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3D Bioprinting for Tissue and Organ Regeneration

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Abstract: **Organ implantation is a significant treatment for a number of end-stage organ disorders. But there are only a few donors accessible. Tissue and organ shortages may one day be eliminated thanks to the development of tissue biofabrication technologies. Cells and biomolecules are often placed into a scaffold with a porous structure to mimic the properties of extracellular matrix (ECM). It has been possible to engineer a number of tissue structures, including bone, skin, and cartilage, by using scaffold-based approaches. Rapid manufacturing, also known as additive manufacturing or three-dimensional (3D) printing, has the ability to totally eradicate these problems . The concept of 3D printing was improving in the early 1980s . Inspiring by recent advancements in tissue engineering and regenerative medicine, 3D printing has been successfully used for tissue biofabrication. Bioprinting is the layer-by-layer implantation of biological elements and living cells using computer-aided transfer techniques. This technique allows for the creation of tissue constructions with a variety of cell locations and vascular patterns that replicate the structural features of human tissues and organs. The three stages of 3D bioprinting are preprocessing, processing, and postprocessing. Inkjet, extrusion, laser, Tissue and Organ, Skin tissue, Cardiac Tissue , Microchannels , etc. are just a few examples of the various forms of 3D bioprinting.**

Keywords: **3D Bioprinting, organ regeneration, skin tissue, cardiac tissue.**

I. INTRODUCTION

One of the important medicaments for various end-stage organ diseases is Organ implantation [1]. However, there are limited donors available [2]. The development of tissue biofabrication technology will one day help resolve tissue and organ shortages [3]. In order to imitate the qualities of extracellular matrix (ECM), cells and biomolecules are typically seeded within a scaffold with a porous structure [4]. Engineering several tissue structures, like bone, skin, and cartilage, has been accomplished by using scaffold-based techniques [5, 6]. Nevertheless , these methods usually fail in reproducing the intricate structures of native tissues and are unable to arrange various cell types in the required places or in a systematic way [2, 7]. Rapid methodology or additive manufacturing, typically called to as three-dimensional (3D) printing, has the potential to completely abolish these issues [8]. Early in the 1980s, the idea of 3D printing was changing in the better way [9]. 3D printing has been effectively adopted for tissue biofabrication, with inspiration from current developments in tissue engineering and regenerative medicine [10]. The layer-by-layer deposition of biological components and living cells utilizing computer-aided transfer methods is known as bioprinting [11]. This method is scalable, reproducible, and has a large throughput. It makes it possible to arrange several cell types in the required structure [11]. By using this method, it is possible to produce tissue constructs that mimic the structural characteristics of human tissues and organs while having diverse cell placements and vascular patterns [1]. Preprocessing, processing, and postprocessing are the three phases that are included in 3D bioprinting [12]. A component of preprocessing is the creation of a computer-aided design (CAD) of a target tissue or organ. Using medical imaging techniques like computer tomography (CT) or magnetic resonance imaging (MRI), a blueprint of the tissues and organs can be created [13, 14]. The blueprint is then transformed into a heterogeneous model that describes the composition and distribution of materials and cells [15]. By breaking down the specific prototypes into a two-dimensional (2D) layers, the 3D structures are rebuilt [16]. The layer-by-layer precision deposition techniques used in the printing process allow for the concurrent deposition of cells and biomaterials [17]. The incubation of the printed

tissue constructions in a bioreactor is the postprocessing [17]. This article aims to explore the benefits arising from the use of 3D bioprinting for tissue and organ regeneration. For example the working mechanism of a 3D Bioprinting, Different types of 3D Bioprinting: Inkjet, Extrusion, and laser.

II. 3D BIOPRINTING STRATEGIES

The precise layering of biomaterials forms the core of the 3D printing process [18]. The procedure is broken down into three simple steps: the preparatory phase, the printing phase, and the post-handling phase [19]. Anatomically accurate 3D models are created using computer graphics tools like CAD/CAM and rendered into a stack of 2D layers with user-defined thicknesses before being fed into a bioprinter for printing [20]. The option of the bio-ink material was also chosen in this step [21]. The actual printing of the tissues using additive manufacturing methods is the processing step [21]. The maturation of the manufactured construct in a bioreaction and its structural and functional characterisation are referred to as post-processing steps [21, 22].

III. INKJET BIOPRINTING

In inkjet bioprinting, droplets of cells and biomaterials are patterned onto the appropriate surfaces [16]. Droplets are continually dropped at predesigned spots to create structures [17]. There are several kinds of inkjet bioprinters that have been created, including thermal, piezoelectric, and pneumatic microvalve-based inkjet printers [19]. Small air bubbles are produced in thermal inkjet bioprinting by heating in the printhead [1]. When the bubbles burst, pressure pulses are created that cause drops of bioink (a combination of cells and biomaterials) to shoot out of a nozzle [23]. The imposed temperature gradient, the frequency of the current pulse, and the viscosity of the bioink, will make the droplet volume range from 10 to 150 pL [24]. A thermal printer's localized heating only lasts for about two seconds [25]. Hence, this has less of an impact on the cells while printing [20]. When a voltage is supplied, a piezoelectric crystal causes a form change and generates an acoustic wave in piezoelectric inkjet bioprinting [26]. Due to a momentary pressure from the nozzle, the sonic wave fragments the bioink into numerous droplets that are ejected [27]. The bioink is controlled by a constant pneumatic pressure in pneumatic microvalve printing [28]. The benefits of inkjet bioprinting are contactless printing. Inkjet bioprinting makes it simple to change the size, rate of deposition, and location of droplets, enabling the fabrication of precise patterns and concentration gradients of chemicals and cells [29, 30]. Inkjet bioprinting can produce a 3D structure with complicated or asymmetrical geometries, such as branch or tubular structures [31]. High spatial resolution is provided by inkjet bioprinting, however the bioink must adhere to various specifications [32]. To prevent clogging and droplet ejection, inkjet bioprinting employs a biomaterial solution with a low viscosity. As a result, its ability to handle highly viscous biomaterials is limited [33, 34]. The viscosity's maximum value is in the range of 0.1 Pa/s [15]. Small scaffolds are currently the main product of inkjet bioprinting [15]. To boost printing speed and create larger cellular constructions, inkjet printheads with numerous nozzles have been adapted [35]. When combining direct ink printing with microvascular multinozzle printheads, the throughput printing capabilities can be enhanced [2].

IV. EXGRUSION BIOPRINTING

Extrusion bioprinting involves the continuous extrusion of bioink from a nozzle tip in the form of line structures that are propelled by mechanical or pneumatic pistons [36]. Instead of producing droplets, extrusion printing creates filaments [37]. Extrusion bioprinting requires biomaterials with high enough elastic moduli and adequate loss moduli [38, 39]. So that they may be extruded via the tip, they should be almost fluid in form [38, 39]. The extrusions, meanwhile, ought to be sturdy enough to keep their shape both before and after printing [38, 39]. The microextrusion printing, biomaterials are adaptable [38, 39]. For example, it is possible to print thermoplastic biomaterials by heating and extruding them as a liquid, which is then solidified to take the form of the desired shape [38, 39]. Typically, extrusion-printed scaffolds have higher structural stability [40, 41]. A high viscosity cell suspension could be printed using the microextrusion process [42]. However, as compared to inkjet and laser bioprinting, its resolution is quite low [42]. Other restrictions include the selection of materials and stress-induced cell deformation [42, 43].

V. LASER BIOPRINTING

Droplets are transported by a laser-induced forward transfer in laser bioprinting [17]. A source film with the printing chemicals, a "ribbon" with a donor transport support, and a pulsed laser beam are the basic components of laser printing [38]. A ribbon that supports the source film and absorbs laser energy [25]. A high-pressure bubble created by the laser pulse's high energy generates material droplets from a source film onto a substrate for collection [16]. Laser bioprinting was initially used to create two-dimensional patterns in cells or polymers [27]. A 3D structure can only be printed by alternately

depositing hydrogels and cells over a number of cycles, utilizing droplets produced by laser pulses in recent decades [1, 20]. Nozzles are absent in laser bioprinting [30]. It helps get rid of the blockage that happens more frequently in extrusion and inkjet bioprinting[12]. High-viscosity bioinks could be printed using it [44]. The viscosity of the bioinks, the printing speed, the energy, and the pulse frequency of the laser all affect the printing resolution of laser bioprinting [24]. Single-cell manipulation and precise positioning of cell droplets are made possible by laser bioprinting [45]. Even so, because laser light causes cell death during bioprinting, the cell viability is frequently lower than that of inkjet bioprinting [33].

VI. TISSUE AND ORGAN BIOPRINTING

Due to its limited capacity for seeding, cell seeding to casted scaffolds has drawbacks but these difficulties can be solved via 3D bioprinting [3]. High-density cells can be precisely positioned in the appropriate spot via 3D bioprinting [41]. Its capacity to arrange many cell types in a systematic way mimic the diverse architectures of natural tissues [16]. The acute cell death caused by the dispensing pressure and nozzle diameter is one of the main issues of printed cell-laden scaffolds [1, 12]. The printing conditions must be improved to increase cell viability. Moreover, the substances that are used to create bioinks must be carefully chosen [19]. Alginate-HA hydrogels with Schwann cells were printed by Rajaram et al. for the regeneration of peripheral nerves [28]. In their study, a polyvinyl alcohol and low amounts of polyethylenimine supplemented calcium chloride crosslinking solution with an alginate-HA strand [46]. The hydrogels that were printed out demonstrated enhanced structural integrity and great cell survival [2]. *In vivo*, the bioprinted constructions created bone tissue with sufficient vascularization [47]. For the encapsulation of bone-related SaOS-2 cells, Neufurth et al. used a sodium alginate hydrogel supported with gelatin [30]. The amount of cell proliferation significantly increased by the addition of agarose and the calcium salt of polyphosphate [48]. All of this suggests that 3D bioprinting can produce tissue constructs that closely resemble the heterogeneous structure and cell distribution of genuine tissue structures [37]. 3D bioprinting has been extensively used to biofabricate cartilage tissues by printing chondrocytes into hydrogel scaffolds [49]. Alginate and chondrocytes were used by Cohen et al. to create 3D implants with variable shapes [49]. The printed scaffolds had a high cell viability [49]. The hydrogels were used to individually encapsulate osteoblasts and chondrocytes [50]. Throughout cell culture, a high cell viability was maintained. Human articular chondrocytes printed with poly(ethylene glycol) dimethacrylate have also been used to create cartilage tissues (PEGDMA) [51]. In these printed structures treated with growth factors and chondrogenic agents, functional neocartilage development was found. Pescosolido et al. printed hydrogels with semi-interpenetrating networks of HA and dextran derivatized with hydroxyethyl-methacrylate (dex-HEMA) [52, 53]. The hydrogel is stable physically and photocrosslinkable [52, 53]. High cell viability was seen in the encapsulated chondrocytes. Recently, hydrogels infused with cell-filled microcarriers were used to print bilayered osteochondral models [54]. The hydrogels' mechanical strength was improved by the inclusion of microcarriers [54]. Higher cell viability was achieved in the printed hydrogels as a result of the establishment of a microcarrier-cell complex [52, 53]. The epidermis is the top layer of the multilayered structure that makes up human skin, and the dermis is the bottom layer [52, 53]. The primary cell types in the epidermis and dermis, respectively, are keratinocytes and fibroblast [22]. Bioprinting of heart valves has proved effective. The heterogeneous structure of the aortic valve root wall and trileaflets is mimicked by the printed structures. Researchers have printed an aortic valve that mimicked the mechanical heterogeneity of an aortic valve using a PEGDA hydrogel [53]. It was found that the aortic valve could sustain high cell viability for up to 21 days [3]. Alginate-gelatin hydrogels with sinus smooth muscle cells (SMCs) in the valve root and aortic valve (leaflet) interstitial cells (VICs) in the leaflet were used by Duan et al. to print aortic valve conduits [55]. In vitro, SMCs and VICs both exhibit high rates of survival, efficient spreading, and phenotypic retention [56]. Additionally, a trileaflet aortic valve was manufactured utilizing photocrosslinkable hydrogels made of GelMA and methacrylated HA [16, 27]. The hydrogels that were manufactured contained human aortic valve interstitial cells (HAVICs), which demonstrated high cell viability and remodeling capacity [57]. By printing cells and useful materials simultaneously, 3D bioprinting can produce biological tissues with useful features [41]. By combining designed ears with functioning electronics. Bionic ears was developed using alginate hydrogels seeded with chondrocytes were printed into the shape of a human ear [3]. An inductive coil antenna coupled to cochlea-shaped electrodes supported on silicone was manufactured with conducting silver nanoparticle-infused silicone [6]. The *in vitro* cell culture allowed the printed ear to keep its shape [58]. The inductive coil antenna was surrounded by cartilage, and the neocartilage displayed good morphology and tissue-level survival [46]. Furthermore, the printed ear could pick up electromagnetic signals and play stereo audio [59].

VII. SKIN TISSUE 3D BIOPRINTING

The human epidermis and dermis have developed into a complex structure, with subcutaneous tissue being the third zone [6]. Such a structure protects the body from exposure to UV rays, keeps skin from drying out, and functions as a barrier

against the entry of poisons and infections [44]. Skin is also recognised as the immune system's initial line of protection [60]. The epidermis grows from the inside to the outside, with mature cells at the surface and growing keratinocytes in the basal layer [61]. The majority of the top layer of the epidermis is composed of keratinocytes organised into the keratinised stratified squamous epithelium [33]. This basement membrane also separates the epidermis from the dermis [62]. In the stratum corneum, proliferative cells undergo differentiation sequentially, with newly undifferentiated cells at the bottom and terminally differentiated cells at the surface [11]. The purpose of melanin, which is released by melanocytes, is UV protection [22]. It is also responsible for the skin's pigmentation, which gives it its colour [63]. In addition to nerve endings and glandular ducts, the epidermis contains immune system cells such as Langerhans and T-cells [5]. The dermis, the second layer of skin, is also composed of two layers: the upper papillary dermis, which consists of loose, areolar connective tissue and dermal papillae that protrude through the epidermis to form ridges that leave fingerprints when sweating; and the lower reticular dermis, which is composed of dense, irregular connective tissue [63]. The papillary area is rich in type III collagen, whereas the reticular region has more type I collagen [63]. This variation in the ratio of collagen in the extracellular matrix of the dermis is responsible for the skin's elasticity and mechanical strength [62]. Due to the numerous cell types, it contains, including vasculature, neurons, and hair follicles, the dermis links the rest of the body and the skin [62, 63].

VIII. CARDIAC TISSUE 3D BIOPRINTING

Cardiovascular diseases (CVDs) are one of the leading causes of death globally, especially in industrialised nations [21]. One estimate says the annual incidence of myocardial infarction is around eight million people [13]. Heart valves are impacted by diseases such as stenosis and others [27]. The loss of irreplaceable cardiomyocytes is a big issue with these heart-related disorders, as these cells lack a repair or regeneration mechanism [64]. The loss of cardiomyocytes is compensated for by the creation of non-functional scar tissue, dramatically raising the risk of acute cardiomyopathy [64]. Currently, these disorders are treated with coronary artery bypass grafting, cell therapy, and left ventricular assist devices, with heart transplantation as the last choice [65]. This gets us to the second big issue, which is the dearth of organ donors and the hazards connected with the transplantation procedure, such as immunological rejections, which result in a dismal success rate. Tissue engineering mitigates various issues associated with healing injured blood arteries and heart valves [64]. Traditional cardiovascular tissue engineering techniques involve stem cells' development, maturation, and proliferation on a functional biomaterial scaffold that facilitates stem cell differentiation [64].

Autologous and allogeneic stem cells are the cells of choice for cardiac tissue engineering due to their broad availability and a lower likelihood of immunological rejection of grafts [65]. Due to their biocompatibility and resemblance to the native tissue matrix of the cells, decellularised tissue matrices and synthetic and natural hydrogels have been attempted for cardiovascular tissue engineering scaffold creation [64]. Due to the intricacy of heart tissue, which needs the integration of cells from diverse stem cell sources such as cardiomyocytes, fibroblasts, and endothelial cells, forming a functioning cardiac construct is difficult. Also problematic is achieving the autorhythmic characteristics of the myocardium [66]. 3D bioprinting can be used to overcome these obstacles [67]. It is capable of layer-by-layer construction of a functioning cardiac construct, layer-by-layer [56]. Several attempts have been made to make the myocardium work again using 3D printing to make scaffolds and "tissues-on-a-chip" [56, 67].

IX. MICROCHANNELS 3D BIOPRINTING

The vascularisation of thick tissue constructions remains a significant obstacle, despite significant progress in the fabrication of 3D tissue constructs with acceptable cell density and defined structure [68]. Although vascularisation can occur in response to biochemical stimulation by growth factors, the angiogenesis process could take one to two weeks [69]. There is widespread usage of sacrificial materials to generate microchannels and cavities [70]. Sucrose, glucose, and dextran have been printed into microchannels that may be dissolved in cell culture, producing vascular channels. These channels can be lined by endothelial cells [71]. Blood can be perfused via the channels using a high-pressure pulsatile flow [71]. This vascular casting method provides independent control over the shape of the template and the production of complicated vascular systems [72]. Additionally, microchannel networks may be printed using agarose that can be aspirated under a vacuum or manually extracted [73, 74]. Numerous cell types and chemical stimuli are involved in the angiogenesis process [75]. Studies have been conducted to evaluate if 3D bioprinting can include the controlled release of these elements. Research has shown that VEGF may be introduced into bioinks by combining gelatin microparticles (GMPs) with bioinks [76]. The prolonged-release of VEGF from GMPs resulted in a considerable increase in scaffold vascularisation compared to the rapid release group created by combining VEGF with hydrogels [44, 60].

X. CONCLUSION

In the realm of tissue and organ regeneration, additive manufacturing in bioprinting has enormous potential. It allows for synthesising physiologically relevant tissue with improved and consistent functional outcomes for patients. Such approaches are preferable to autografting and allografting since autologous grafts generate needless patient stress, and there is a severe lack of allograft donors. 3D bioprinting provides a unique possibility since it creates tissue from the bottom up, eliminating the danger of immunological transplant rejection and alleviating donor shortage difficulties. 3D bioprinting might potentially result in a patient-specific therapy that yields superior therapeutic outcomes and is visually beautiful. Nevertheless, despite the field's advancements, there are still several obstacles to biocompatibility and integration of the printed construct with the body. Maintaining cell viability in the bio-ink formulation and then printing them in exact geometries necessitates standardising printing procedures and stringent quality control to preserve the printed construct's quality.

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