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Advancements and Challenges in 3D Printing of Electroconductive Hydrogels for Cardiovascular Bioprinting

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ABSTRACT

Cardiovascular diseases (CVDs) are the most prevalent cause of fatalities worldwide, affecting both cardiac and vascular tissues. Tissue engineering is a promising treatment alternative for people with end-stage CVDs; however, it has disadvantages such as poor scaffold design control and insufficient vascularization. 3D bioprinting, a recent advancement, has overcome these restrictions by creating layer-by-layer structures such as organs, scaffolds, and blood vessels. This method enables precise control over cell distribution, architectural structure, and compositional correction. Furthermore, since cardiac tissue is electroactive, incorporating electroconductive nanomaterials into the scaffold facilitates intracellular communication, mimics the heart's biochemical and biomechanical microenvironment, and prevents arrhythmia in the heart. In addition, these electroconductive materials can improve the quality of 3D-printed scaffolds. In this study, we will review the different techniques of 3D printing hydrogels after evaluating the many types of hydrogels employed for cardiac tissue engineering (CTE). Then, we will discuss the influence of incorporating electroconductive fillers into hydrogels on printed scaffold quality. Finally, we will briefly discuss the challenges and potentials.

Keywords: 3D bioprinting, Cardiac tissue engineering, Electroconductive hydrogels

INTRODUCTION

Cardiovascular diseases (CVDs) were the primary cause of mortality for 19.9 million people worldwide in 2021, which included 37% of deaths under the age of 70 globally [1]. CVDs are chronic pathological conditions affecting cardiac and vessel tissues, such as coronary heart disease, rheumatic heart disease, and cerebrovascular disease. Myocardial infarction (MI) is the leading cause of CVDs, occurring when plaques in the coronary arteries rupture or erode, producing blood clots and restricting circulation in the heart, completely or partially. This results in hypoxia-induced cardiomyocyte (CM) death and severe inflammation, leading to cardiac tissue degeneration and the creation of scar tissue in the infarcted area [2-5]. Cardiac transplantation is the gold standard therapeutic procedure for patients with end-stage heart failure. While it has achieved clinical success, there are still many challenges and drawbacks to this method, such as the lack of donors, the possibility of organ rejection by the immune system, and the need for immunosuppressive drugs [6]. As a result, innovative treatment procedures such as cell transplantation have been assessed as promising novel treatments for cardiac tissue regeneration for more than two decades. However, cell engraftment and the viability of transplanted stem

cells in the host body are major constraints of stem cell treatment. Furthermore, stem cells cannot develop into more adult-like phenotypes and are unable to generate extracellular matrix (ECM) and vascularization after injection, resulting in sluggish conduction and spontaneous activity, which can lead to fatal arrhythmias after transplantation [7-9]. Tissue engineering is a promising treatment strategy for end-stage CVDs; it can reduce complications and overcome the limitations of cell-based therapeutics. An optimal scaffold for cardiac tissue engineering (CTE) should be morphologically, electromechanically, and biologically similar to the original ECM [10]. Construction procedures for this 3D architecture range from the simple use of hydrogels to electrospun nanofibers and decellularized tissues. However, poor scaffold architecture control, incomplete vascularization, and a lack of cell types that can aid in damaged tissue reconstruction have restricted their efficacy [11]. The 3D bioprinting method is one of the recent promising procedures that has allowed us to overcome these restrictions. Using various components such as biomaterials, living cells, growth factors, and bioactive substances, this approach produces structures layer-by-layer in the form of various organs, scaffolds, and blood vessels. These structures can partially or completely replace natural organs in the targeted host location [12-16]. Bioprinting allows for precise control over the geographical distribution of cells, architectural structure, and compositional correction. Furthermore, it can provide a highly continuous and stable biological structure, allowing for a high-resolution simulation of the heart and paving the path for innovative research into cardiac tissue repair and regeneration using novel technology. Nevertheless, 3D bioprinting-based heart regeneration technology is still in its early phases of development, requiring collaboration among experts to study bioink characteristics at physical and chemical levels to better define myocardial architecture and physiological diversity [17].

Because the heart is an electroactive tissue that can transmit electrical signals, an electromechanical connection between CMs is required for synchronous response to these electrical signals and regulation of cardiac cell processes (such as adhesion, proliferation, and migration) [18, 19]. Nowadays, a number of electroconductive biomaterials are used in the field of CTE. The materials include carbon-based nanomaterials [20], gold-based nanomaterials [21], conductive polymers [22], piezoelectric polymeric materials [23], melanin [24], silicon nanowires [25], silver nanoparticles [26], and selenium nanoparticles [27]. Some studies have previously reviewed the potential applications of 3D printing technology in CTE, focusing on the type of bioink and the type of cells [28-30]. In this study, we will reference the types of hydrogels utilized in CTE and subsequently review the various methods of 3D printing hydrogels. The influence of incorporating electroconductive elements into the hydrogels on the printed scaffolds and the print quality will also be discussed in this review.

Hydrogels as a bioink

Hydrogels are 3D structures of cross-linked hydrophilic polymers that have excellent water absorption capabilities. Hydrogel-based 3D bioprinting has the benefit of developing living structures from bio-inks that contain live cells, growth factors, and other biocompatible components [31]. Bio-inks are categorized into both naturally derived and synthetic materials. Naturally produced materials are more widely employed because of their inherent biocompatibility and near resemblance to the ECM. Natural polymer hydrogels mostly involve polysaccharide-based (agarose, alginate, chitosan), protein-based (collagen, gelatin, fibrin, Matrigel), glycosaminoglycan-based (hyaluronic acid, heparin), or even decellularized ECM [16, 32-37]. It is noteworthy that among naturally derived hydrogels, alginate and collagen are the most commonly used in bioprinting, followed by gelatin methacrylate (GelMA), fibrinogen, and gelatin [38]. The recognition of this group as the most common application component of bioinks stems from their high biological activity and their natural presence in the human body, such as collagen. However, their shortcomings for 3D bioprinting include insufficient mechanical strength, poor rheological characteristics, the tendency to generate immunological responses, and batch-to-batch variability [39]. On the other

hand, synthetic hydrogel polymers generally consist of polyacrylic acid derivatives, polyethylene glycol copolymers, polyvinyl alcohol, polyphosphazene, and synthetic peptides [40, 41]. The biocompatibility of synthetic hydrogels is inferior to that of naturally extracted hydrogels, but their powerful mechanical properties, simplicity of control, and low immunogenicity are their advantages, which attract much attention in fields of tissue engineering such as cardiovascular regeneration [42, 43].

3D bioprinting techniques

The literature describes a variety of techniques for fabricating 3D scaffolds, including solvent casting, molding, electrospinning, and 3D printing [44]. In recent years, 3D printing of hydrogels has received increasing research interest as a reasonable manufacturing strategy for biomedical applications, especially in tissue engineering. Innovative technology for 3D printing of living cells and biomaterials to produce complex scaffolds in CTE comprises droplet-inkjet [45], extrusion-based [46], and laser-assisted bioprinting [47], which will be explained in the next section.

Extrusion-based printing

Extrusion-based bioprinting is a common approach for constructing cardiovascular tissues by distributing cells across a hydrogel matrix altogether and depositing them layer-by-layer, as well as the deposition of a melted polymeric filament known as fused deposition modeling (FDM) [29]. In this method, the pneumatic or mechanical (piston or screw-based) dispensing mechanism extrudes bioinks through nozzles and deposits them on the printed platform [29]. A wide range of choices of materials, ranging from pristine polymers to composite materials, have been used in this procedure [48, 49]. Interestingly, this is the only printing process that has been effectively utilized for 3D bioprinting of electroconductive hydrogels [50]. As the bio-ink should have a low elastic shear modulus under high shear stress to move through the nozzle and a high static elastic modulus to preserve its form after deposition for printing several layers, the rheological properties of the bioinks, such as shear-thinning features and viscosity, play a vital role in the extrusion-based printing method [51]. Nevertheless, the death of cells due to the induced shear stress during the printing technique and also the poor print resolution are the main shortcomings of this technique [52].

Droplet-inkjet printing

Inkjet bioprinting is a popular 3D printing technology for biological purposes. The process involves delivering a regulated volume of bio-inks, often including cells, to preset areas via a thermal or piezoelectric mechanism [53]. This approach promotes exact control of implanted cells, growth factors, genes, and medications and is compatible with a wide range of biomaterials. The great resolution of the droplet-inkjet structure, due to the creation of small droplets, is the key benefit of this technique that allows for precise geometry and scaffold size management. Furthermore, because of its high-speed printing and cost-effectiveness, this technology is frequently employed in the field of blood vessels [54, 55]. However, inkjet bioprinting needs bioinks with low viscosity (3.5-20 mPa·s), resulting in produced constructions with poor structural integrity and mechanical strength. Other drawbacks of this method involve irregular droplet size, low droplet directionality, mechanical and shear stresses on the cells, and repeated nozzle blockage. In addition, controlling the number of cells to be enclosed in a single droplet is another issue that would be considered [52, 56].

Laser-assisted printing

In contrast to extrusion and inkjet bioprinting, where bioinks rely primarily on continuous external mechanical force and gravity to construct a 3D structure, laser-assisted bioprinting relies on precise optical guidance [17]. A pulsed laser beam, a ribbon containing bioink, and a receptive substrate create a laser-assisted bioprinter system. The laser interacts with the ribbon, ejecting droplets of bioink, usually containing cells, onto the substrate. Laser-assisted printing is a nozzle-free technique that eliminates nozzle clogging and maximizes cell viability by reducing mechanical stress during the printing process. The deposition of high cell densities and excellent resolution are

among its advantages. In addition, this method can employ a wide range of biomaterials with varying viscosities (1–300 mPa·s). Despite these benefits, it is mostly utilized for 2D bioprinting (monolayer) and is often ineffective for depositing multiple cell types. Because it is time-consuming, expensive, and works with small-sized constructs, its clinical applications are limited [56].

Stereolithography (SLA) is another 3D printing method based on a laser. SLA is the technique of converting a photocurable polymer solution into a photopolymerized solid in a layer-by-layer form with light energy, such as a UV or laser beam. This procedure solidifies a liquid resin, producing the desired pattern in a resin bath. SLA is employed for high-resolution electroconductive hydrogel printing and is compatible with a wide range of materials [57-60]. The primary advantage of this approach is the shape fidelity of hydrogels after printing. Because of its contactless method, SLA has great cell viability and also allows for the construction of complex patterns with high resolution, such as vascular networks with various scales (50–250 μm) [54, 59, 61]. However, SLA has limitations due to the need for photosensitive materials, which can restrict bioink selection. In addition, the requirement of incorporating a cell type into a hydrogel limits other possible bioinks. Moreover, laser diodes are typically more expensive than nozzles, and there is the possibility of cell side effects after laser exposure during manufacturing [54, 59, 62]. Considering the variety of 3D printing methods and the fact that the ideal bio-inks for each method require prerequisites, selecting the appropriate 3D printing approach might vary depending on the type of hydrogel and the intended application.

Influence of incorporating electroconductive biomaterials in the printability of hydrogels

As previously stated, electroconductivity is critical for biomaterials in CTE because the heart has a conduction system and beats spontaneously at a rhythm. Additionally, CMs require a conductive microenvironment for electromechanical coupling. Despite non-conductive materials being used in research and clinical trials, efforts are ongoing to find effective electroconductive biomaterials for improving CTE [63]. On the other hand, blockage of conduction by non-conductive scaffolds implanted in the desired location, similar to the fibrotic tissue formed after MI, leads to asynchronous contractions of some parts of the heart, which may lead to progressive heart failure [19]. Therefore, the regeneration of a similar biostructure as the ultimate objective of 3D bioprinting needs to be performed in a manner that can retain and establish the requisite biophysical stimulations, including electrical. A varied range of electroconductive biomaterials like carbon-based, gold-based, conductive polymers, piezoelectric polymeric materials, melanin, silicon nanowires, silver nanoparticles, and selenium nanoparticles are widely used in CTE [64]. Also, MXenes and liquid metals have recently attracted increased study attention in the field of biomedical engineering because of their desirable collection of features, including hydrophilicity or high fluidity, metallic conductivity, and strong biocompatibility [65, 66]. Recently, our group published a comprehensive overview that thoroughly examined the advantages and challenges associated with graphene-based nanomaterials in the field of CTE [63]. Such conductive filler materials not only provide the capacity to tune the desired structure and physicochemical characteristics of printed hydrogels, but they also alter the rheological properties of inks during 3D scaffold creation, and these additives can affect the ultimate quality of the printed scaffold [67-69]. Designing a bioink involves evaluating important factors such as printability, stability, biology, rheology, viscosity, gelation, and crosslinking capacities to ensure optimal performance. In addition, the design deviation is determined by bioink parameters like viscosity, shear stress, and fidelity. While increasing viscosity enhances shape fidelity, the increased shear stress may damage cells and produce deceptive biophysical signals. The fidelity of a 3D-constructed structure is dependent on the rapid transition to a solid state following deposition, and also decreasing gelification time enhances structural resolution [58, 70]. As a result, selecting the proper electroactive fillers for bioinks is critical as it affects the main

characteristics, including rheological and mechanical features. Table 1 presented some recent studies about applying the electroconductive hydrogels in 3D printing for CTE. In a study, Moya and his colleagues assessed the influence of combining alginate-based hydrogels with graphene oxide (GO) for 3D printing of scaffolds. The obtained results indicate that with the addition of GO, a significant reduction in recovery time was established. This could be because the GO sheets can interact with the polymer chains through hydrogen bonding, and the GO acts as a physical binder, thus it can improve the viscosity recovery time [71]. Additionally, a study has been conducted in the field of heart tissue repair, during which carbon nanotubes and a scaffold constructed of alginate, methacrylate collagen, and human coronary artery endothelial cells were synthesized using 3D printing method. The obtained results indicated that the existing cells had proper proliferation, migration and differentiation and created patches with vascularization potential before implantation in the body environment. The electromechanical properties of these scaffolds were also suitable [69]. A composite of alginate and GO was fabricated for bone tissue engineering by extrusion-based 3D printing. It was shown that the addition of GO to alginate hydrogel significantly enhanced the printing performance of the prepared bioink. 3D printed scaffolds showed significant improvement compared to those printed with mesenchymal stem cells and alginate. Furthermore, shear thinning behavior in hydrogels containing GO was observed. Among the other findings were a decrease in the pressure required for printing as well as a decrease in the thickness of the printed strands after adding GO, which can be very useful for cell-laden printing because it reduces the shear stress applied to the cells and can improve the exchange of nutrients and oxygen to the encapsulated cells. The resulting composites are suitable in terms of printing ability, structural stability, induction of bone formation, and use in the repair of other tissues [68]. In another study, Serafin et al. created a scaffold from the composition of gelatin-hyaluronic acid containing polypyrrole as a conductive polymer. They 3D printed their scaffolds with an extrusion-based printer. They discovered that the printed scaffolds had high shape fidelity. They stated that adding polypyrrole nanoparticles to hydrogels effectively acted as a filler and that all bioinks had solid-like behavior. Furthermore, the viscosity of bioinks rose with the addition of different quantities of polymer additives, and the homogenous viscosity recovery profile for those with additives happened 5–10 seconds after eliminating the shear stress. Also, the electrical conductivity of the scaffold increased with the addition of polypyrrole. In vitro experiments also demonstrated that the samples containing the additive had 87% viability. They suggested that the created scaffold might be used for electroactive tissue engineering applications such as nerve tissue, cardiac tissue, and spinal cord [72]. In a study, a cardiac patch was developed using aerosol jet printing technology, in which conductive titanium carbide (Ti3C2T*x*) MXene nanoparticles were printed in the form of an aerosol on a PEG substrate with regular patterns. And then were seeded induced pluripotent stem cell derived CMs on the Ti3C2T*x* MXene-PEG composite in a regular pattern. They claimed that their research was a breakthrough in 3D printing with cellular-level resolution. This fabricated structure has no signs of cytotoxicity 7 days after cell culturing. Furthermore, it demonstrated that the gene expression of MYH7, SERCA2, and TNNT2 increased significantly. And also the synchronous beating as well as electroconductivity improved [73].

Methacrylated collagen (MeCol). Carbon nanotubes (CNTs). Human coronary artery endothelial cells (HCAECs). Chondroitin sulfate A (CS). Human adipose tissue derived mesenchymal stem cells (hADMSCs). Polyethylene glycol diacrylate (PEGDA). Polyaniline (PANI). Mouse cardiac progenitor cells (mCPCs). Titanium carbide (Ti3C2T*x*). Human induced pluripotent stem cell derived cardiomyocytes (iCMs). Methacrylated Hyaluronic Acid (MeHA). Human umbilical vein endothelial cells (HUVECs). Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-derived CMs).Polypyrrole (PPy). Mesenchymal stem cells (MSCs). Neuronal stem cells (NSCs). Poly(vinyl alcohol) (PVA). Gallic acid (GA).

Table 1. Some recent studies about applying the lectroconductive hydrogels in 3D printing for CTE.

Biomaterials	Electroconductive nanomaterials	3D printing technique	Cell type	In vitro/in vivo	Main results	Ref
MeCol/ Alginate	CNTs	UV-assisted 3D bioprinting	HCAECs	+/-	↑ Stiffness ↑ Storage modulus ↑ Electrical conductivity ↑ Proliferation, migration, and differentiation of cells	[69]
(Alginate/ CS/ Gelatin) methacrylate	GO	Micro-extrusion 3D bioprinting	hADMSCs	+/-	↑Shape fidelity ↑ Recovery of viscosity after printing ↑ Resolution of 3D printed structures ↑ Cell viability and directed proliferation ↑ Printability ↑ Mechanical strength	[71]
PEGDA	PANI	Micro-stereolithography	mCPCs	+/-	Mimicking the bio-structure of myocardium Rough geometry and irregular pore size due to the ineffective photo-polymerization reaction in hydrogel solution depending on PANI concentration ↑ Strand diameter and pore size ↑ Electrical conductivity ↑ Cell viability	[74]
PEG	Ti3C2T*x*-MXene	Aerosol jet printing	iCMs	+/-	Possibility of cell-level resolution in printing ↑ Expression of MYH7, SERCA2, and TNNT2 ↑ Cell alignment and viability ↑ The synchronous beating of cells	[73]
MeHA	Thiophene	Stereolithography 3D printing	HUVECs and hiPSC-derived CMs	+/-	↑ Viscosity of bioink ↑ Storage modulus ↓ Loss modulus after 3D printing process Solid and stable printed scaffold ↑ Beat rate of cells Biocompatible scaffold	[47]
Gelatin/HA	PPy	Extrusion 3D bioprinting	MSCs and NSCs	+/-	Shear-thinning behaviour of hydrogel Solid-like behaviour of hydrogel ↑ Storage and loss modulus ↑ Viscosity of bioink ↑ Shape fidelity Homogenous viscosity recovery profile High cell viability and attachment	[72]
Fibrinogen	GO	Extrusion 3D bioprinting	MSCs	+/+	Mechanical and regeneration support for the infarcted area ↑ Expression of connexin 43 ↓ Cell apoptosis after MI ↑ Cardiac function	[75]
PVA/ GA	PEDOT	Extrusion 3D bioprinting	Mouse CMs	+/+	Solid-like behaviour of hydrogel ↑ storage and loss modulus ↑ Elasticity Be able to propagating the electrical signal ↑ Cardiac function	[76]
PGS/ TOCNF	PPy	Extrusion 3D bioprinting	H9c2	+/-	↑ Young's modulus and Elongation Shear-thinning behaviour of hydrogel Approved cell biocompatibility, proliferation and attachment	[77]
Alginate/ Gelatin	CNFs	Extrusion 3D bioprinting	NIH/3T3 fibroblasts	+/-	↑ Young's modulus Shear-thinning behaviour of hydrogel Solid-like behaviour of hydrogel ↑ Viscosity of bioink Recovery of viscosity for all hydrogel after printing Approved cell biocompatibility	[78]

Poly(3,4 ethylenedioxythiophene) (PEDOT). Poly(glycerol sebacate)(PGS). 2,2,6,6-tetramethylpiperidine-1-oxyl radical-oxidized nanocellulose (TOCNF). carbon nanofibers (CNFs). NIH 3T3 mouse embryonic fibroblast cells (NIH/3T3 fibroblasts)

CONCLUSION AND PROSPECTS OF FUTURE

3D printing of electroconductive hydrogels is a key research area in bioelectronics, implants, and medical devices, particularly in cardiovascular bioprinting. The 3D printing technique has the potential to produce structures on a very small scale, such as vascular networks, as well as print cardiac patches and even complete organs; however, the latter has a very long way to go to achieve clinical applications. Bioinks are created by combining various cell types, hydrogels, and biochemical factors to mimic the structure and function of heart tissue. Despite the outstanding advancement in 3D printing in recent years, 3D bioprinting-based heart regeneration technology is still in its early stages of advancement, requiring collaboration among experts to study bioink characteristics at the physical and chemical levels to better define myocardial architecture and physiological diversity. The investigation of emerging technologies such as 4D printing in the field of 3D printing of electroconductive hydrogels still needs to be studied. However, some studies have been conducted on the development of 4D printing by using materials sensitive to infrared light and temperature, pH-responsive, and electric field-responsive, which require more serious efforts to reach clinical step [67, 79-81]. Also, the combination of other fabrication methods, such as electrospinning and 3D printing, can pave the way for more precise control of the geometry of printed structures for tissue engineering. There are four ways to approach this combination: 1- 3D printing of the scaffold on the electrospun membrane; 2- 3D printed scaffold covered with electrospun fibers; 3- Infusion or crosslinking electrospun fibers into the printed structure; and 4- Layer-by-layer placement of the electrospun membrane and 3D printed grid [67]. To sum up, 3D printing technology is an emerging technology with many potentials in various fields such as medicine, industry, electronics, etc., which has attracted the attention of tissue engineering researchers, especially cardiac tissue engineering, due to its many advantages. Nevertheless, it can be said that it is still in the early stages of its development and requires more serious research. Furthermore, finding a bioink with suitable biological, rheological, and mechanical properties that is printable and suitable for cardiac tissue engineering applications is still a challenge that requires more studies in this field.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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