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3D Bioprinting for Tissue Engineering Application Review

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Abstract – Three-dimensional (3D) printing (rapid prototyping or additive fabricating innovations) has gotten significant consideration in different fields in the course of recent many years. Tissue engineering uses of 3D bioprinting, specifically, have attracted the attention of numerous researchers. 3D platforms delivered by the 3D bioprinting of biomaterials (bio-inks) empower the recovery and rebuilding of different tissues and organs. These 3D bioprinting methods are helpful for creating platforms for biomedical and regenerative medication and tissue engineering applications, allowing quick production with high-accuracy and control over size, porosity, and shape. In this review, we present an assortment of tissue designing applications to make bones, vascular, skin, ligament, and neural structures utilizing an assortment of 3D bioprinting strategies.

Keywords – Bioprinting, Scaffold, Bio-ink, Tissue engineering.

I. INTRODUCTION

Recently, regenerative medication and tissue engineering research has been coordinated toward the recovery, substitution, or reclamation of injured functional living tissues and organs, like bone, vascular, skin, neural, and ligament [1,2,3, 4, 5]. These tissue engineering applications require the experiences of researchers from various fields, just as particular information on biomaterials, cell science, biocompatibility, imaging, and the characterization of platform surfaces [6, 7]. Perhaps the main parts of tissue engineering are the manufacture of permeable three-dimensional (3D) frameworks that give the suitable climate to recovering tissues and organs. 3D platforms for use in tissue engineering field are created utilizing a different assembling techniques and biomaterials [8,9]. A several significant qualities should be considered in these applications [10]: First and in particular, a tissue engineering framework should be biocompatible. Second, created 3D frameworks ought to be biodegradable or bioabsorbable so that tissue at last replaces the platform. Third, the ideal platform ought to have mechanical properties reliable with the tissue to be embedded. Fourth lastly, the 3D platform ought to be promptly manufacturable in an assortment of shapes and sizes. A several strategies have been produced for manufacturing 3D platforms utilizing engineered and characteristic polymers, including gas foaming, phase separation, electrospinning, and melt molding [11, 4, 12, 13].

These framework manufacture strategies can't exactly control the pore size, shape of the platform, or the inward channel design inside the platform. In addition, there are limits on the ability to create platforms utilizing cells.

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In recent years, 3D bioprinting techniques that can control the size and state of a 3D framework and the ability to create platforms along with cells have stood out [14, 15].

In this review, we portray 3D bioprinting and its use in adjusting shape and size of a 3D framework. 3D bioprinting was first presented by Charles W. Frame in 1986 [16].

3D bioprinting is the way toward making 3D strong or gelation objects utilizing 3D demonstrating programming dependent on computer aided design (CAD) or computer tomography (CT) scan imaging [17]. 3D bioprinting refers to the method related with making a 3D construction utilizing metals, pottery, artificial materials, or natural polymers [18]. Commonly, 3D bioprinting equipment comprises of a X-, Y-, Z-axis drive machine, PCs, 3D demonstrating programming, and bioprinting materials. Subsequent to making a plan utilizing CAD or CT pictures, the 3D bioprinting hardware associated with a PC makes a 3D platform structure [19]. 3D bioprinting techniques ordinarily comprise of stereolithography (SLA) [20], digital light processing (DLP) [21], multi jet modeling (MJM) [22], fused deposition modeling (FDM) [23], specific laser sintering (SLS) [24], and laminated object manufacturing (LOM) [25].

The SLA and DLP techniques utilize fluid photopolymer resins and ultraviolet (UV) lasers to make 3D constructions [21, 20]. The MJM technique includes uses materials through a small diameter nozzle in a material injection process that operates in a manner similar to typical inkjet printers [22]. MJM is an inkjet bioprinting measure that utilizations print head advances to deposit photograph curable plastic resins or casting wax materials in a layer-by-layer strategy. The FDM 3D bioprinting strategy is a typical and basic technique for utilizing thermoplastic fibers as a bioprinting material [23]. Fibers are softened in the top of a 3D printer by warming and afterward used to make 3D designs. The SLS bioprinting innovation combines little particles, like polymers and ceramics, by warming utilizing a powerful laser to shape a 3D construction [24]. LOM is a strategy for making 3D models by stacking layers of characterized sheet materials, like polymers, plastics, and metals [25]. These techniques are utilized in an assortment of fields, including design demonstrating, arts, lightweight machinery, and in 3D biomaterials utilized in tissue engineering.

Among the 3D bioprinting strategies accessible SLA and DLP laser-based bioprinting techniques, inkjet bioprinting, and the FDM bioprinting of 3D construction utilized in tissue engineering field have pulled in the most consideration from scientists.

II. Bone Tissue Engineering Using 3D Bioprinting

Bone has a profoundly particular organic- inorganic structure that can be classified into a micro and nano-composite construction that is kept up neighboring complex cell segments [26]. Scientists have researched efficient ways to deal with replacing lost or defective bones and developing good bone substitutes for an extremely long of time [27]. The 3D artificial bone platform is utilized in the clinic for bone recovery. Bone displays superb self-recuperating capacities if defects are small; in any case, enormous scope bone losses or deformities can't be totally mended by the body's intrinsic recovery frameworks [28]. In such cases, surgery is expected to supplant or fix lost or defective bone. Researchers have attempted to produce bone substitutes and to create restoration techniques [29, 30, 31, 32]. Bones might be characterized into two sorts of constructions: cancellous bone (inner part of the bone), which has a supple design with a porosity of 50–90%; and cortical bone, which frames a thick external layer with a porosity of under 10%. Differences between the inner and outside constructions of bone require cautious thought for framework plan for use in bone recovery. Platforms can be utilized to convey biomolecules, for example, TGF-beta, BMP, IGF, FGF, or VEGF, to advance bone recovery [33]. As of late, researchers have been keen on 3D bioprinting advances that can make structures in a single step utilizing a various of biomaterials [34, 35].

Bone tissue designing field is dependent on different 3D bioprinting methods. Rath et al. performed preliminary in vitro tests to assess the impacts of dynamic versus static 3D culture conditions during the cultivating of osteogenic cells (bone marrow-inferred stromal cells and osteoblasts) onto biphasic calcium phosphate (CaP) 3D frameworks under osteo- inductive and basal culture conditions [36]. Dong et al. coordinated poly(ε-caprolactone) (PCL) and chitosan thermo gels to frame a hybrid 3D framework for bone recovery [37]. The 3D PCL frameworks were manufactured by FDM bioprinting strategy. The in vitro study shows that the cross-breed 3D framework could upgrade cell multiplication and improve the osteogenesis of rabbit bone marrow mesenchymal stem cells (MSCs). They theorized that the PCL/chitosan 3D frameworks could improve osteo-inductivity, cell cultivating viability, and give phenomenal mechanical properties contrasted with the PCL or chitosan-thermo gel 3D platform alone. Corcione et al. built up a solvent free process for creating a hydroxyapatite and poly (lactic corrosive) composite material reasonable for 3D bioprinting measures (utilizing the FDM technique) to acknowledge customized platforms for bone tissue engineering [38]. In their

investigation, a clinical picture of maxillary sinus acquired by cone beam computer tomography were changed over into a reasonable arrangement and effectively used to create a 3D maxillary sinus model utilizing 3D bioprinting of the composite material. Wang et al. clarified that a cell-loaded collagen/alginate platform could be enhanced with bio glass particles, an all-around created, permeable, hard material used to manufacture bone substitution frameworks, to build the material solidness and animate cell development and mineralization [39].

III. Neural Tissue Engineering Using 3D Bioprinting

In excess of a billion people around the world, are thought to experience the ill effects of sensory system issues [40]. Chronic degenerative diseases or traumatic injury of the sensory system influence central sensory system (CNS) function. Neurodegenerative diseases because of aging are additionally getting progressively significant. Notwithstanding numerous examinations, treatments that can completely reestablish neuronal capacity are not yet accessible, and our molecular understanding of pathogenic mechanisms is restricted. These limits emerge from the absence of models appropriate for recreating complex conditions in vivo. 2D cultures are fundamentally utilized for their expense viability, simplicity of taking care of, and relevance to different cell types; nonetheless, 2D cultures can't uphold the cell–cell and cell–extracellular matrix (ECM) interactions present in vivo [41,42, 43, 44]. Paradoxically, 3D tissue engineering is thought to give a more human-like climate for cells. An assortment of 3D tissue engineering frameworks has been read for their capacity to incorporate various cell types and make complex neural tissue organization structures [45, 46, 47, 48]. 3D bioprinting can precisely imitate the complexity of our bodies utilizing precisely positioned cells and biomaterials dependent on an ideal plan [62].

Xu et al. utilized fibrin as a bio-ink to make 3D cell structures. Whole cell patch-clamp recordings and immunostaining examination showed that embryonic hippocampal and cortical neurons kept up their capacities and fundamental cell properties, including normal, healthy neuronal phenotypes and electrophysiological characteristics, subsequent to being printed through warm inkjet nozzle [49].

Ilkhanizadeh et al. utilized an inkjet bioprinting framework to print biologically active macromolecules onto poly(acrylamide)-based hydrogels that were accordingly cultivated with essential fetal neural stem cells (NSCs). They tracked down that the printed macromolecules remained biologically active when imprinted onto poly(acrylamide)- based hydrogels and affected the separation of multipotent essential fetal NSCs in an effective and spatially very much controlled way [50]. Lee et al. detailed a tissue engineering framework for bioprinting murine NSCs, VEGF-delivering fibrin gels and collagen hydrogels in the development of an artificial neural tissue. They affirmed the morphological changes showed by the printed murine NSCs installed in the collagen and its relocation toward the VEGF-delivering fibrin gel. The murine NSCs showed high practicality (92.9%) subsequent to bioprinting, a suitability comparable to that of physically plated cells. Murine NSCs printed inside 1 mm from the boundary of a VEGF-delivering fibrin gel showed development factor-prompted morphological changes. The cells printed inside this range relocated toward the VEGF-delivering fibrin gel, with a complete movement distance of 102 ± 76 μm following 3 days of culture. The outcomes show that 3D bioprinting of VEG-delivering fibrin gel upheld supported arrival of the development factor in the collagen framework [51]. Owens et al. printed fully biological grafts composed exclusively of cells and cell secreted material. They printed grafts in a rodent sciatic nerve injury model of both motor and sensory function. Specifically, they thought about the regenerative limit of the 3D bio printed grafts with that made out of hollow collagen tubes or autologous grafts by estimating the compound action potential (for motor function) and the adjustment in the mean arterial pressure as a result of electrically evoking the somatic pressor reflex [52]. Hsieh et al. utilized a thermo-responsive hydrogel as a bio-ink and 3D bio printed NSCs. The stiffness of the hydrogel could be effectively calibrated dependent on the solid substance of the dispersion. The NSCs in the PCL/poly-DL-lactide (PDLLA) hydrogels showed separation and excellent proliferation yet not in the PCL/poly-L-lactide (PLLA) hydrogels. In addition, the NSCs-loaded PCL/PDLLA hydrogels infused into a zebrafish embryo neural injury model protected the capacity of the impeded sensory system; be that as it may, the NSCs-loaded PCL/PLLA hydrogels just showed minor repair impacts in the neural injury model. The function of an adult zebrafish with traumatic brain injury was saved in the wake of embedding the 3D bio printed NSCs-loaded PCL/PDLLA develops [53]. Gu et al. delivered neural tissue by 3D bioprinting human NSCs that were supporting neuroglia and separated in situ into functional neurons. The bio-ink consolidated novel clinically pertinent polysaccharide-based biomaterials including agarose, alginate and carboxymethyl chitosan. Differentiated neurons formed synaptic contacts, set up networks, were immediately active, showed a bicuculline-induced increased calcium reaction, and prevalently expressed gamma-aminobutyric acid [14, 15].

IV. Vascular Tissue Engineering Using 3D Bioprinting

3D tissue engineering to copy human body functions has been sought after for quite a while. As the thickness of a 3D tissue increases, blood vessels become fundamental parts. In a 2D cell culture having a cell population thickness of roughly 20~30 μm, nutrients and oxygen promptly diffuse. Be that as it may, when the 3D tissue thickness surpasses 100 μm, it is hard for oxygen and supplements to diffuse to each side of the tissue [54]. Hence, vascular tissue guided into the 3D tissue serves to supply nutrients, oxygen and remove waste products. new blood vessel formation in highly vascularized tissues (e.g., liver, kidney, lung, spleen, heart, pancreas, or thyroid) is fundamental [55, 56]. Along these lines, there is general agreement that the ability to remake complex vascular networks is vital to 3D tissue engineering [53]. Be that as it may, this issue stays a significant hindrance to endeavors to make 3D engineering constructions with the volume and complexity of human organs [57, 58, 59]. 3D bioprinting advancements that make objects of desired shape utilizing an assortment of bio-inks and cell types have arisen as attractive ways to deal with planning small diameter vessels. The advantages of 3D bioprinting technique are that researchers can deliver hetero cellular tissue constructs that promptly control the cell thickness. This gives scientists better tools for tending to angiogenic issues in 3D tissue engineering [57]. These methods can make biomimetic microenvironments in 3D tissues and produce vessels with ideal functions and constructions.

Cui et al. manufactured fibrin microchannels utilizing an inkjet-based bioprinting technique. While bioprinting human microvascular endothelial cells (HMVEC) loaded fibrin hydrogel, they affirmed that the cells adjusted themselves inside the fibrin channels and multiplied to frame intersecting linings. A 3D tubular construction was acquired from the printed patterns. They inferred that all the while bioprinting both the cells and platform advanced HMVEC proliferation and microvasculature formation [60]. Norotte et al. printed small diameter-multilayered tubular vascular grafts that were promptly perfused for additional maturation. Agarose was utilized as a bio-ink to print smooth muscle cells and fibroblasts, aggregated into discrete units, either multicellular spheroids or cylinders of a controlled diameter (300–500 μm). The post-bioprinting fusion of the discrete units brought about single-and double layered small diameter vascular tubes [61]. Wu et al. utilized an omnidirectional bioprinting technique to manufacture 3D microvascular networks inserted inside a pluronic F127 hydrogel framework. Utilizing this technique, they created 3D microvascular networks utilizing a various leveled, 3-generation branching topology to frame microchannels of diameter 200–600 μm, in which two large parent channels were partitioned into numerous smaller microchannels [62]. Kolesky et al. revealed another 3D bioprinting technique for manufacturing 3D tissue develops packed with vasculature, ECM and different kinds of cells. They affirmed the printability of these designs utilizing two materials, a silicone elastomer and Pluronic F127, and affirmed cell feasibility utilizing a cell-loaded gelatin methacryloyl (GelMA) hydrogel. They tracked down that human neonatal dermal fibroblasts and human umbilical vein endothelial cells (HUVEC) multiplied over time [63]. Jia et al. [64] created perusable vascular constructions with exceptionally requested courses of action in a single step process. 4-arm poly (ethylene glycol)- tetra acrylate (PEGTA), GelMA, and alginate were utilized in blend with a diverse coaxial expulsion printing framework to accomplish direct 3D bioprinting. The rheological properties of the bio-ink and the mechanical qualities of the subsequent develops were tuned by presenting PEGTA, which encouraged the precise deposition of complex multilayered 3D perusable hollow tubes. This mix bio-ink likewise showed good biological characteristics that upheld the proliferation and spread of typified endothelial and stem cells inside the 3D bio printed builds, prompting the exceptionally coordinated, development of organically pertinent, perusable vessels [64]. In different methodologies, perusable frameworks and 3D bioprinting have been coordinated to accomplish 3D tissue vascularization. Pimentel et al. [65] created thick (1 cm) and thickly populated tissue develops utilizing a 3D 4-arm branch network with firmness practically identical to that of soft tissues. This build could be straightforwardly perfused on a fluidic stage over long time (>14 days). They utilized poly (lactic corrosive) (PLA) as the help structure and poly (vinyl liquor) (PVA) as the water-dissolvable principal material. The PLA was specifically eliminated, and the PVA structure was utilized to make an artificial 3D vascular network inside the ECM that impersonated the solidness of the liver and encapsulated hepatocellular carcinoma (HepG2) cells. These hybrids builds were straightforwardly perfused with medium to instigate the expansion and development of HepG2 spheroids. In this examination, the most noteworthy spheroid thickness was gotten with perfusion, however generally speaking, the tissue develops showed two distinct zones: one with a high cell death rate and practically no cell division and one of fast expansion. The model, in this way, produced tissue angles inside necrotic tumor regions [65].

V. Skin Tissue Engineering Using 3D Bioprinting

In human body, the skin is the biggest organ and shields different tissues from outer external stimuli. Skin damage prompting infections, or other genetic or actual illnesses, can create chronic ulcers. Skin injuries can expose different tissues to the external

environment, including bacteria and viruses. Skin injuries can upset internal heat level guideline. Pathological components of ordinary skin flora can multiply within the sight of a messed-up skin hindrance. Skin misfortune in people can lead to death in extreme cases [66]. Subsequently, skin damage is a significant issue with broad impacts on different tissues. Autologous grafts got straightforwardly from the patient are frequently used to avoid immune rejection and reestablish skin function and wound healing. Unfortunately, skin damage wounds over large areas or with a significant depth are not adequately healed utilizing autologous grafts [67, 68, 69]. Consequently, there is a need to create artificial skin substitutes utilizing novel ways to deal with skin recovery [70, 71]. These studies have created complex skin substitutes that associate with human tissues after in vitro development and transplantation [72, 73, 74]. It has been difficult to mimic the skin of an individual while obliging nerve endings, vessels, multilayered 3D designs, and the various subordinate constructions, like sebaceous glands, sweat glands, and hair follicles. Complex skin structures require accurate signaling frameworks. Without such frameworks, the skin structure is lost [75]. In this regard, 3D bioprinting is an attractive strategy that empowers the development of human skin structure mirrors utilizing the spatio-temporal designing of different bio-inks and cells.

Koch et al. create a skin model containing dermis and epidermis layers utilizing 3D laser-based bioprinting framework. They utilized an alginate hydrogel as a bio-ink and printed fibroblast, keratinocyte, and hMSC. They at that point assessed the impact of the laser-put together bioprinting framework with respect to the cell expansion, survival rate, and apoptotic action. Changes of the cell surface markers and DNA damage were surveyed and genuinely assessed more than a few days. The cells survived the exchange strategy with a viability surpassing 98%. All cell types tried kept up their expansion capacity after 3D bioprinting [76]. A collagen bio-ink, keratinocytes, and fibroblasts were printed to frame a basic skin structure. The 3D bioprinted cell builds were passed after various culture times utilizing immunohistological techniques. The presence of cell–cell channels, which showed tissue arrangement, was explored in the indispensable 3D designs [77]. Michael et al. manufactured a skin substitute utilizing 3D laser-based bioprinting. The skin substitutes were made utilizing fibroblasts and keratinocytes. These 3D designs were in this way tried in vivo. The bioprinted keratinocytes shaped a multifaceted epidermis with beginning separation and layer corneum following 11 days of culture. Their multiplication was predominantly identified in the suprabasal layers. E-cadherin, a pointer of adherent's intersections and, subsequently, tissue arrangement, was found in the epidermis in vivo just as in vitro. In mice, some veins were found to develop from the injury edges and the injury bed in the bioprinted bearing of the cells [78]. Lee et al. exhibited the possible utility of 3D bioprinting in tissue engineering utilizing skin model as a prototypical human model. They printed collagen as a bio-ink, and fibroblasts and keratinocytes were utilized as constituent cells to frame the dermis and epidermis. Immunohistological portrayal uncovered that the 3D bioprinted skin tissue was naturally and morphologically illustrative of human skin tissue in vivo. Compared with traditional strategies for tissue engineering for skin, 3D bioprinting offers a several benefits as far as structure maintenance and shape, reproducibility, adaptability, and a high culture throughput [57, 58]. Cubo et al. printed bilayer skin utilizing fibrin bio-inks containing human primary keratinocytes and fibroblasts and human plasma. The structure and function of the 3D bioprinted skin was analyzed utilizing immunohistochemical strategies, both in 3D cultures in vitro and after long -term transplantation into immunodeficient mice. In the two cases, the recovered skin was very much like human skin and, besides, was indistinct from the bilayered dermo-epidermal counterparts that were hand made in laboratories [79].

Nanoparticles have recently, arose as a transdermal delivery framework. Their surface properties and size decide their efficacy and efficiency in infiltrating the skin tissue. Hou et al. used 3D bioprinting innovations to create a worked on artificial skin model valuable for quickly screening nanoparticles for their transdermal entrance limit. A collagen hydrogel was utilized as a bio-ink, and fibroblasts were printed into the construction. The adequacy of this stage was assessed by utilizing a 3D framework utilizing one layer of fibroblasts sandwiched between two layers of a collagen hydrogel to screen silica nanoparticles with various surface charges for their entrance capacity. Emphatically charged nanoparticles exhibited further infiltration, predictable with perceptions from past investigations including living skin tissue [80]. (Figure1.1)

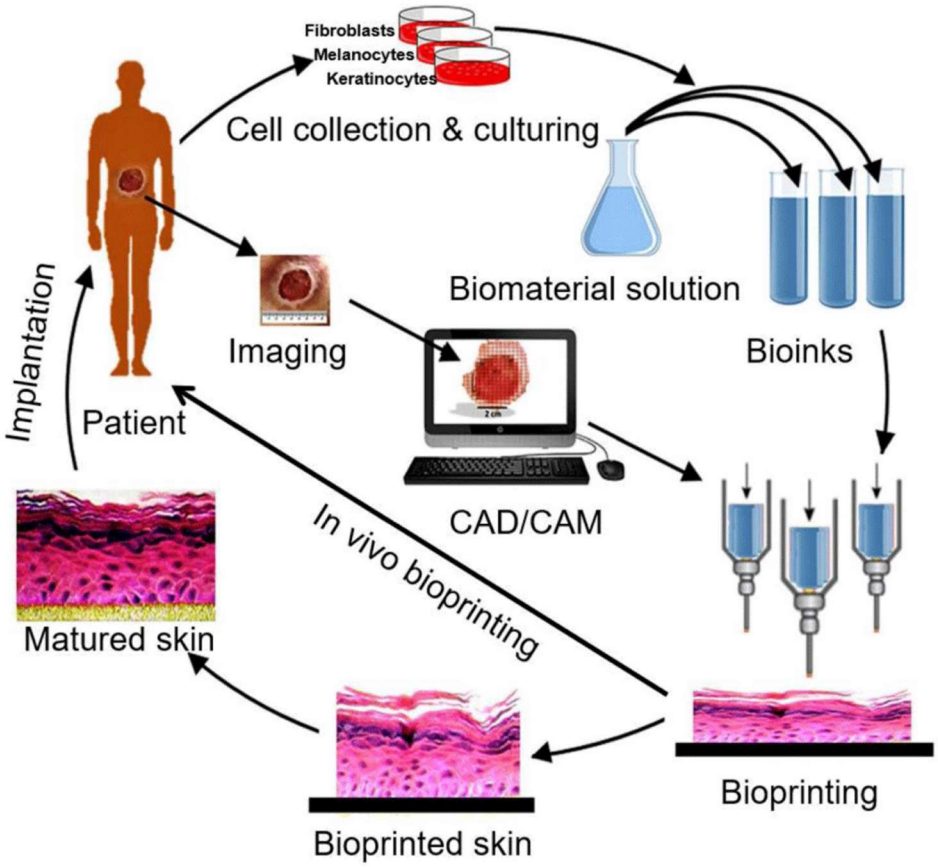

Figure 1.1: The 3D bioprinting process of skin tissue [81].

VI. Cartilage Tissue Engineering

Using 3D Bioprinting Ligament, which is a couple of millimeters thick, forestalls grinding among joints and perseveres through outrageous burden stresses during limb movements. The cartilage defects because of aging, degenerative diseases, trauma or other a several factors unavoidably lead to arthralgia and chronic disorders [82,83]. Regardless of various attempts, artificial cartilage that can completely mimic the composition of the tissue, ECM, and mechanical properties has not yet been created [84]. 3D bioprinting, which can create results of an ideal shape utilizing different materials and cells, presents an incredible open door in cartilage tissue engineering.

Kundu et al. created cell-printed 3D platforms utilizing the PCL and chondrocyte-embodied alginate hydrogel. Cell-based biochemical in vitro examines were performed to measure the DNA, complete collagen content, and glycosaminoglycans (GAGs) in the distinctive alginate/PCL gel builds. Alginate/PCL gels containing transforming factor-β (TGF-β) showed more prominent ECM arrangement. The cell-printed 3D alginate/PCL gel platforms were embedded in the dorsal subcutaneous spaces of female mice. Immunohistochemical analysis uncovered enhanced cartilage tissue and collagen type II fibril arrangement in the alginate/PCL gel (TGF-β) mixture platform following a month [85]. Kesti et al. built up a cartilage specific bio-ink for use in 3D bioprinting applications dependent on a mix of alginate and gellan blended in with commercially available BioCartilage particles. They imaged bioprinted frameworks utilizing magnetic resonant imaging (MRI) to compare the 3D shapes and the first model, and assessed the utility of MRI in identifying changes in the water relaxation times as they identified with ECM creation in engineered grafts. To assess cartilage development, cell-loaded Bioink and Bioink/BioCartilage plates were refined for about two months in vitro with and without TGF-β3 supplementation. The entirety of the attributes of the 3D bioprinted platforms were better than those of local articular cartilage [86]. Ren et al. utilized collagen hydrogel as a bioink and created 3D cartilage builds. The chondrocyte thickness slope demonstrated a zonal conveyance all through the ECM. They assessed the impact of the chondrocyte thickness inclination on the development of the regional distribution of ECM in the bioprinted 3D construction. The ECM creation was

emphatically corresponded with the cell thickness during the beginning phases of culture, and the biosynthetic ability of chondrocytes were influenced by both the cell thickness and the cell dispersion in the bioprinted 3D structure [87].

VII. Cancer Model Using 3D Bioprinting

Malignant growth is a main source of disease and death all through the world. Notwithstanding progresses in malignant growth treatment, numerous difficulties remain, and the attributes of the tumor microenvironment should be considered [88, 89]. Malignant growth research regularly depends on 2D cultures and animal models; in any case, it is hard to copy 3D human tissues in 2D cultures, and animal model outcomes can't really be extrapolated to the human reaction [90, 91, 92, 93, 43, 44]. These issues might be tended to by facilitating our comprehension of complex cancers tissue utilizing 3D tumor models that can emulate the microenvironment of local malignant growth.

Snyder et al. created a 3D liver disease model and examined the radiation protection functionalities and drug change of living liver tissue analogs. They utilized Matrigel as a bio-ink onto which were printed human hepatic carcinoma cells. Cell-loaded bioprinted Matrigel and microfluidic chips were utilized to assess the radiation protecting properties of the liver cells utilizing the amifostine pro-drug. The advantages of radiation protection and the transformation of pro-drug by numerous cell types were best acknowledged utilizing a dual tissue model [94]. Huang et al. made an in vitro 3D computer chip in a hydrogel utilizing 3D projection bioprinting. The computer chip included a honeycomb fanned design that mirrored a 3D vascular morphology valuable for checking, and investigating differences in the behavior of malignancy cell lines (Hela) versus normal cells (fibroblasts). Fibroblasts displayed more noteworthy morphological changes because of channel width than HeLa cell lines; in any case, the channel width impacted fibroblasts movement, while HeLa cells relocation expanded as the channel width diminished [95]. Zhao et al. revealed a HeLa cells loaded 3D bioprinting alginate/fibrinogen/gelatin hydrogels to develop in vitro cervical tumor models. Cell reasonability was surpassing 90% utilizing the characterized bioprinting innovation. Examinations between the 2D and 3D culture models uncovered that the HeLa cells showed a higher multiplication rate in the bioprinted 3D culture model and would in general frame HeLa cells spheroids, though they shaped monolayer cell sheets in the 2D culture framework. HeLa cells in the bioprinted 3D models additionally showed higher chemoresistance and higher grid metalloproteinase (MMP)expression than those in the 2D cultures [96].

VIII. Challenges and Future Directions

Hitherto, an assortment of 3D bioprinting advances have been utilized to study tissue engineering applications expected to mimic an assortment of tissues and organs. 3D bioprinting has prepared consolidating biomaterials, imaging, displaying, and computational advancements in the biomedical and tissue engineering field. The 3D bioprinting advances licenses acclimations to the shape, porosity, and size of the 3D frameworks, drawing in much consideration in the tissue engineering field. A several difficulties stay, for instance the advancement of biomaterials (bio-inks) for use in bioprinting tissues or organs. Regular 3D bioprinting centers around 3D construction creation without cells, though late 3D bioprinting innovations have rapidly and precisely delivered 3D designs utilizing cells in a single step. 3D bioprinting with cells requires materials with brilliant biocompatibility and cell partiality. Therefore, the advancement of bio-inks is vital for bioprinting with cells. Later on, it will be important to grow new biomaterials and increment the exactness of bioprinting hardware to rapidly make precise 3D designs.

Table 1.1: Most common bio inks and their applications.

bio inks	Cell/Tissue Type	Reference
Alginate	Chondrocyte, cartilage	[97,98]
Collagen	Hepatocytes	[99,100]
Fibrin	Skeletal Muscle, Neural Tissues	[101,102]
Hyaluronic acid	Fibroblasts, bone cells	[103,104]
Poly (ethylene glycol)-PEG	Fibroblasts, cartilage	[105,106]
Gelatin	Bone, cartilage	[107–108]

IX. Conclusion

Three-dimensional (3D) printing of biological structures has great attention in last ten years and their application have started to utilize in regenerative medicine. This technology is in early progression step but it has huge possibility to implement in tissue

engineering, drug delivery, cell/tissue and organ products, and other biomedical applications [109]. Despite of increasing articles, patent and researches of 3D bioprinting of living tissues or organs, it still faces significant challenges as unstable cellular behavior and more complexities compared to non-biological printing process. In order to avoid these challenges, researchers need to work in multidisciplinary teams with engineers, biomedical scientists, basic scientist and medical doctors [110].

One of most important challenges of 3D bioprinting relies on in situ bioprinting as known as printing cells and biomaterials directly onto or in a patient [111]. However, the bioprinting technology is still being developed, it will be necessary to improve with ethical issues and related laws in order to use of 3D printers in industry, hospitals and academia. New regulations must be made before adopting developed prototypes into clinical application [112,113].

In the future, it is foreseen that researches will focus on enhancing 3D printing materials with respect to biocompatibility, mechanical properties, in situ bio printability and sustainability of printed cells, tissues and organs via vascularization process [114]. Maintaining of cell viability, vascularization and cell migrations are the main limiting factors in generating of large sized, fully functional, tissues and organs. Especially vascularization is related to the nutrient diffusion process so it must be developed for realization of in vivo applications of 3D bioprinting [115]. Also, novel bioinks were still developing in order to use as new scaffolds or unique cell, tissue applications. With these improvements, 3D bioprinting technology will be a revolutionary approach in medicine.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

Authors have equally participated and shared every item of the work.

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