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Three-dimensional Bioprinting with the Use of Induced Pluripotent Stem Cells in Regenerative Medicine

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Abstract: The field of regenerative medicine has witnessed significant advancements in recent years due to the emergence of three-dimensional (3D) bioprinting technology. One promising approach is the utilization of induced pluripotent stem cells (iPSCs) as a cell source for bioprinting, allowing for the construction of complex tissue structures. iPSCs hold immense potential as they can be derived from a patient's own cells, enabling personalized therapies, and eliminating the risk of immune rejection. The integration of iPSCs with 3D bioprinting technology expands the possibilities of regenerating damaged tissues and organs. iPSCs can be programmed to differentiate into various cell types, offering the ability to generate specific tissue structures. The bioprinting process involves the precise deposition of cells, growth factors, and biomaterials in a layer-by-layer manner, mimicking native tissue architecture. This spatial control allows for the creation of intricate tissue constructs with high fidelity, enhancing their integration within the host tissue upon implantation. This review aims to review the current progress and challenges in utilizing 3D bioprinting with iPSCs in regenerative medicine.

Keywords: 3D bioprinting; iPSCs; tissue engineering; regenerative medicine

1. Introduction

In recent years, the emerging field of regenerative medicine has unveiled promising possibilities for developing effective therapies to treat various diseases and injuries. One remarkable advancement within this field is the combination of 3D bioprinting technology with the utilization of Induced Pluripotent Stem Cells (iPSCs) [1]. iPSCs have revolutionized the field of regenerative medicine by providing a readily accessible, patient-specific source of cells that can be used for tissue engineering and regeneration. Integrated with the precision and versatility of 3D bioprinting, iPSCs offer a potential approach for creating functional and personalized tissues [2]. The use of scaffold materials is currently mainly limited to non-cellular carriers such as bone fillers and absorbable stents. However, recently there is a growing interest in the use of cellular carriers for the creation of artificial tissues. When choosing a biomaterial, surface chemistry, charge, the possibility of chemical interaction, protein adsorption, and other factors must be in consideration. In addition to chemical aspects, physical properties achieved during fabrication, such as geometry, stiffness, and surface roughness, can affect the way cells adhere, proliferate, and differentiate [3].

2. Induced Pluripotent Stem Cells

Induced pluripotent stem cells are genetically reprogramed adult cells, and their use has advantages in tissue engineering. The benefits are relatively simple extraction from cutaneous sources, such as dermal fibroblasts, compared to invasive extraction from

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bone marrow or adipose tissues; and autologous transplantation which avoids immunogenicity and enhances in vivo survival. iPSCs were first generated from mouse fibroblasts by the introduction of four transcription factors (Oct4, Sox-2, c-Myc, and Klf-4) through genetic reprogramming where retrovirus was used [1]. Since human iPSCs are created without destroying an embryo, the ethical concerns are significantly reduced. More importantly, iPSCs offer a key advantage over embryonic stem cells (ESCs) by allowing the use of mature somatic cells from patients with genetically defined diseases. The resulting iPSCs reflect the donor's unique genetic changes, enabling the characterization of specific phenotypes in patient-derived stem cells and their differentiated progeny. These disease-specific differentiated cells can be utilized for drug screenings to identify compounds that specifically reduce or reverse the observed phenotypes. Achieving a straightforward, efficient, and rapid reprogramming protocol presents several challenges. One key decision is selecting the appropriate reprogramming method [4]. Currently, the most widely used technique involves integrating reprogramming factors into the genome via lentiviral or retroviral transduction. This method is the easiest and most efficient to date. However, future focus will likely shift to other methods, as cells produced through permanent and random integration of foreign genes pose a certain oncogenic risk, making them unsuitable for therapeutic applications. To circumvent the use of integrating viruses, alternative reprogramming approaches have been developed, such as the use of Sendai viruses, plasmids, modified RNA, or small molecules [5].

3. Three-dimensional Bioprinting

3D bioprinting is an emerging technology that combines three-dimensional (3D) printing techniques with biotechnology to create functional living tissues and organs. This technology has the potential to revolutionize the field of medicine and significantly impact various industries, including healthcare, pharmaceuticals, and regenerative medicine [6]. At its core, 3D bioprinting is a layered manufacturing process that involves the deposition of living cells, biological materials, and biomaterials in a controlled and precise manner to construct three-dimensional structures [7] (Fig. 1).

The actual printing of the tissue or organ starts with the selection of appropriate bioinks that can provide structural support and a suitable microenvironment for cell growth and differentiation. Low compartmentation compared to natural tissues/organs is one of the main disadvantages of current bioprinting techniques. Most tissue/organ structures are more delicate than current bioprinting devices can achieve. One of the main questions in 3D bioprinting techniques, in comparison to regular additive manufacturing methods, is attributed to the direct involvement of living materials during the fabrication process [8]. The development of more realistic in vitro culture can be achieved by combination of 3D bioprinting and iPSCs. However, one of the setbacks in the bioprinting of undifferentiated iPSCs is their sensitivity to mechanical forces occurring during the printing process. For this reason, the parameters must be precisely optimized, as the cells undergo high shear forces, laser radiation, or electric/thermal stress [8,9].

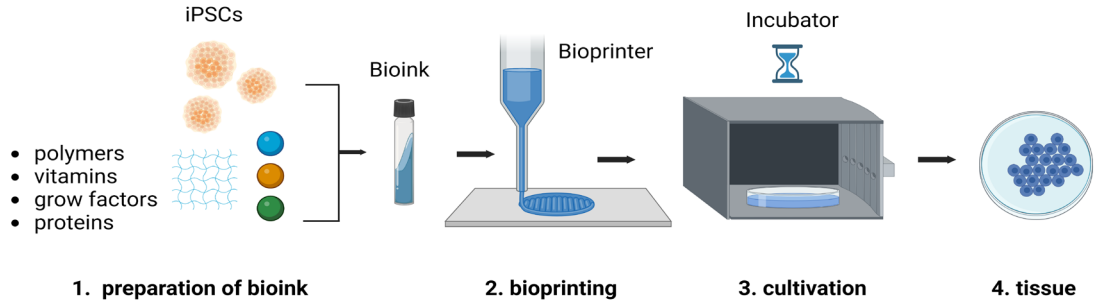

Figure 1: Basic steps of the process of bioprinting with iPSCs (Biorender.com)

2.1. Bioink

Bioink is a specialized type of ink that is used in the field of biofabrication to create three-dimensional structures, such as tissues and organs, using 3D bioprinting techniques. This ink is typically composed of two main components: biological materials and a support matrix. The biological materials can vary depending on the specific application, but they commonly consist of cells, growth factors, and other biomolecules that are essential for tissue development and regeneration. Growth factors are proteins that play a crucial role in regulating cell behavior and tissue formation, while other biomolecules, such as extracellular matrix components, can provide structural support and mimic the natural environment of the target tissue [10,11]. For the different bioprinting technologies, several unique properties of the bioink are required. Higher viscosities may improve the stability of the construct however highly viscous bioinks may have negative outcomes on extrusion pressure since higher pressure is required for greater viscosities. Mechanical improvement of bioink can be achieved by addition of polysaccharides. Gu et al. in their study combined alginate with carboxymethyl-chitosan and agarose when preparing extrudable porous ink, which showed improvement in bioink formulations as well as encapsulation of spheroids. The viscosity of bioink should be modifiable, to support the usage of the same bioink in diverse available bioprinters. There is a requirement for a specific shear thinning property in extrusion and droplet-based printers to compensate for the high shear stress created during the printing. The printed structure should support the cellular behavior and needs enough stiffness to maintain the printed 3D structure. Viscosity can be regulated by toning of the molecular weight, polymer concentration, the mass of additives, temperature, and precrosslinking [12,13].

2.2. Inkjet bioprinting

Inkjet bioprinting uses the method of placing droplets of bioink-containing cells and biomaterials at the exact spot layer by layer [14]. There are two types of actuators: thermal and piezoelectric. Thermal actuators use temperatures up to 300 °C for a few microseconds to generate bubbles of picolitre size that are consequently released through the nozzle onto the substrate. The actual change in temperature of the substrate must not be more than 10 °C, for cells to stay viable. Piezoelectric crystal generates pulses and so acoustic bio-printer ejects nanolitre eventually picolitre droplets [15]. However, there is a possibility of acoustic cell disintegration with frequency from 15 to 25 kHz that may damage cell membranes and escalate to cell lysis. Viability is about 70 – 95% depending on cell type and printing parameters. Acoustic depositions provide a gentler approach to cell viability than thermal ones. One of the main drawbacks is that cells can sometimes form aggregates and clog the nozzles. This can be prevented with chemical additives, although not toxic to the cell cultures. It is essential to know how the jetting process affects biomechanics, metabolism, and physiology of cells through thermal and mechanical stress, such as shear, tension, and compression [16]. iPSCs have been successfully three-dimensionally bioprinted into cartilage mimetic structures utilizing a composite bioink composed of nanofibrillated cellulose (NFC). This process involved the co-printing of iPSCs with irradiated human chondrocytes, facilitating the formation of the desired cartilage-like tissue [17]. Vega et al. demonstrated how iPSC-derived neural progenitor cells (NPCs) could be bioprinted into defined structures using Aspect Biosystems' novel RX1 bioprinter in conjunction with a unique fibrin-based bioink. This rapid bioprinting process, which required less than five minutes to print four tissues, preserved high levels of cell viability (greater than 81%) and maintained the differentiation capacity of the NPCs [18].

2.3. Extrusion bioprinting

In extrusion bioprinting, bioinks are extruded from the nozzle in form of filaments, which are deposited layer-by-layer to construct the final product. Thanks to its cost-effectiveness and ease of operation in comparison with inkjet and laser bio-printers it is arguably the most commonly used bio-printing method [19]. One of the main advantages of extrusion bioprinting is sidestepping the harsh conditions (heat, shear, shock, chemicals, etc.) that cells can encounter in other bioprinting methods. Other benefits are the use of wide-ranging viscosities of bioinks, high cell density, and various concentrations of cells. Disadvantages include deformations of hydrogel, comparably lower resolution, the potential of clogging of the nozzle, and an apoptosis of cells due to improper pressure used [20]. This technique is commonly used in manufacturing of orthopedic tissue thanks to its ability to precisely deposit multiple cell types and biomaterials and cre-

ate cell-laden heterogeneous and hierarchical structures [21]. Dogan et al. demonstrated that human induced mesenchymal progenitor cells (hiMPCs), after being formulated into an alginate/collagen type I bioink and subsequently extruded, retained their ability to form complex vessels displaying a hierarchical network. This process mimicked the embryonic steps of vessel formation during vasculogenesis [22]. Bilkic et al. assessed the feasibility of bioprinting NPCs in three-dimensional hydrogel lattices using a fibrinogen-alginate-chitosan bioink, which had been previously optimized for neural cell growth and subsequently modified for structural support during extrusion printing. The mechanically robust three-dimensional constructs promoted the formation of neural progenitor NPCs clusters and preserved their morphology and viability throughout the entire culture period [23].

2.3. Stereolithography bioprinting

Stereolithography (SLA) bids numerous benefits in the form of high resolution and rapid printing of greatly complex scaffolds. The ability to fabricate implantable scaffolds with anatomically accurate geometry, controlled surface characteristics, and tunable physical and chemical assets make SLA highly relevant for clinical applications [24]. Significant importance in SLA 3D bio-printing is the illumination source. It is the key element to define the performance of the printer and the definition of printed parts, and the properties of the scaffold can be adjusted by controlling the illumination source. Bioinks in stereolithography need to be biocompatible, biodegradable, and photocurable which leaves us with a relatively limited range of materials. Frequently used bioinks include PEGDA, poly(propylene fumarate) (PPF), PTMC, poly(e-caprolactone) (PCL), GelMA, and poly(D, L-lactide) (PDLLA) [25]. To improve cell performance and induce specific differentiation, combination of photocurable materials and EMC is used. However, the viability in SLA bioprinters is lower in comparison with other methods since these bioinks tend to be less biocompatible and photoinitiators may negatively impact cell survival [26]. Wang et al. built a low-cost stereolithography system around a commercial projector, incorporating a simple water filter to prevent harmful infrared radiation from the projector. Visible light crosslinking was achieved using a mixture of polyethylene glycol diacrylate (PEGDA) and gelatin methacrylate (GelMA) hydrogel with an eosin Y-based photoinitiator. Experimental results with NIH 3T3 fibroblast cells showed that this system could produce highly vertical three-dimensional structures with a 50 μm resolution and maintain 85% cell viability for at least five days [27]. Grigoryan et al. addressed the limitations of hydrogel stereolithography in capturing spatial heterogeneity within mammalian tissues. They developed and characterized a multi-material stereolithography bioprinter that enabled controlled material selection and precise regional feature alignment while minimizing bioink mixing [28].

3. Results and Discussion

In most tissues and organs an extensive vascular network is required to provide nutrients and oxygen to the cell. Vascularization is critical during the biofabrication process for the engineering of tissue constructs. One of the most complicated challenges in 3D bioprinting is the incorporation of a vascular network that can sufficiently provide nutrients even deep within the tissue (Fig.2). With current technology, it is still technically challenging to print functional capillaries at the micrometre scale. One alternative is to let capillaries develop by first fabricating a vascular network that then matures in vivo or in bioreactors [29].

Levato et al. discovered that polymer microspheres called microcarriers help in cell adhesion, attachment, and growth. There is a possibility to modify these microcarriers with bioactive molecules to induce stem cell differentiation. Stem cells behavior and phenotype can also be influenced by the surface grooves by being parallel or in channel form. Parallel grooves can cause the cells to be aligned in elongated form which can help differentiation of stem cells into fibroblasts and cardiomyocytes. 3D bio-printing can be extend-ed into 4D printing which works on time-dependent and predetermined change within scaffold with controlled differentiation [30,31].

Traditional bioprinting methods often rely on creating cell-loaded scaffolds to support tissue growth. However, scaffold-free bioprinting techniques are emerging, which enable direct printing of cells without the need for supporting structures. This approach eliminates the potential issues associated with scaffolds, such as poor cell distribution and biocompatibility concerns [32,33].

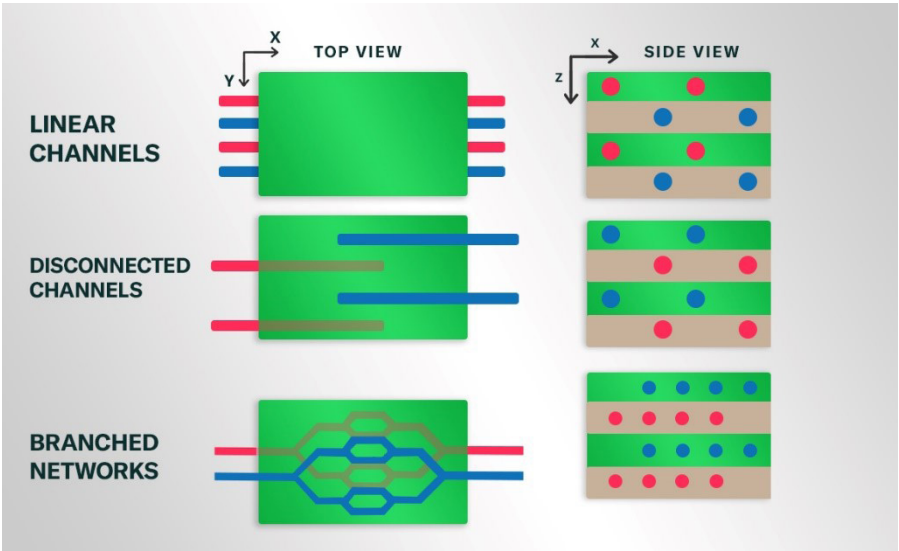

Figure 2: Figure shows top and side view of vascular networks. Bioprinting can produce tubular hollow constructs that form linear or disconnected channels of the complex branched network. These channels can be prefunded with a substrate with growth factors and oxygen or be a base for vascularization.

4. Conclusions

It is the simplicity of 2D cell culture that undoubtedly attributes to its failure compared to the natural in vivo environment. Particularly gradients in nutrients, oxygen, and growth factors are almost nonexistent in 2D cultivation and their deficiency can significantly change the cell behavior. The main goal is to develop a 3D culture technique that enables the fabrication of complex, multifaceted constructs that can resemble a complex natural environment. Additionally, the compatibility of iPSCs with various bioinks and biomaterials needs to be thoroughly investigated to achieve optimal cell viability, proliferation, and functionality within the printed constructs. The integration of induced pluripotent stem cells into 3D bioprinting has brought us closer to the realization of functional and personalized tissue and organ engineering [34]. The advancements in iPSC generation, targeted differentiation, and scaffold-free bioprinting have laid the foundation for this transformative field. However, challenges pertaining to resolution, vascularization, maturation, and immune response continue to demand innovative solutions. Further research, interdisciplinary collaborations, and technological advancements are paramount to overcoming these hurdles and unlocking the full potential of 3D bioprinting with iPSCs.

Acknowledgments

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