterials and biological molecules to print the structures. Bioprinting must produce scaffolds with appropriate micro architecture to provide mechanical stability and promote cell in growth, while also taking into account the impact of manufacturing cell viability

such as chemical cytotoxicity, caused by solvent use and pressure induced apoptotic affect produced during material extrusion. Because cell placement is included during fabrication, bioprinting eliminates the homogeneity issues associated with post-fabrication cell seeding. The benefit of homogeneously distributed cell-laden scaffolds has been demonstrated by faster integration with host tissue, rejection risk, and most importantly uniform, tissue growth In-vivo. Traditional cell seeding techniques are either static or dynamic, and while the latter improves seeding efficiency and cell penetration into the scaffold. It is known to have an impact on cell-morphology. The immediate vascularization of the implanted scaffolds is critical. With proper vascularization, the scaffolds receive an influx of oxygen/nutrients and an efflux of carbon dioxide/byproducts, preventing core necrosis. Vascularization also helps the implants remodel. Bioprinting techniques have been used to create micro-vascular like structures, which have the potential to position endothelial cells within the 3D structures as a pre-vascularization step prior to implantation (1). The growing interest in 3D printing is a direct result of its advantageous circumstances, such as quick adjustment of prototyping of adjustable parts. The technique is now considered as a standard method of production. Several materials like composites, polymers and ceramics could be used in ventures such as automation, aviation, medical services and clinics, gadgets, food and others. In the current manufacturing trends, 3D printing represents a cutting-edge innovation in the field of prototyping and component improvement. Furthermore, to overcome then goals of industry 4.0 disruption, mutually in the academic and industrialized sectors, interest in 3D printing is growing dramatically to utilize the method's merits. It is now possible to construct the geometry of the confounded structure, which was very monotonous in the subtractive production method, using 3D printing fabricating methods (18). 3D printing is defined as the process of completing a 3D CAD model into a component by saving material layers over each other. The 3D printing process or AM was developed in the 1980s. 3D printing has quickly gained popularity as a developing production method. As a result, it has been widely adopted in a variety of fields, including design gems, polymer printed materials, applied autonomy and mechanization, tissue and frameworks, and gadget items.3D printing aided application fields due to few characteristics such as short timeframe process, minimal effort, customization, and material reduction.

1. Process of 3D bioprinting:

1.1 Material jetting:

Material jetting is the process of selectively depositing droplets of build material on to a build bed to create a 3D object. The process entails the formation and deposition of droplet material, as well as the displacement of material with or without cells to desired position. Droplet-based bioprinting technologies include piezoelectric or thermal ink jetting, acoustic wave jetting, electro-hydrodynamic jetting, and laser-induced forward transfer (LIFT). (9)

1.2 Material extrusion:

Biomaterials are extruded from the nozzle in material extrusion techniques, resulting in defined structures. Extrusion-based printing is classified into three types based on the mechanism used to extrude materials. Mechanical extrusion uses a motor to drive a piston downwardly, with the amount of material extruded determined by the rate of motor displacement; pneumatic dispensing systems supply air pressure into the syringe cartridge, with the difference between supplied air pressure and ambient pressure driving the flow of material; and the third setup uses a rotatory screw, with the amount of material extruded determined by the angular turn of the screw. For dispensing cell-hydrogel in extrusion-based bioprinting, both pneumatic and mechanical systems have been widely used. Rotary screw extrusion has only been used in bioprinting when a high viscosity material, such as PCL melt is used. Alternatively, valves at the nozzle can be used to control the flow of the hydrogel. This type of printing is also known as micro valve bioprinting. Micro valve bioprinting can dispense droplets or strands of hydro gel. (9, 17)

1.3 Vat polymerization printing:

A container filled with cell-hydrogel suspension is subjected to selective polymer curing to form 3D structures in vat polymerization printing (VPP). The components of the vat polymerization printing system used for bioprinting are very similar to those of their additive manufacturing counter-parts.VPP systems include an energy source that initiates the polymerization process selectively within the entire vat containing photosensitive polymer. In stereo lithography (SLA), three-dimensional constructs are formed point by using laser curing. Alternatively, UV light can be area-projected into a photopolymer vat using a digital micro-mirror device light processing (DLP). (9, 17)

1.4 Picking and placing:

In addition to methods that require the delivery of spheroids via suspensions, robotic arms have been designed to directly manipulate spheroids in a pick and place fashion. The kenzan method is one such pick and place method. The apparatus consists of needles arranged in arrays for the placement of cell spheroids. The spheroids are picked and transferred from wall plates to the needle arrays using suction. The spheroids are arranged in a specific pattern. Spheroids on needle arrays are incubated for several days before being removed from the array platform (9).

2. Production methods for 3D printing:

Traditional manufacturing techniques necessitate a significant amount of graceful supply chain management, require a massive amount of work supremacy 3D printing process to be robotized, and rely on CAD programming to print items utilizing a variety of materials, significantly reducing the amount of supply chain management. When all is said and done, 3D printing does not require any expensive any expensive moulds or instruments for machining, structures, or punches, and it is a cost-effective method. Depending on the source, there are various 3D printing production techniques. Any of the techniques can be chosen based on the applications of the components.

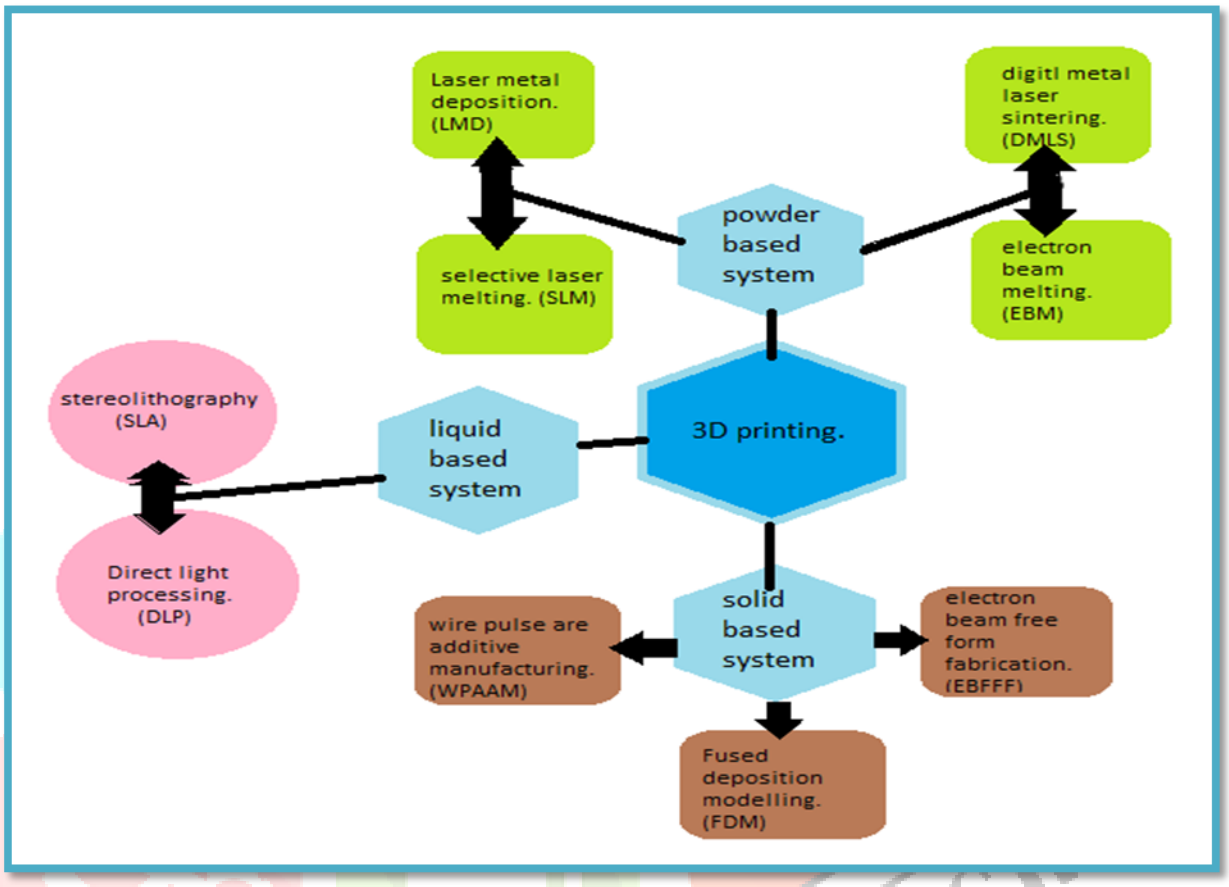

Figure 1: classification of 3D bioprinting techniques.

2.1 Powder based systems:

Powder-based 3D printing processes are widely used in both polymer and metal 3D printing. It covers only 3/7th of 3D printing categories: binder jetting, PBF, and directed energy deposition. Metal powder-based 3D printing employs both powder injection (or blown powder) and powder bed feedstock mechanisms. For the time being, the only mechanism used for polymer powder-based 3D printing is a powder bed feedstock mechanism. An energy source is used in powder injection-based 3D printing, such as powder directed energy deposition (PDED), to melt the blown deposited by a nozzle. The PDED process is typically used with metal powders only in this scenario (19). Laser metal deposition (LMD), digital metal laser sintering (DMLS), selective laser melting (SLM), and electron beam melting (EBM) are types of powder based systems. (16, 17, 18)

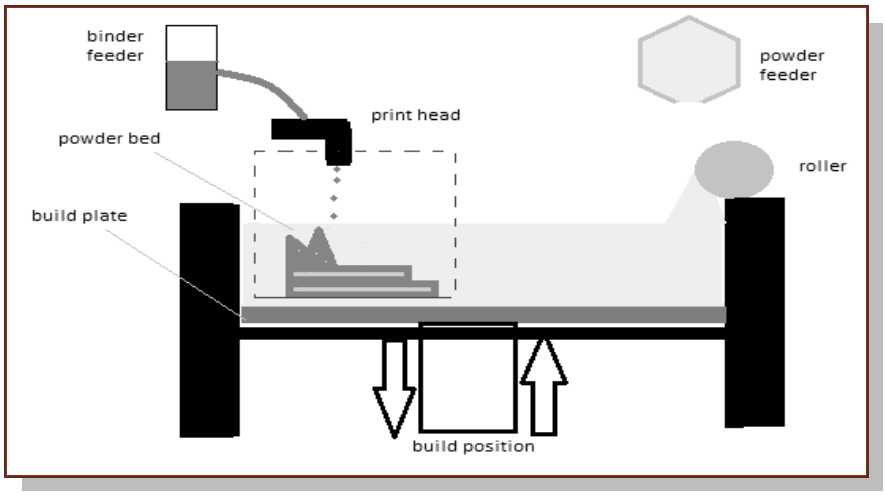

Figure 2: Powder based 3D bio printing

2.2 Liquid based systems:

Most liquid-based additive manufacturing (AM) systems construct parts in a vat of photo-curable liquid resin, an organic resin that cures or solidifies when exposed to light, typically in the ultraviolet range. The light cures the resin near the surface, resulting in the formation of a thin hardened layer. Once the entire layer of the part has been formed, an elevation control system lowers it to allow the next layer of resin to be coated and similarly formed over it. This process is repeated until the entire section is finished. If necessary, the vat can be drained and the part removed for further processing. The various vendors variations on this technique are dependent on the type of light or laser, method of scanning or exposure, type of liquid resin, and type of elevation and optical system used. Another method is to use a print-head to spray drops of liquid photo polymer onto a build tray, similar to inkjet printing, and then cure them with UV light. Again, the technique varies depending on the type of resin, exposure, elevation, and so on (20). Stereo-lithography and direct light processing are liquid based systems. (17)

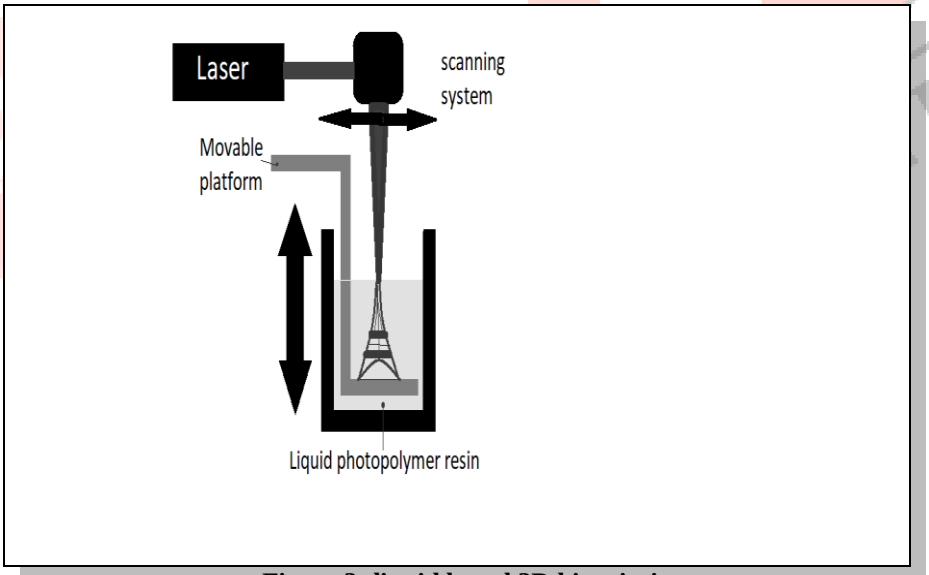

Figure 3: liquid based 3D bioprinting

2.3 Solid based systems:

A solid-based system is a type of 3D printing manufacturing process. Solids are used as the primary medium in solid-based 3D printing methods to create the component or model. They are not the same as the fluid-based photograph restoration process. They are also distinct from one another in that the primary type of strong materials in some methods may come as fibers or wires, a few as sheets or rolls, and others as pellets. A unique collection of strong-based 3D printing methods that use powder as the intermediate will be protected independently. Fused deposition modeling (FDM), electron beam freeform fabrication (EBFF), and wire pulse arc additive manufacturing (WPAAM) are the three major types of solid-based system.

2.3.1 Inkjet-based bioprinting:

The first attempts at printing live cells were made with specially adapted commercially available inkjet printers. The cells died during printing due to instantaneous drying out once on the substrate, which was an early problem encountered when developing inkjet bioprinting. Encapsulating the cells in a highly hydrated polymer solved the problem, leading to the development of cell-loaded hydro gels. Inkjet bioprinting enables precise cell positioning, with some studies achieving as few as a single cell printed droplet. (2) Thermal inkjet printing creates a bubble by heating an element. The bubble causes pressure to build up within the print-head, resulting in the expulsion of a droplet. The thermal element has a temperature range of 100 °C to 300 °C. Initially, there were concerns that such high temperatures would harm the cells, but research has shown that the high temperatures are localized and only exist for short period of time. Acoustic waves are used to eject the bioink from the piezoelectric apparatus. This mechanism restricts the use of highly concentrated and viscous bioinks because their viscosity dampens the applied acoustic/pressure waves, preventing droplet ejection. Low viscosity is obtained by using low concentration solutions, which is a limiting factor in the production of 3D structures. Induced sheer stress have the potential to damage cells, most research indicates that this is not the case. The advantages of inkjet-based bioprinting include high print speed, low cost, and widespread availability, however, drawbacks include low droplet directionality and unreliable cell encapsulation due to ink concentration. Because of its high resolution and cell variability, inkjet bioprinting is very appealing. This method allows for precise positioning of multiple cell types. However, due to the limitations of vertical printing and restricted viscosities, inkjet bioprinting may need to be combined with other printing techniques in the future. (5, 1l)

2.3.2 Laser assisted 3D bioprinting:

n this process a pulsed laser beam is used to deposit bioink, including cells, onto a substrate. The use of a laser for material deposition provides a non-contact direct writing process for 3D printing. A ribbon coated with bioink and a receiving substrate on which the bioink is to be deposited is visible in the source. The energy source is UV or near UV wavelengths lasers with nanosecond pulse wavelength. The laser is responsible for the volatilization of the heat-sensitive bioink from the “ribbon”. The bioink is applied to a target plate made of quartz or that allows laser transmission through it. Depending on the optical properties of the laser and the ink, laser-absorbing, sacrificial interlayer between the bioink and the ribbon aids viable cell transfer. To facilitate the deposition process and sustain cell growth, the substrate on which the ink is to be deposited is also coated with either natural polymer, nutrient medium or a biopolymer. Because the bioink is volatile, when a laser pulse is applied, a high speed jet of cell-laden bioink is propelled onto the substrate. Researches created “absorbing film-assisted laser induced forward transfer (AFA-LIFT)” or “biological laser processing (bioLP) as well as “matrix-assisted pulsed laser evaporation direct writing (MAPLEDW)” (12).

2.3.3 Extrusion-based bioprinting:

In recent years, extrusion-based bioprinting, also known-+ as direct writing, has been widely used in bio fabrication and tissue engineering. The extruded bioinks are widely distributed as strands due to two main mechanisms: pneumatic force (gas or pressurized air) and mechanical force (screw or piston). In fact, it is made up of dispenser system that is placed on o robotic stage and is controlled by a stage controller. The bioinks are deposited on a building substrate, and the piston-drive deposition setup controller. The bioinks are deposited on a building substrate, a building substrate, and piston-driven deposition setup controls bioink overflow via screw-driven systems. This is a critical issue in the deposition of highly viscous biomaterials.

2.3.4 Fused deposition modeling:

Fused deposition modeling (FDM), also known as 3D printing, is one of the oldest AM techniques. According to the printing mechanism, it produces the melted thermoplastic designated as a support for bioinks by feeding a thermoplastic polymer into a liquefier and then extruding a filament. Several parameters influence the property of FDM parts. Researchers attempted to focus on key parameters in order to optimize the fabricated parts and eventually obtain a combination of them.

2.3.5 Stereo lithography:

Stereo lithography (SLA) is based on selective polymerization of a liquid, photosensitive resin by a light source, such as UV light or a laser. In the early 1980s, the first study on the fabrication of the 3D structure, through the photo-polymerization of the liquid-based resin utilizing UV light, was achieved by Kodama, who developed two approaches, one utilizing a mask for each layer to do the exposure through, and the other using an optical fiber to cure the photopolymer selectively. A predefined design was created by controlling the fiber movement along the X and Y axis, contributing to this by the addition of movement along the z-axis to produce 3D scaffolds in a layer-by-layer approach via UV light.

In essence, stereo lithography is a dynamic version of photolithography that employs a narrow beam of light to cure the polymer and produce the desire pattern, as opposed to photolithography, which employs a static photo-mask to create micro-pattern. Light selectively polymerizes the resin in this system using a computer-aided design (CAD) model. After the first layer has been formed, the platform is lowered and a new resin material is added to polymerize and create the second layer. It is also possible to accomplish

this by moving the product in Z-direction after dipping into the liquid medium. Finally, the uncross-linked resin between the layers is washed, and the construct is post-cured with UV to complete the polymerization reactions and increase the product's stability.
Laser-based stereo lithography and digital light projection (DLP) can be used to cure the resin of the two different irradiation approaches. A laser beam controlled by a computer directly writes an object in a bottom-up manner in the laser-based method. A digital micro-mirror device (DMD) uses micro-scale mirrors aligned in an array to control the required light intensity for printing. In this array, each mirror can be rotated independently to switch between on and off states. As a result, only the desired area is illuminated and polymerized (21).

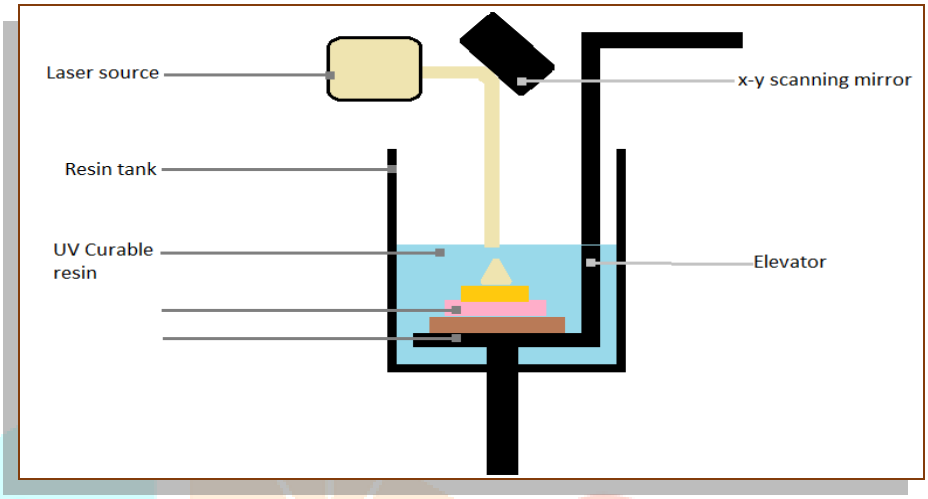

Figure 4: liquid based printing by stereo lithography

2.3.5 Scaffold-free bioprinting:

Scaffold-free bioprinting relies on the tissues autonomous self-assembly as it grows. The idea behind autonomous self-assembly is that tissue do not need a template or scaffold because they have innate mechanisms for producing surrounding tissue architecture. By allowing cells to assemble autonomously, this approach attempts to replicate embryonic environmental and structural development. Prefabricated multi cellular building blocks such as cell pellets, spheroids, or tissue strands are used to generate 3D constructs in scaffold-free bio printing. (12)These "building blocks" are printed at high cell densities, fusing together and releasing the desired tissue ECM components. When compared to scaffold-based bioprinting, this cell-friendly approach avoids the use of exogenous material, reducing toxicity, improving cell viability, increasing cell-to-cell interactions, and shortening the length of post-bioprinting maturation. It does, however, necessitate higher cell densities, limiting printer selection, has low scalability, and lacks mechanical integrity due to the lack of scaffolding or physical support. (13)

3. Biomaterials used in 3D bioprinting: (2,6)

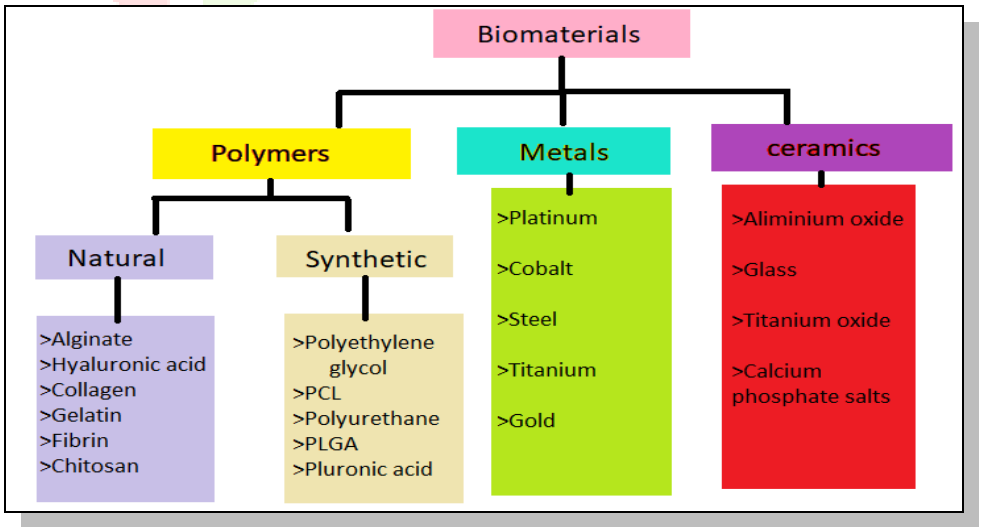

Figure 5: the classification of biomaterials used in 3D bioprinting

3.1 Natural Polymers:

Polymers found in nature can be synthesized using physical, biological, or chemical methods. Natural polymers are compatible, can hold fluid, and are easily dissolved in various solvents such as phosphate buffers and cell culture solutions, making them more tissue friendly. Because of these characteristics, it is possible to print it layer by layer, resulting in a model that, when placed in a stable environment, will mimic a natural organ. Following are some commonly used natural polymers. (2)

a. Alginate:

Alginate is derived from the cell walls of phaeophyceae (brown algae) and used as alginic acid salts. Wang first used sodium alginate, but the gelation point was 0ºC, and 3D bioprinting was done at room temperature, thus, it was cross-linked with other metals such as calcium and barium to increase its compatibility and mechanical strength. (2)

b. Hyaluronic acid:

Hyaluronic acid is a component of the extracellular matrix that plays an important role in cell proliferation and angiogenesis. It can be used to change the viscosity of other polymers, such as gelatin, due to its high cell adhesive property and water-absorbing quality. Hyaluronic acid, like other natural polymers, is cross-linked with synthetic polymers like methyl acrylate to increase its compatibility. (6)

c.Collagen:

Because of its resistance and toughness, collagen is widely known to support the skin, ligaments, bone, tendon, and cartilage. Type1 and 2 are the most commonly used in musculoskeletal repair with 3D printed models. Collagen has been shown to promote bone and cartilage cell proliferation, maturation, and differentiation. Collagen, like other polymers, works best in bioprinting when combined with other polymers to increase viscosity and decrease degradation when compared to using collagen alone. Cross linking with alginate, agarose, hyaluronic acid, and fibrin is common. (2,6)

d.Gelatin:

Gelatin is a linear molecule formed by the breakdown of collagen. Because it is a natural substance, it is not toxic, has low immunogenic properties, is hydrophilic, and is highly degradable, making it a unique polymer. Gelatin is combined with culture media before printing to make it denser. Many agents, including hormone growth factors, can crosslink with gelatin molecules. Heparin acts as best combination other than other naturally occurring polymers. (2)

e. Fibrin:

Fibrin is natural polymer that forms in the presence of thrombin in blood due to the rapid polymerization of fibrinogen. Although fibrin has been found to be superior in properties such as compatibility when compared to other natural polymers, it is combined with other natural polymers to overcome its low strength, less viscosity, high degradation, and gelation properties when used alone. (2)

f.Chitosan:

Chitosan is typically derived from prawn shells through the hydrolysis of chitin. It, like other natural polymers, has low strength and degradable properties; thus a similar combination of cross linking with chemical agents is done with collagen, alginate, and gelatin to increase viscosity and biodegradability, and it is used to repair rigid structures such as skin, bone, and cartilage to make it more compatible. (2)

3.2 Synthetic polymers:

Synthetic polymers are created artificially in a laboratory by humans using chemicals in appropriate environment; they have high strength and resistance. The main advantage of synthetic polymers is that we can easily modify them because they can withstand temperature and pH changes and can be processed according to our needs due to their increased resistance and mechanical strength. Because synthetic polymers have shallow gelation temperature compared to natural polymers with very high melting temperatures, they are ideal for 3D bioprinting models; thus, formed polymers are inert, difficult to degrade, and have a high tensile strength.

Polyethylene glycol:

Polyethylene glycol is a linear synthetic polymer that is immunogenic, has affinity for water, and is thus well suited for bioprinting. Polyethylene oxide is another name for polyethylene glycol. Polyethylene glycol cannot adhere to cells properly; therefore, it is cross linked with other molecules such as carboxyl group, acrylate or thiol group to make it more suitable for use in soft tissue repair.

Polycaprolactone:

Polycaprolactone is a partially crystalline polymer that degrades easily in our bodies. It is a thermoplastic polymer that changes its mechanical structure and degradation rate when combined with other agents at 60ºC. It is an ideal material for use in 3D bioprinting fused deposition modeling technology. Polycaprolactone, like all other synthetic polymers, is cross linked with other bio-agents such as polycaprolactone-alginate to increase its cell adhesive property for cartilage regeneration.

Polyurethane:

Polyurethane is a linear biodegradable polymer with excellent compatibility and mechanical properties. Polyurethane is inert and cannot be degraded when used alone. As a result, it is cross linked with other materials and bio agents like adipose stem cell-fibrin-alginate-gelatin and cryoprotectant.

Polylactic-co-glycolic acid:

Copolymerization of two polymers, lactic acid and glycolic acid, yields polylactic-co-glycolic acid. The transition temperature of polylactic-co-glycolic acid is typically around 40-60ºC, and glycolic acid and lactic acid are used in a 1;3 ratio. The degradation rate of polylactic-co-galactic acid has been found to be dependent on the concentration of glycolic acid used during the synthesis it can also be combined with other agents such as growth factors or adipose stem cells to make it more compatible for creating the complex structure of 3D bioprinted organs.

4. 3D Bioprinting of various tissues and tissue engineering:

Bioprinting technology has sparked widespread interest due to its potential for biomaterial fabrication in tissue and organ bioengineering. Skin is one of the body's complex multi-layered organs, and the use of bioprinting in construction or regeneration of burned skin is becoming increasingly important in human life. In this regard, Kim et al. created a new model for 3D human skin construction by combining extrusion and inkjet modules. By combining two different bioprinting techniques, this effort was found to be time-effective. The 3D bio printing of various tissues is discussed below.(5,7)

4.1 3D Bioprinting of cardiovascular tissues:

Various 3D bioprinting techniques have been used to create 3D cardiovascular tissue constructs using different proliferative cell types. When co-cultured with endothelial cells, these tissue constructs display synchronous macroscopic beating and self-assembly of vessel-like conduits. Laser direct write bioprinting technology has been used to form embryonic bodies (EBs) from mouse embryonic stem cell (ESCs) and can be used to control and direct EB formation and size, allowing for directed cardio genesis. The LIFT cell bioprinting technique was also used to print mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECS) onto a cardiac patch to promote angiogenesis and improve cardiac function, as determined by left ventricular catheterization 8 weeks after an acute myocardial infarction (MI). (7)

4.2 3D bioprinting of musculoskeletal tissue:

Several techniques have been used to bioprint musculoskeletal tissue using stem cell. C2C12 myoblasts, which can proliferate indefinitely and differentiate into multinucleated myotubes, have been used to create precisely patterned 3D skeletal muscle constructs using bioprinting techniques. The bioprinted cells were highly viable and responded to electric pulses synchronously. Another study used inkjet bioprinting to pattern grown factors onto fibrin-coated, highly oriented sub-micron polystyrene fibers created with a spinneret-based tunable engineered parameters technique. This enabled the simultaneous differentiation of embryonic fibroblasts into myocyte, tenocyte, and osteoblast cell lines, resulting in a muscle-tendon-bone bio mimetic tissue construct. (5, 7)

4.3 3D Bioprinting of nerve tissue:

Neurons with high cell viability were bioprinted using inkjet and micro extrusion 3D bioprinting technology, and bioprinted neurons maintained basic cellular phenotypes and functionality for more than two weeks after printing, with successful development of voltage-gated potassium and sodium channels. Neural stem cells (NSCs), ESCs, IPSCs, adipose-derived adult stem cells, and adult MSCs have all been used to engineer neural tissue. Because glioma stem cells are thought to be the main cell type underlying the poor prognosis associated with high grade glioma, they were used to create a glioma tumour model. NSC differentiation can be controlled using biologically active macromolecules and transcription factors, making these cells especially useful in the fabrication of 3D constructs with precise spatial patterning. (5)

4.4 3D Bioprinting of hepatic tissue:

Several studies have been conducted to investigate 3D bioprinting of liver tissue using stem cells or immortalized hepatic cell lines. Stem cells are studied in particular because they have shown great promise in expressing hepatocyte-like phenotypes, and because adult hepatocytes are scarce, difficult to isolate, propagate poorly, and undergo rapid functional deterioration in vitro. Nerve-based bioprinting was also used to print iPSCs and ESCs into clinically relevant thickness structures in an alginate hydrogel matrix, and the cells were differentiated into hepatocyte-like cells. The bioprinted cells expressed hepatic markers and were phenotypically similar to native hepatocytes, as evidenced by albumin secretion and morphological analysis.

4.5 3D Bioprinting of adipose tissue:

Adipose tissue was created using 3D bioprinting techniques combined with stem cell technology. For example, Gruene *et al.*, created 3D grafts using laser bioprinting techniques with human adipose- derived stem cells in algenate and blood plasma hydrogel scaffolding. The printing methodology has no effect on the stem cells' proliferation or differential potential, and the stem cells maintained their triggered lineage behavior, as measured by the expression of adipogenic markers. Confocal microscopy was used to show that the laser bioprinting technique allowed for the micro-scale arrangement of adipose stem cell, which could allow for the generation of multi cellular tissue grafts with the complexity of native adipose tissue. Adipose tissue constructs were also created by bioprinting a de-cellulised extracellular matrix bioink with encapsulated adipose-derived stem cells and a polycaprolactone framework at the same time.

4.6 3D Bioprinting of skin tissue:

The use of 3D bioprinting and stem cell technology in skin tissue engineering holds a lot of promise. By seeding and cultivating cells in bioactive, printed scaffold templates, scaffold-based tissue engineering methods have been used to reconstruct skin tissue. Techniques that print cell-laden bioinks, on the other hand, can avoid problems associated with scaffold-based tissue engineering methods, such as lack of uniform cell distributions. Furthermore, the incorporation of stem cells into these printed constructs would allow for the introduction of multiple cell types with positional specificity. Full-thickness skin equivalents were printed using 3D inkjet bioprinting, with altering layers of a printable and cytocompatible bio-ink and a dermal fibroblast cell suspension that demonstrated high viability after printing. Keratinocytes were seeded and allowed to proliferate on the dermal cell construct, and a stirring system was installed to prevent sedimentation.

5. Current issues and prospects of 3D bioprinting: (15)

The FDA has issued a document titled "technical considerations for additive manufactured devices" that provides guidelines for additive manufacturing, including 3D printing. With the advancement of printing technology and the development of efficient and cost effective printing methods, it has become necessary to regulate the quality control standard prior to transplantation in each step of the process, such as when designing a model, selecting bioink, printing validation, maturation of post-printing, and assessing product quality. Furthermore, one of the major issues with3D bioprinting is number of components involved in the printing process. Bioprinting is hampered by a lack of software that can virtually define the placements of cells, biomaterials, and biological molecules after a robust designing and translation that drive downstream manufacturing operations. Another challenge is that during transplantation, a sufficiently stable and mechanically inflexible 3D construct is required. During hard tissue repair, the porosity and structure created by 3D bioprinting should have a high elastic modulus so that natural cell growth can occur during implantation. Because of scaffold deformation, newly formed tissues are likely to fail if the scaffold does not provide proper structural maintenance and mechanical support. Another critical requirement for a bioprinted construct in TE that provides growth factors, oxygen, nutrients, and waste removal is proper In-vivo vascularization. In-vivo capillaries present within 100 mm of maximum cells exhibit sufficient diffusion required for cell survival.

SUMMARY:

Bioprinting is a new regenerative medical field that involves in-vitro bio-manufacturing of tissues and organs for organ transplantation and accurate tissue models. It can provide patient specific spatial geometry, microstructures and positioning of different cell types for the fabrication of tissue engineering scaffolds. The advantages of homogeneously distributed cell laden scaffolds has been demonstrated in terms of faster integration with host tissue, reduced rejection risk and most importantly uniform tissue growth in-vivo. It is a standard method of production. Material jetting, material extrusion, vat polymerization, and pick and place are all part of 3D bioprinting process. Traditional manufacturing techniques necessitate a significant amount of graceful supply chain management, necessitate a massive amount of work supremacy 3D bioprinting process to be robotized, and rely on CAD programming to print items using a variety of materials, significantly reducing supply chain management. There are various 3D printing production techniques available depending on the source. Depending on the applications of the components, any of the components, any of the techniques can be chosen. Powder based 3D printing processes are widely used in polymer and metal 3D printing. It only covers 3/7ths of the 3D printing categories: binder jetting, PBF, and directed energy deposition. Metal-powder based 3D printing employs both powder injection (and blown Powder) and powder bed-feedstock mechanisms. Liquid-based additive manufacturing (AM) systems create parts in a vat of photo-curable liquid resin, an organic resin that cures or solidifies when exposed to light, typically in the UV range. The light cures the resin near the surface, causing a thin hardened layer to form. A solid- based system is a type of manufacturing process used in 3D printing. To create the component or model, solids are used as the primary medium in solid-based 3D printing methods. They are not the same as the fluid-based restoration process of photographs. They also include laser-based, extrusion based, and inkjet based bioprinting, as well as stereo lithography. Natural polymers such as collagen, gelatin, alginate etc., synthetic polymers such as polyethylene glycol, polyurethane, PLG, and metals and ceramics are the biomaterials used in 3d bioprinting. Tissue engineering using 3D bioprinting includes cardiovascular tissues, musculoskeletal tissues, nerve tissue, hepatic tissue, adipose and skin tissues. The Food and Drug Administration (FDA) has issued a document titled "technical considerations for additive manufacture devices" that provide guidelines for additive manufacturing, including 3D printing. With the advancements of printing technology and the development of efficient and cost-effective printing methods, it has become necessary to regulate the quality control standard prior to transplantation in each step of the process, such as when designing a model, selecting bioink, printing validation, post-printing maturation, and evaluating product quality.

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