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3D Bioprinting Innovations: Pioneering Solutions for Cardiac Disease

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Abstract. Cardiac disease persists as one of the leading causes of morbidity and mortality worldwide and therefore there is a critical need for the establishment of innovative treatment approaches. This review updates the innovations in 3D bioprinting technology and its applications in cardiac tissue engineering, a fast-developing field that aims to overcome the limitations of the current treatments of cardiac diseases. The 3D bioprinting technique enables the layer-by-layer construction of complex tissue structures emulating natural cardiac tissue in architecture and function using bio-inks composed of living cells, biocompatible materials, and growth factors. The paper reviews inkjet-based, extrusion, and laser-assisted bioprinting techniques, all having different advantages and challenges. It further identifies the need for proper biomaterials, namely natural and synthetic polymers, to facilitate cellular growth and differentiation, as well as the 4 main cell types utilized in the bioink. The 3D bioprinting applications will be reviewed in the fabrication of heart patches and prosthetic heart valves and their potential to improve cardiac repair and regeneration will be described. Although these developments are quite promising, challenges relating to scalability, cell viability, and regulatory considerations remain. The conclusion drawn is that further research in the refinement of bioprinting methodologies and integration with advanced technologies is required so that cardiac care can be revolutionized with personalized and effective therapeutic solutions.

Keywords: 3D bioprinting technology, cardiac disease, innovations.

1. Introduction

Within the last years, 3D bioprinting technology moved to the forefront of biomedical innovation, offering robust tools to create complex structures of tissue individualized in architectural and human organ function mimicry. Using bioinks made from living cells, biocompatible materials, and growth factors, 3D bioprinting enables new paths of research and therapeutic application by layer-by-layer deposition [1]. Among the myriad applications that are possible, cardiac tissue engineering stands out as one of the most promising lines of research, as this has an impact on the improvement of treatments for cardiovascular diseases, which are the leading cause of death in the world [2]. The cardiac tissue is structured and constituted mainly by cardiomyocytes, a complex entity responsible for heart muscle cells undergoing rhythmic contraction. Other relevant constituents are the fibroblasts and endothelial cells, which contribute to the architecture of the heart and the blood flow [3]. Despite the sophisticated design, there is only a limited degree of self-healing in the heart following damage, such as that caused by a heart attack—a process that normally leaves behind scarring rather than restoring healthy heart

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muscle [4,5]. This suggests a serious therapeutic challenge to the re-establishment of the complete function of the heart following its damage using available treatments. This has produced a problem limit those existing medical treatments cannot totally surmount, which results in the gradual loss of heart functionality, culminating in heart failure. 3D bioprinting, as a novel technology was utilized to help counteract this kind of challenge allowing for the creation of very fine heart tissue that mimics the heart structure and functions in a natural way. Recent advances in this field have enabled the creation of heart patches and other constructs more closely approximating the properties of the heart, promising better integration into the host tissue and recovery of cardiac function. Progress on the development of biocompatible scaffolds and methods to enhance cell survival upon printing are also key to translational applications of 3D bioprinting.

2. 3D bioprinting techniques

The three most common types of 3D bioprinting technology for fabricating cardiac tissues include Inkjet-based bioprinting, Laser-assisted bioprinting, and Excursion bioprinting [6]. Each of them has a different working principle and corresponding advantages and disadvantages, and each type of printing will be described and compared subsequently.

2.1. Inkjet-based

Inkjet-based bioprinting technique works by delivering controlled quantities of bioinks to a printing surface by droplets, which are then stacked by layering as needed to form 3D structural scaffolds [7]. Compared to the original 2D inkjet printers, the contents of the reserve cartridges have been changed to cellular bio-solutions and control of the Z-axis has been added to the electronic control of the inkjet printers, which makes the overall flexibility and maneuverability easier for the construction of the 3D scaffolding, as well as being an efficient and inexpensive approach for scaffolding building in general [8]. There are two types of energy support for inkjet printing, thermal and voltage printing. Voltage printing is more widely used due to concerns about the effect of high temperatures on the cellular appearance in bio-solutions [9]. However, it still has strict requirements for the viscosity and fluidity of the printing biomaterial, which must be in a liquid state to facilitate the delivery of tiny nozzles to be ejected without damaging the activity of their cellular contents [10]. In addition to this, what also needs to be taken into account in cardiac tissue printing is that inkjet-based printing products do not keep a high cell density, which can make it challenging to form highly organized tissues [11].

2.2. Excursion

Extrusion bioprinting utilizes either pneumatic or mechanical pressure to eject biomaterials through a nozzle for structural construction [6]. Bioinks are printed continuously and uninterruptedly in cylindrical lines, as opposed to the dripping droplets style in inkjet, and are more widely used in projects where the structural integrity of the scaffolding is required [7]. The main advantage of extrusion bioprinting in cardiac tissue printing is the wider choice of biomaterials [12]. It works with a large range of cellular solution viscosities, and the compatible bioink is used to stabilize the structure of its cellular encapsulation when printing extrusion [13]. Meanwhile, the CMs cellular deposition density produced by extrusion bioprinting is also considered to be conducive to cardiac tissue construction [11]. In addition to this, micro extrusion-based bioprinting can achieve compatibility with the use of multiple printheads corresponding to multiple bioink types in the same printer [6]. Compared to inkjet bioprinting, however, the activity and functionality of the cells tend to be reduced in this technique process, as the streamlined jet of ink needs to be cut during the printing process to achieve the corresponding assembly.

2.3. Lser-assisted

Laser-assisted bioprinting utilizes laser pulses transmitted on a glass-based ribbon [6]. The interaction of the laser causes the biomaterial to evaporate and then be adsorbed onto the ribbon for droplet formation, accomplishing the positional movement of the cellular content. Laser-assisted bioprinting allows for high-precision pattern formation and composition of 3D structures [7]. It is worth noting that

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the biggest difference between it and the previous two techniques is that it does not require a nozzle, which is a good way to circumvent the damage that shearing stress of the print biomaterial may cause to the cells inside, and it also facilitates the sedimentation of high-density cells [11]. In addition to this, the high cellular activity output of laser-based printing is also attributed to the fact that there is no direct contact between the bio-ink and the dispenser during the printing process and no additional pressure is applied [7]. But at the same time, this new technology also faces some problems, the biomaterials utilized in this technique need to be photocurable for final construction, and common lighting stimulation like UV light may damage the cell DNA and affect their function [14]. The high cost of equipment and maintenance as well as the long printing time also need to be considered, but it still has a promising development prospect due to its special nature.

3. Biomaterials for cardiac bioprinting

Bioinks as a key foundation for 3D tissue fabrication are usually made of natural or synthetic polymers that play a role in supporting cell growth, while also assisting in cell reproduction and mature differentiation [11]. From the point of view of printing requirements, the chosen bioink material needs to possess high stability [15]. Stability in this context refers not only to its structural composition, but also to the sedimentation and solidification properties it provides in the printing technique. When considering practical applications such as the modelling of myocardial tissue, it is also necessary to take into account the fact that human ECM properties should be reverted as much as possible in order to maximize the level of differentiation and functionality of the cells.

Nowadays the main choices for 3D heart tissue printing bioink are from natural biomaterial sources. These materials are usually isolated and washed directly from the target biological tissues, and their biggest advantage is that they maintain a large degree of biocompatibility similar to that of the tissue of interest. For example, in cardiac tissue bioprinting, the most commonly used decellularized extracellular matrix (dCEM) is a naturally derived material [14]. It retains most of the cellular cues in heart tissue and does a remarkable job of supporting cell adhesion and differentiation, which are crucial in tissue formation [14]. However, its weaknesses are the low viscosity of the solution, which needs to be improved for better 3D scaffold structural maintenance, and There is also the concern of immunogenic problems from potential chemical residues of biomaterial extraction [11].

Synthetic bioinks, on the other hand, have a strong scope to manipulate molecular control and achieve the desired physical and chemical properties to fulfill the needs. For example, they can have better integrity and printability to support better robust 3D structural stacking, in addition to reducing immunogenicity issues through molecular modulation [11]. The functionalized polymers under consideration are mainly biodegradable hydrogels, which are designed to interact with native cardiovascular tissues after synthesis with good tension and flexibility and can be metabolized promptly after in vivo assisted repair and construction [14].

Lastly, hybrid bioinks consist of various combinations of natural and synthetic polymers discussed previously. Each biomaterial possesses distinct advantages and disadvantages; thus, hybrid bioinks are designed to harness the benefits while mitigating the downsides to create more effective bioinks for 3D bioprinting. A material called polycaprolactone (PCL) helps increase the strength of printed designs, while doesn't support cells well. To solve this problem, the researchers added tiny particles called carbon nanotubes (CNTs) to help the cells grow better on the PCL [14]. In addition, the researchers explored the integration of human decellularized extracellular matrix (hdECM) with gelatin methacryloyl (GelMA) hydrogels to create cardiac patches for heart repair. Compared to GelMA alone, the inclusion of hdECM enhanced the differentiation of neonatal human cardiac progenitor cells (hCPCs) while decreasing their proliferation [14].

In the end, the most important component of the printing material is the cell source, as they will be integrated for the actual tissue regeneration. The cell types utilized in CTE could be mainly summarized in the following four types, which also have their own advantages and disadvantages. (1) CM cell lines such as H9c2 cells, are derived from embryonic rat ventricular tissue and are used as a substitute for primary cardiomyocytes. They have similar properties as CM but do not undergo autonomous

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contraction [14]. (2) Primary cardiomyocytes, on the other hand, are more widely used for CTE. They are isolated directly from neonatal rat hearts, so the actual cardiac tissue is more similar, however, the issue of immune-matching during cross-species transplantation can be a concern. (3) Cardiac Progenitor Cells (CPCs) are derived from pediatric patients undergoing cardiac surgery, and this class of cells has a high ability to improve cardiac functions but is in limited supply. (4) stem cell-derived cardiomyocytes, as a product of transcription factor editing technology, have high medical potential and can be used to create and supply a variety of cell types including cardiomyocytes. It is also possible to customize the treatment to the individual and avoid immune reactions [16].

4. Applications in cardiac tissue engineering

4.1. 3D Bioprinting application in heart valve disease

Recent developments in the area of 3D bioprinting have dramatically improved the ability to model cardiac tissue, with a particular focus on valve diseases such as calcific aortic valve disease. In this respect, van der Valk et al. introduced a breakthrough 3D bioprinting model that mechanically replicated the human aortic valve leaflets in vitro [17]. By incorporating methacrylated gelatin (GelMA)and methacrylated hyaluronic acid in a hydrogel, they engineered a model that could replicate the aortic valve's characteristic mechanical properties based on its layers and encapsulate human valve interstitial cells. Using this novel approach, the VIC mechanobiology response to hydrogel stiffness was studied, and one was able to demonstrate that under conditions of osteogenic differentiation, VICs in fibre-like hydrogels exhibited considerable pathological differentiation and microcalcification [17]. Another development of a prototype of prosthetic heart valves is the TRISKELION, whereby three-dimensional printing was used in the fabrication of a polymeric heart valve that could combine the good hemodynamic properties of a biological valve with the durability of the mechanical prosthesis. Tschorn et al. showed that their prototypes designed and printed to high precision in FreeCAD had silicone prototypes with good performance metrics. The regurgitation fraction was 22.26% ± 4.34%, which demonstrates an effectively high regurgitation fraction against the standard biological and mechanical valves with values of 8.55% ± 0.22% and 13.23% ± 0.79%, respectively. Also, the mean systolic pressure gradient for TRISKELION was 9.93 ± 3.22 mmHg, which offers a competitive hemodynamic performance compared to the biological and mechanical ones. Such results presented an opportunity for 3D printing not only in rapid prototyping but also for the structural and functional optimization of the heart valves themselves [18]. As this field continues to evolve, further advances in biomaterials can be integrated to enhance both performance and device longevity for these bioprinted cardiac devices, ultimately improving the prognosis for the patient undergoing cardiovascular surgery.

4.2. 3D bioprinting of heart patches

3D bioprinting in heart valves has shown a good faith effort to replicate biomechanical and biological properties required to function. Similar advancements in technology also show quite strong potential for cardiac patches. These patches are used to repair and regenerate damaged heart tissues, mainly after injuries such as heart attack or myocardial infarction. It seems to show a very promising approach towards the enhancement of heart functioning. Noor et al. published a compelling study on the use of materials derived from patients in an innovative way for the generation of thick, vascularized cardiac patches that anatomic and biochemically adhere to the recipient's heart. The study reported that the thickness reached about 2 mm with the printed vascularized cardiac patches, which is important for achieving structural integrity and functionality in a clinical setting. Also, the calcium imaging results showed that the signal propagation velocity in such bioengineered patches reached more than 10 cm/s within the parenchymal tissue, demonstrating efficient electrical coupling among the cardiomyocytes [19]. Another investigation on an adjunctive cardiac patch, consisting of polycaprolactone and gelatin methacrylate, showed that it had the potential to repair myocardium post-myocardial infarction. Their patch was shown to support human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) for a period of 14 days in culture with cell viability over 90%, demonstrating a good environment for

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cell survival, which is necessary for tissue regeneration [20]. On top of that, the study reported that the iPSC-CMs embedded within had high expression of cardiac-specific genes like connexin 43 and cardiac troponin T, suggesting that they were maturing and beginning to develop a cardiac phenotype that was critical to the recovery of myocardial function [20]. These developments in 3D bioprinting highlight, more than anything else, the possibilities of cardiac patch development as one of the game-changing developments to come in the near future, promising an unprecedented clinical transformation in individual cardiac therapies.

5. Challenges and limitations

The field of 3D bioprinting in cardiac tissue engineering has a lot of potential, but several key challenges and limitations can be highlighted, which ought to be addressed to push this technology further towards clinical application. One main technical challenge for all bioprinting processes concerns scalability. Current technologies fail to balance high-resolution printing against volumetric scalability, which is needed to create full-size functional cardiac constructs [21]. This is often at the expense of extended production times, which further diminishes the possibility of fabricating tissues of clinically relevant sizes at the required resolution.

Another critical challenge relates to cell viability during and after the bioprinting process. Under conditions like shear stress involved in extrusion, possible exposure to hostile environmental conditions, and time for maturation post-printing, the survivability of the printed cells can get drastically negatively impacted, thus affecting their functionality [1]. Thus, the high viability of the cells while maintaining the integrity and functionality of bioprinted tissue is the key to success for cardiac constructs but remains an ongoing challenge.

Several important regulatory and ethical challenges impede the translation of 3D bioprinted cardiac tissues into the clinic, beyond technical and biological. A clear system of guidelines and standards should guarantee safety and efficacy in a clinical setting of advanced bioprinting applications, which now maintains a very complex dynamic in its regulatory landscape. Besides this, ethical concerns regarding the origin of cells must be addressed according to the cannons of medical ethics [22].

6. Conclusion

In conclusion, this review has pointed out how 3D bioprinting can be a game-changing technology in cardiac tissue engineering by abdicating its applications, challenges, and future directions. While progress in using bioprinting techniques to fabricate biomimetic cardiac tissues has been well, considerable technical, biological, and regulatory challenges still stand in the way. Eventually, further research is required in the refinement of these technologies and interdisciplinary collaboration to see these 3D bioprinted cardiac tissues finding applications in clinical practice and practical applications in patient care. With integrations of new technologies in the field, and refinement of existing methodologies, surely 3D bioprinting in cardiac tissue engineering will help shape the future. These include bioreactors that would simulate, in an advanced manner, the physiologic environment required for the maturation and functioning of tissues. Such systems may provide mechanical, electrical, and biochemical cues more appropriately to develop functionality and more integration of the engineered cardiac tissue. Another recent technology that enables new possibilities for cardiac tissue bioprinting is an organ-on-a-chip system. Heart microenvironments can be simulated much more accurately by such microfluidic device chip systems, for example, testing the tissue's response to pharmaceuticals and stressors, hence leading toward applications in personalized medicine. In addition, the post-grafting viability and functionality of the bioprinted cardiac tissues are also relevant to the in vitro attempts at the integration of vascular and neural networks. As these developments further unfold, it is believed that they will not only improve the repair and regeneration abilities of cardiac tissue but also provide truly new and personalized solutions that would change the nature of cardiac care.

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