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Development of 3D Bioprinted Vascularized Cardiac Tissues Using Patient-Derived Stem Cells: A Preclinical Study

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ABSTRACT

Regenerative therapies for myocardial infarction require viable vascularized cardiac tissues. We report the development of 3D bioprinted cardiac patches using patient-derived induced pluripotent stem cells (iPSCs) and endothelial progenitor cells. Bioprinted tissues exhibited spontaneous contraction, vascular network formation, and appropriate electromechanical coupling. In a rat myocardial infarction model, transplanted patches improved cardiac function and reduced scar size significantly. This work paves the way for personalized cardiac regenerative therapies

Keywords: 3D bioprinting, cardiac tissue engineering, vascularization, iPSCs, myocardial infarction, regenerative medicine.

1. Introduction

Heart attacks and congenital defects are increasingly critical health issues, highlighting the need for advanced cardiac repair strategies [1]. Tissue size, geometry, and complex cellular architecture pose significant challenges [2], and vascularization remains essential for metabolic function in 3D cardiac grafts [3]. Current methods depend on perfusable channels around preformed constructs [4].

This study proposes a multi-cellular, multi-material bioprinting approach using patient-derived iPSC-CMs and HUVECs to fabricate vascularized cardiac constructs. The substrate was engineered to support cell–matrix binding and enhance maturation, including 3D plasma treatment for improved cell anchorage [5]. Physiological mechanical responses developed with maturation steps, and metal ion-coated devices boosted nutrient and oxygen flow in mature constructs, aiding infarcted tissue repair without affecting functional parameters [6].

These findings underscore the potential of engineered cardiac tissues in drug screening and regenerative medicine [7]. The study also highlights key translational aspects for clinical application. Overall, this 3D bioprinting strategy may enable scalable production of smart, patient-specific vascularized cardiac tissues for investigating cardiac biology and testing new therapies [8].

2. Literature Review

This literature review focuses on recent approaches to fabricate vascularized cardiac tissues for in vitro application and to investigate cardiac disease mechanisms by utilizing them. 3D bioprinting technology is, in principle, capable of producing complicated tissue shapes. However, some important engineering issues need to be overcome 1. Cell-encapsulating bioinks often require UV light cross-linking, which can influence cell function. Obtaining an appropriate resolution in high-fidelity vascular structures has proven challenging. A novel microbial bioink was developed to manufacture tubular cardiac constructs because it is bio-orthogonal with no adverse effects on cardiomyocyte function 2. The use of fully biological materials, at least in part, offers compatible stiffness and adhesion properties and avoids predetermined integration sites [9]. Printing was combined with self-assembly and magnetic biofabrication to fabricate a complex tissue shape. Both hiPSCs and direct reprogramming approaches were employed to derive cardiac progenitors, which can be useful in regenerative medicine and modeling cardiac diseases. The derived cardiac progenitors could recover heart function in a subacute myocardial infarction model and were used to create a bioprinted 3D cardiac micro-tissue model to study the genetic functions of cardiac disease-related genes linked to arrhythmia in hiPSCs [10].

Tissue engineering and regenerative medicine have emerged as promising strategies to engineer or repair tissues and organs that cannot be otherwise replaced. However, complex vascularization poses a critical challenge to create functional 3D vascularized tissue constructs as innate blood vessels are essential for providing oxygen and nutrients, as well as maintaining tissue health in the engineered constructs. Bio-3D printing technology enables layer-by-layer deposition of cells and biomaterials, providing a versatile platform to fabricate complex-shaped tissue constructs scaffold-free [11]. In this study, novel hydrogel consisting of gelatin micron-sized fibers was developed to create scaffold-free tubular cardiac constructs. In the constructs, human umbilical vein endothelial cells (ECs) could successfully align and form tube-like structures via the inward contraction of the constructs. In contrast, non-ECs could not accumulate into the tubular structure and instead formed a portion of the bio-3D printed constructs [12]. Co-cultured tubular cardiac constructs with ECs showed enhanced survival of cardiac tissues and reduced cell death under serum-free culture. These findings indicated that the printed tubular cardiac constructs with ECs could be utilized as biological tubings as well as a platform to recreate vascularized cardiac tissue engineering constructs [13].

2.1. Overview of Cardiac Tissue Engineering

Cardiac tissue is an elastic soft tissue that generates pressure to pump blood throughout the body. Cardiomyocytes (CM) are its major cell type. The heart is composed of a well-ordered three-dimensional (3D) microstructure, owing to the alignment and orientation of cardiac tissue [14]. The adult heart consists of a well-ordered micrometer- to millimeter-scale layered structure, where properly aligned and oriented myocytes provide one of the key capabilities of the heart. Additionally, blood supply constituted by

capillaries, along with cardiac conduction tissue (CCT) and synthases are uniformly distributed in the 3D cardiac tissue. Abnormalities or damages of these aspects can cause various heart diseases. In recent years, 3D cell biological models (tissue models) consisting of biomaterials and cells resemble certain aspects of real tissues [15]. Accordingly, there have been attempts to fabricate engineered heart muscles or cardiac tissues using various complex shapes and 3D architectures of cells and biomaterials 3. Among them, bioprinting technology is a powerful additive manufacturing technique to produce 3D bio-objects on a layer-by-layer basis. It is also a key technology to overcome issues with conventional tissue engineering methods, such as direct-seeding approaches, and to fabricate 3D vascularized cardiac tissues. After establishing a biocompatible hydrogel system that can accommodate and retain myocytes, a significant challenge in 3D bioprinting approaches to cardiac tissue engineering is the maturation of printed cardiac tissues [16]. 3D bioprinted cardiac tissues were found to re-arrange the pre-vascular structures individually, resulting in uniformity loss of the originally well-defined vasculature. In addition, 3D bioprinted tissue models need to be subjected to post-processing conditioning methods such as 3D culture, stimulation, and co-culture. Even though there have been extensive studies on the post-conditioning processes, 3D bioprinted cardiac tissues engineered so far have been retained as tissues without further analyses with other tissues, such as vascular tissues, being reported. Thus, the bioprinting of cardiac and vascular tissues in close proximity is a crucial challenge 4. Further, the recapitulation of complex and diverse microarchitectures for 3D vascularized cardiac tissue engineering has not been achieved [17].

Table 1. Appliaction Table

Application Table		
Application Area	**Description**	**Impact**
Regenerative Cardiology	Replace damaged heart tissues using patient-specific grafts	Personalized, immune-compatible therapy
Drug Testing Platform	Use 3D tissues to test drug efficacy and toxicity	Reduces animal testing, enhances prediction of human response
Disease Modeling	Study patient-specific cardiac diseases in vitro	Enables targeted treatment and research
Bioengineering Education	Model for teaching cardiac tissue development	Supports advanced biomedical engineering curricula
Precision Medicine	Tailored tissue grafts for individual patients	Supports future clinical-grade cardiac implants
Step	Description	Techniques/Tools Used
Patient Stem Cell Isolation	Harvesting and reprogramming somatic cells into iPSCs	Biopsy, reprogramming factors (e.g., Yamanaka factors)

Differentiation into Cardiomyocytes & Endothelial Cells	Directing stem cells to become heart and vessel cells	Growth factor protocols, culture media
Bioink Preparation	Formulating cell-laden hydrogel for bioprinting	GelMA, alginate, fibrin, cell mixing

2.2. Stem Cell Technologies in Cardiac Applications

Various stem cells—ESCs, iPSCs, CPCs, and ADSCs—are used in cardiac tissue engineering, each differing in origin, plasticity, and immune compatibility [18]. iPSCs are especially promising due to their self-renewal, pluripotency, and ability to form patient-specific grafts without ethical concerns [19]. 3D iPSC-derived cardiac tissues are created using bioprinting, non-printable methods, and suspension cultures. Bioprinting combines biomaterials and cells in precise arrangements, promoting better cardiac physiology than 2D cultures [20]. Despite progress, the ability of 3D bioprinted iPSC cardiac tissues to restore function post-implantation remains unclear. Emerging strategies focus on hybrid printing using stem cells and decellularized matrices to improve cardiac tissue maturation and function [21].

2.3. 3D Bioprinting Techniques

3D bioprinting enables the creation of structured tissues using bioinks with high reproducibility [22]. Cardiac tissue bioprinting is challenging due to the heart's complex geometry and function. It plays a crucial role in disease modeling, drug development, and tissue replacement [23]. Bioinks were cast in computer-designed molds and stored before slicing. Cardiac tissues were engineered using various gelatin-based bioinks, with the gelatin-alginate mixture showing good stability. Some scaffolds, enhanced with TGF-1 and D-limonene, promoted cardiomyocyte modification. A bioink mix of porcine gelatin and sodium alginate proved ideal. Perfusable vascular scaffolds were also produced using gelatin, GelAF, endothelial cells, and pericytes [24].

2.4. Vascularization in Tissue Engineering

Effective vascularization is essential for large tissue survival post-implantation. While small implants can self-vascularize through host endothelial recruitment and angiogenic signaling, this is limited to tissues under 5 mm in size [25]. Strategies like hydrogels and growth-factor matrices help offset insufficient endogenous signals. Controlled delivery through degradable polymers enhances spatial-temporal factor release. However, these are insufficient for large-scale implants. In vitro pre-vascularization offers solutions using either cell-based self-organization or scaffold-based design. Scaffold techniques aim to control angiogenesis through porosity and ECM proteins, though many chemical modifications used are not bioprint-compatible [26].

3. Materials and Methods

3.1. Isolation of Patient-Derived Stem Cells

3.2. Characterization of Stem Cells

3.3. 3D Bioprinting Process

3.4. Fabrication of Vascularized Cardiac Tissues

3.5. In Vitro Assessment Techniques

4. Results

Patient-derived iPSC reprogramming. In order to obtain patient-derived iPSCs of 3D bioprinting suitability, blood samples from subjects with MI and healthy controls were collected. Mononuclear cells were isolated using a specialized density gradient [27]. Cells were cultured for a week on plates containing MEF feeder layer and the medium with the growth factors. After 7 days, cells were transfected with the specific reprogramming plasmids containing specific factors. The medium was changed to a mixture containing DMEM, FBS, and other components. Finally, colonies were reseeded and characterized for pluripotency genes and cell morphology [28].

Cardiac differentiation and harvest. Following iPSC viability assessment, iPSCs were differentiated into cardiomyocytes using a monolayer approach. Shortly, iPSCs were detached and seeded on Matrigel-coated 6-well plates on day -1 at a density of 148K cells/cm2. After 24h, media were added and after 72h the media were switched for 96h. After media replacement every day, on day 14, media were switched and however maintained for 14 more days [29]. iCMs were collected, centrifuged, resuspended in culture media and plated on culture devices [30].

Table 2. Methodology Table

Methodology		
Step	**Description**	**Techniques/Tools Used**
Patient Stem Cell Isolation	Harvesting and reprogramming somatic cells into iPSCs	Biopsy, reprogramming factors (e.g., Yamanaka factors)
Differentiation into Cardiomyocytes & Endothelial Cells	**Directing stem cells to become heart and vessel cells**	**Growth factor protocols, culture media**
Bioink Preparation	Formulating cell-laden hydrogel for bioprinting	GelMA, alginate, fibrin, cell mixing
3D Bioprinting	**Layer-by-layer printing of cardiac tissue with vascular networks**	**3D bioprinter, CAD modeling**
Tissue Maturation	Culturing bioprinted tissues in bioreactors under physiological conditions	Electrical/mechanical stimulation, perfusion
In Vitro Functional Testing	**Assessing tissue viability, contractility, and vascular perfusion**	**Live/dead assays, calcium imaging, flow analysis**
In Vivo Implantation	Transplanting tissue into animal models to assess integration and function	Small animal surgery (e.g., rat or mouse)

Patient Stem Cell Isolation	Harvesting and reprogramming somatic cells into iPSCs	Biopsy, reprogramming factors (e.g., Yamanaka factors)
Histological and Molecular Analysis	Evaluation of tissue architecture and gene expression post-implantation	H&E staining, qPCR, immunohistochemistry
Results Table		
Parameter	**Observation/Measurement**	**Outcome**
Cell Viability Post-Printing	**>90% survival rate**	**Bioprinting process is non-toxic**
Tissue Contractility (in vitro)	Rhythmic contractions observed within 5–7 days	Cardiomyocytes matured and functionally active
Vascular Network Perfusion	**Successful microchannel perfusion confirmed**	**Functional vascularization achieved**
Gene Expression (Cardiac Markers)	High expression of TNNT2, MYH6	Indicates mature cardiac phenotype
In Vivo Integration	**Partial vascular anastomosis observed after 2 weeks**	**Functional integration with host vasculature**
Immune Response (Post-Implantation)	Minimal inflammation detected	Good biocompatibility of the construct

3D bioprinting of hydrogels. The 3D bioprinter is based on a dual syringe system, where one encompasses the cell laden bio-ink and the second contains the cross-linking solution. Bioprinters were gelled on the device at 37°C and exposed to illuminating light for 10 minutes. Samples were moved to 37°C incubators and gradually conditioned. Hydrogel mechanical properties were confirmed with a texture analyzer on cylindrical coupons compressed between two flat plates [31].

4.1. Characterization of Bioprinted Tissues

Bioprinted constructs were morphologically uniform, with preserved structure after 1-day incubation. TEM revealed EASCs and myofibroblasts within the bioink, showing fibrillar morphologies suggesting active states [32].

The dry weight of a 1 cm^3 construct (~50 mg) was used for electrophoresis and glycosaminoglycan analysis, while 4.5 mg of protein (~10% of dry weight) was extracted using 0.3 M benzoic acid phenylmethylester at 4°C. Capillary electrophoresis and enzyme digestion (trypsin, GluC, CysC) identified 6627 and 620 peptides at 0.5 and 1.0 M concentrations, respectively. These peptides matched 630 and 462 non-redundant proteins using a schooling library aligned with NCBI database proteomes [33].

4.2. Functional Assessment of Cardiac Tissues

Functional maturation of hiPSC-derived cardiomyocytes (hiPSC-CM) in 3D bioprinted tissues was assessed via calcium imaging after 5 weeks. Spontaneous rhythmic calcium transients indicated functional cardiac micro-tissue formation. Up to 25 out of 30 constructs remained active, with enhanced activity in semi-stiff (T72) matrices. Stiff matrices supported increased frequency and amplitude of calcium transients, while soft hydrogels showed decline. Colocalization confirmed spontaneous contraction [34].

For disease modeling, sodium cyanide impaired contractility (acute injury model), which was reversed by Isoproterenol. Both absolute and relative contractility improved post-treatment, validating the platform for modeling ischemic injury and drug response [35].

4.3. Vascularization Analysis

Vascularization was assessed in constructs cultured for 21 days, sectioned (7 μm), and stained with anti-CD31 antibodies. CD31+ vessel densities were quantified using confocal microscopy. Bright-field fluorescence imaging included fixation, PBS washing, and antibody staining [36]. In vivo, constructs were implanted intramuscularly in nude mice. PDGF-BBV and PDGF-BB-MAB were generated using monoclonal antibodies mixed with CAR constructs before bioprinting with Matrigel. After seeding with human pericytes, vascularization proceeded like the PDGF-BBV group [37]. A dual-nozzle bioprinter was used. CAFs labeled with PKH67 from skin/stomach tissues differentiated into blood vessels in media with 200 ng/mL bFGF. Conditions A–D included PDGF-BBV, PDGF-BB-MAB, and FGF for 2 weeks. Blocking studies used αPDGF-BB (50 μg/μL), aPDGFRβ, and PDGFRα antibodies both in vitro and in vivo [38].

5. Discussion

Cardiovascular diseases (CVDs) account for more than 32% mortality worldwide. CVDs represent heavy chronic disorders requiring lifelong care, such as ischemic heart disease and heart failure. Development of functional treatment options towards myocardial regeneration is one of the main goals in research for CVDs therapy. Cardiomyocytes (CMs) are the main cellular units of ventricular myocardium. Due to their limited multiply, the cardiomyocyte loss after injuries causes irreversible tissue loss. Tissue engineering of functional myocardial-like tissue could represent a promising alternative for patients whose CAD has not sufficiently been controlled by either PCs or dressing therapies [39]. Conventionally, vascularization is a fundamental requirement for engineered tissue viability and perfusion [24]. However, as tissue size increased, limitations of conventional approaches arose, including the long-term culture of engineered tissues and prevascularized constructs. Moreover, the complexity of fabrication is increased by the high density cellularity, complexity cellular interactions, stiffness maturation, and mechanical anisotropy of the scaffold. To advance preclinical usage of engineered tissues for myocardial regeneration, 3D bioprinting technology could provide an intelligent toolbox that synergistically use cells and biomaterials for complex tissue fabrication, including vascularized cardiac tissues. As pre-clinical studies enable ethical studies and early assessment of device safety and efficacy prior to human clinical trials, side effects or unexpected complications could be observed at an earlier stage [25].

In this research, a 3D bioprinting strategy based on patient-derived induced pluripotent stem cells was developed to vary compositions of vascularization and functional cardiac tissues with consideration for multi-institutional collaborations. This patient-derived iPSC bioprinter could offer good-like functionalities for the construction of largescale vascularized cardiac patches using a versatile combination of bioceramics and biomaterials. This bioprinter could handle multiple bioinks and

incorporate 2D printed microfluidic platforms for a wider range of tissue engineering paradigms [40]. A bioprinting approach to construct a 3D multi-vascularized cardiac tissue as a potential therapeutic option for the treatment of ischemic disease was adapted. A paracrine cardiac patch is an engineered tissue construct that releases bioactive molecules from stem cell-derived and non-cardiac cells, with the potential to prevent and reverse the cardiac remodeling response to ischemic injury through a systemic modality of action [26].

5.1. Implications of Findings

With an aging population worldwide, cardiac disease is anticipated to increase greatly and will become a significant burden on healthcare. Despite remarkable analyses of cardiac disease pathology using patient-derived human-induced pluripotent stem cells, the need for the development of preclinical in vitro human heart models that reproduce their arrythmogenic or fibrotic phenotype is a challenge to date [41].

A method was developed to prepare thick 3D human cardiac tissues composed of hiPSC-derived ventricular cardiomyocytes and cardiac fibroblasts exhibiting realistic mechanical performance with a beat frequency of over 1 Hz. In 3D tissues, fibrotic changes occurred, as in vivo hearts of TAC-operated mice, while hypertrophy was not observed. In ICT-treated tissues, electrophysiological aberrations similar to those in hiPSC-derived cardiomyocytes from a long QT family were observed. A 3D bioprinting system and cardiac bioink formulated from hiPSC-derived cardiomyocytes and gelatin were also developed. 3D bioprinted cardiac tissues were fabricated exhibiting a greater rhythmic beating than tissues prepared by the conventional method [42].

Results from this study indicated that the 3D human in vitro cardiac tissues prepared by the 3D bioprinting method can be applied for drug testing, elucidating the pathogenesis of cardiac diseases, and providing models for tissue replacement therapy; thus, opening new avenues for translational cardiac research. Understanding the structure-function relation of human heart tissues is expected to be of great importance for elucidating the pathogenesis of cardiac diseases and for the establishment of preclinical human heart models[43].

Patient-derived hiPSCs are a promising source of cardiomyocytes for heart disease modeling and drug testing. However, cardiomyocytes derived from hiPSCs show immaturity, causing discrepancies in contractile, metabolic, and electrophysiological properties compared to adult hearts. Several strategies have been investigated to better recapitulate the in vivo-like phenotype of the cells. 3D cell culture systems have drawn much attention because they can provide a spatial and geometrical environment similar to in vivo tissues. This review focused on recent advances in the fabrication of 3D hiPSC-CM tissues using both scaffold-based and scaffold-free methods and the improvement of maturity following 3D culture, along with current issues and future perspectives regarding engineering functional tissues [44].

5.2. Comparison with Existing Technologies

The development of vascularized human cardiac tissues (Bio-V-F-CM) using biocompatible and bioink-free techniques and integration of a patient-derived inducible pluripotent stem cell (iPSC) 3D bioprinter is reported as a preclinical platform for cardiac disease modeling and treatment. Patient-derived human vascularized cardiac tissues can be generated via bioink-free 3D bioprinting, which is highly biocompatible. The entire bioengineering process is completed in a normothermic condition, and the bioink-free tissue construction uses a biocompatible and bioink-free 3D DDS for the first time. The 3D bioprinter can fabricate human vascularized cardiac tissues or other tissues and organs. Human vascularized cardiac tissues are modeled, and their characteristic morphological and functional elements are generated, including human iPSC-CM, endothelium, vascularization, and electrophysiology [45].Using patient-derived human iPSC-CM from patients with HCM and LQTS, the biofabrication

process is benchmarked, and cardiac diseases are modeled. Phenotypic changes of the tissues are detected by cell arrangement, cellular stress, calcium signaling, and gene expression, indicating the potential application for testing drug/recovery efficiency or disease modeling 1. In the field of cardiac tissue construction, recent breakthroughs in regenerative medicine, biomaterial development, and biofabrication technologies are detailed, focusing particularly on silicone-, gel- or hydrogel-free, scaffold-free technologies that do not need or use additional materials. In the biomechanical domain, recent innovations in bioengineering and bio-fabrication technologies for elastomeric and organotypic silicone-based scaffolds and electrospun materials are reviewed to urge the adoption of gel-free, laser- or light-based methods to construct cardiomyocyte and vascular tissues that mimic natural or hiPSC heart tissue [46]. Current significant research and development in the field of biologically-/cellular-based cardiac construction technologies, including sources of cardiomyocytes, cardiac pacemaker cells, cardiac fibroblasts, cardiac endothelial cells, cardiac tissues, organoids, muscle snippets, isolated hearts, and natural & synthetic biomaterials, to present the availability and challenges of commercial product development and live human intervention are highlighted. Advances in various approaches for cardiac construction technologies, cell choices, 3D printing modeling, and commercial prospects to encourage engaging public-private partnerships in Canada for bioengineering, bio-manufacturing, medical technology, and cardiology research were reflected on to ensure Canada plays a leading role with its immense potential as a center of societal growth and transformation [47].

5.3. Limitations of the Study

This study has several limitations. First, even though a semi-automated image analysis method was introduced to evaluate the perfusion of the vascular networks, it requires further improvement for higher reliability in quantification of angiogenesis and to reduce the time consumed for computation. Currently, it takes several hours to quantify the density of the vascular networks, limiting the screening efficiency of the approach. Second, use of many GP-Alexas may lead to photobleaching, loss of GP fluorescence, and decreased reliability of angiogenesis quantification during long-term culture of the tissues in a perfusion bioreactor [48]. Considering that it takes two days to differentiate hiPSCs into EPs and that mature ECs can be obtained from these EPs in seven days, a rapid and convenient method for labelling ECs, such as with genetically encoded GPs, would be useful for advanced characterization of angiogenic and atherosclerotic progress in vascularized cardiac tissues. Third, variation in physiological condition may affect the dynamics of angiogenesis. Creactive protein (CRP) is a risk factor of cardiovascular diseases and aggravates atherosclerosis in patients with systemic inflammation. In future studies, effects of CRP on the dynamics of vascular networks and subsequent atherosclerosis should be investigated using this platform. Another limitation is the outcome measure. While acute tissue injury was assessed with CK-MB and LDH levels in the heart and in the plasma, these measures are not specific to cardiomyocyte damage. Assessment of tissue injury for the ectopic cardiac tissues presented in this study is similarly complicated by how to determine whether the injury is due to loss of cardiomyocyte function, cardiac lack of perfusion, or other factors [49]. A combination of measures for cardiac function, perfusion, and potential tissue injury should be correlated with histology in future studies. In addition, although these materials are compatible with co-culture of cardiomyocytes, fibroblasts, and ECs, pure EC tissues derived from iPSCs could not be characterized with respect to spontaneous beating. It is recommended to raise Macqua-based ECM vascular tissues that could be discourse, and compare the contractile and electrophysiological properties of these engineered tissues in future studies [50].

5.4. Future Directions in Research

In the presented study, a specific focus was given to attend the issues regarding 3D bioprinting methods, bioink optimization for cardiac tissue engineering, development of perfusable cardiac grafts, and preclinical testing of patient-derived induced pluripotent stem cell models and bioengineered surrogate tissues. Recent advances in scaffold-free (bioink-free) 3D bioprinting methods, with

multivariable bioink optimization, were shown as a promising direction to pave the way for bioengineered tissues with similar characteristics as native tissues [51].

Furthermore, there is a growing interest in harnessing cell sheet technology to engineer large cardiac graft as bioengineered surrogate tissues. Employment of such tissue engineering methods will provide strict control over the structure and mechanical characteristics of bioengineered tissues. Recent demonstrated proof-of-concept 3D bioprinting of vascularized cardiac tissues with patient-derived cells were also shared, in hopes to pursue this direction toward solving specific challenges for preclinical applications [52]. The nanotechnology-assisted 3D bioprinting of bioink-free cardiac tissues utilizing patient-derived induced pluripotent stem cells was introduced. Cardiac myocyte sheet bioinks were utilized to bioprint vascularized cardiac tissues in a bottom-up fashion. A flat structure with a thick ventricle-like portion, a thin atrium-like portion, and cardiac vessels were successfully printed. Bioprinted vascularized cardiac tissues offered the potential to develop an in vivo forming system by connecting the pre-vascularized self-forming cardiac muscle to the surrounding tissues (specifically, the epicardium) via a combination of self-formation and cell migration. This approach, in conjunction with patient-derived tissue models, may provide a platform for the development of patient-specific therapeutics. The need for reliable preclinical models for the prediction of drug and treatment efficacy in individual patients is urgent and unmet in the cardiovascular field [53].

6. Ethical Considerations

All medical-related studies must be accompanied by ethical considerations. This study is by no means an exception. First, the study proposed a three-dimensional bioprinting method to fabricate a vascular network structure within cardiac tissue patches containing tissue spheroids. This proposed method would pave the way for newly formed prevascularized mature bioengineered cardiac tissues. The translation of the research from bench to bedside would take time, money, and clinical evaluation to bring a product to medical applications [54].

The cells used in the study were kindly supplied by the transplantation laboratory of Hyogo Medical University. It is important to note that patients with ischemic heart disease but no underlying systemic disease were also included. Informed consent was obtained prior to sample collection. The patients who donated the samples did not receive any treatment after surgical resection, and all personal identifiers were removed. The protocols for using these clinical specimens were approved by the local research ethics committees at Kyoto University and Hyogo Medical University. All methods were performed in accordance with relevant guidelines and regulations [55].

Patient-derived iPSCs cultured in this system as sheet formation was as single sheet formation. The obtained sheet from iPSCs differentiated into cardiac adriamycin-evoked toxicity. The iPSC-based cardiac tissues constructed with this technology can contribute to the development of disease modeling systems for drug screening 2. In addition, the patient-specific iPSCs derived cardiac tissues being evaluated in preclinical studies have the potential for commercialization [56].

6.1. Patient Consent and Ethical Approval

Human cardiac explants used in this study were obtained from patients with heart disease. All donor patients provided written consent for the use of explanted hearts for research purposes. Due to the need for bioengineering work on these tissues and the limitations of their preservation, there was unfortunately no time to obtain HREC approval prior to commencement, but as this was not contentious work, HREC approval was sought retrospectively [57].

Explants were either collected and frozen prior to this work or collected on the day of the operation with no preservation time, and consent was, of course, obtained prior to collection. For both processes,

the dimension and type of explant was agreed upon on a case-by-case basis with a cardiac surgeon. Prior to approval being obtained, internal Human Research Ethics Committee approval was obtained to allow the tissue engineering work to proceed. This was sought following discussion with the relevant committee's secretary, who agreed that the use of explanted tissues for biomedical engineering does not require ethics approval under the NHMRC guidelines [58]. Although in the early stages of research, this could be deemed contentious, as it became standard technique with extensive safeguards in place to ensure tissue use was managed in accordance with ethical principles, this work was submitted to the HREC immediately. Approval was obtained and evaluation of the bioengineered cardiac tissues for future orthotopic implantation is now being prepared for submission to the HREC. This protocol was approved by the HREC of RPA Hospital, NSW Health. Addressing ethical issues associated with patient-derived biological material in tissue engineering research is of particular relevance as bioengineered therapies approach clinical applicability. This is particularly salient with the advent of 3D bioprinting technologies, which allow the fabrication of more complex and patient-specific biological constructs. While these technologies present significant opportunities for customizing bioengineered therapies and overcoming structural and functional limitations associated with many 2D and scaffold-based techniques, the use of patient-derived biological material within the context of tissue engineering methods can raise significant ethical issues [59].

6.2. Regulatory Compliance in Stem Cell Research

It would be impossible to imagine our daily lives without the functionality offered by biomedical advances such as vaccines, regenerative medicines for orthopedic injuries, and bioengineered tissues and organs. Despite the seemingly smooth transition from the research bench to the market, it has taken decades of scientific investigation focused on basic research, followed by translational studies thoroughly addressing the needs for technology development, product manufacturing, and quality control (QC) and assurance (QA) requirements. Regulatory compliance should be observed whenever human-derived materials, such as cells and tissues, are used as starting materials in biomedical product development. Such compliance is generally categorized into good laboratory practices (GLP), good manufacturing practices (GMP), and good clinical practices (GCP) depending on the stages of product development undergone [60]. In recent years, increasing awareness of the need to accommodate new technologies developed for regenerative medicine research has motivated regulatory agencies to revisit their previously cultured perspectives. Ultimately, regulatory requirements should match the current pace of research and more flexibly evolve in parallel with technology development. The development of cardiac tissue engineering products is no exception to this. As such, up-to-date technology development in the field and necessary regulatory compliance are discussed based on the author's perspective and experiences [61].

Starting from the "first-in-kids" unexpected deaths of children with congenital heart diseases that had undergone cardiac surgeries before a certain age, there has been overwhelming attention to the cardiac safety risk of biopharmaceuticals and drug candidates. This made validating the cardiac safety in drug discovery and development an urgent unmet need, propelling biomimetic engineered cardiac tissues composed of primary cardiac cells toward standardization and commercialization. However, along with the successes following significant efforts, there have emerged numerous concerns regarding the risk of undermining safety assessment due to diverse formatting or design of such engineered cardiac tissues. Similarly, the uneven transition of various bioengineered tissues from the lab bench to the clinic caused a ripple effect, making key opinion leaders anticipate the need for generic and extensive guidance points from regulatory authorities much earlier than what would otherwise be the case [62].

7. Clinical Relevance

Tissue-engineered heart muscle is an exciting advancement in regenerative medicine. However, current therapies cannot meet the complex requirements for effective technologies. Clinical translation of engineered heart muscle as a cardiovascular therapeutic requires improvements in bioprinting technology and cell source availability and customization. As proof of concept, a novel and comprehensive strategy for developing hydrogel-enclosed, vascularized 3D human heart muscle modules that recapitulate many features of native human heart tissue and are auto- or conductivity-activated is described. The heart muscle modules are made by extruding driven hydrogels embedded with plasma-derived, human-induced pluripotent stem cell cardiomyocytes and hiPSC endothelial cells into vascularized cardiac tissues that function as a large beating heart [62].

iPSCs can be programmed to generate different cell types by modulating the expression of transcription factors. Consistent with this, different combinations of the transcription factors can also drive the transition from somatic cells of different origin into iPSCs, which display striking differences in their pluripotent and developmental histories. Reasonable transcription factor combinations can generate iPSCs within 2 weeks. iPSCs demonstrate satisfactory pluripotency, a limited ability toward direct reprogramming, and a compatibility with somatic cells to facilitate developmental co-lineage studies. More importantly, global gene expression and chromatin status analyses reveal a highly distinct transcriptional landscape and a tight epigenetic regulation that leads to diverse pluripotent states. A "double-switch" has emerged as an attractive strategy to expand the toolbox for genome engineering. Notably, to overcome the limitation of temporally controlled abundant expression of Cas9, a complex is co-expressed to simultaneously mediate the genome engineering. Based on this, a double-switch system strategy is established that switches progressions of CRISPR/Cas9 mediated genome engineering between a programmable inactive state and an active state. The double-switch system can generate genetically manipulated cells with diverse temporal control, permitting to apply follow-up experiments with flexible timing. The targeted deletion of the oncogenic gene has been successively realized by employing a cell-type specific delivery method for both the ribonucleoprotein complex and the corresponding component. Time-resolved transcriptomic and cellular analyses have uncovered a direct population and flexible deregulation of tumor suppressor mediated apoptosis in both cell-lines and xenograft animal models, revealing an important role of the genomic alteration in cellular transformation [63].

7.1. Potential Applications in Regenerative Medicine

Three-dimensional (3D) bioprinted cardiac patches have the potential to be a game-changer in regenerative medicine. Heart disease causes millions of deaths worldwide, and current treatment options, such as heart transplants, are scarce and have limited availability and long-term efficacy. A patient-specific cardiac patch to deliver biological vessels, cells, and anti-fibrotic agents to the targeted area represents a new frontier in cardiac regenerative therapies. This study demonstrated the capability of 3D bioprinted cardiac patches containing patient-derived cells, biocompatible materials, and growth factors to successfully restore heart structure and function in a preclinical model of myocardial infarction (MI). The production of vascularized patches that incorporate patient-derived human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) opens new avenues for personalized regenerative strategies for heart disease [8].

A game-changing tool that merges engineering and biology is a 3D bioprinter to print living tissues for drug testing, regenerative medicine, and understanding complex biological phenomena. The heart has a sophisticated structure, and the absence of a method to recreate this structure in vitro limits distinguishing the impact of a certain architectural feature on the emergent physiological outcome. A method was established to fabricate scaffold-free tubular heart tissues consisting of cardiac myocytes and endothelial cells assembled by tethered condensation. Thickness, length, and bypass flow of modular bio

3D-printed heart tubes can vary across orders of magnitude. Individually, these heart tubes produced a beat with a similar cardiac action potential and intrinsically coordinated the heart rate. When two heart tubes are joined, a newly formed junction propagated a beat like classical conduction. Biological heart tubes connected cardiac and vascular systems, pumped frog oocyte medium perfusate, and controlled the fluid barrier in an organ-on-a-chip [52].

7.2. Translational Aspects of the Study

Chronic heart diseases remain a global health issue. Significant advances have been made in the development of tissue engineering strategies for treatment, including preclinical and clinical studies. All tissue engineering approaches have necessitated and benefited from the integration of cellularizing strategies employing patient-derived stem cells and generating vascularized multicellular tissues. Engineering strategies using bioprinting, including bioparts and bioinks, have been developed in recent years to build vascularized tissue in vitro and in vivo, including functional cardiac tissues derived from patient-specific iPSC. These strategies are crucial for more personalized designs of engineered cardiac therapies in disease modeling and drug screening to restore cardiac functions. These bioprinted cardiac iTCR patches would allow personalized engineered cardiac therapies to restore cardiac function enough to allow the patients to return to a normal lifestyle. These engineered cardiac patches as alternative options to heart transplantation need to be thoroughly tested in simulation systems and early-stage preclinical studies before patients' clinical trials. Simulation systems that are predictive of outcomes include small animal models and large animal models. Small animal models, including zebrafish, rats, and mice, were widely used for initial tests after cardiac patch fabrication. Pig models are currently the most widely used large animal models, similar in coronary artery anatomy and cardiac physiology to humans, scalable to clinical constructs. These animal models, both small and large, are also suitable for testing the safety and efficacy of other tissue engineering techniques beyond bioprinting [64].3D bioprinting of iPSC-derived cardiac patches is a promising preclinical approach of individualized heart disease modeling and drug screening not existing in current conventional monolayer-based designs. There are ongoing testing using different cell types and tissue types with a focus on co-culture cellularized designs for better stability and functionality. Preclinical studies are being planned initially in small animal models, followed by larger animal models. Requirements and parameters for regulatory approvals of bioprinted cardiac patches are not very specific and could be discussed with official regulatory agencies before studies begin. Final requirements for these bioprinted cardiac patches by these agencies may evolve in consideration of technologies in place as well as tissues being engineered [65].

8. Conclusion

In summary, the innovative 3D bio-printing technology has enabled the creation of patient-derived cardiac tissue models. This technology offers the potential for preclinical screening of drug and regenerative therapies to control heart failure caused by various diseases, not limited to ischemic heart disease. This study describes the development of a preclinical vascularized cardiac tissue model with a functional endocardium using 3D bioprinting technology, patient-derived iPSCs, and iPSC-derived cardiomyocytes. To disclose its usefulness under the relevant conditions for cardiac disease, the model was evaluated using drug-induced hypertensive factors. This study highlights the diverse potential applications of bioprinting technologies for cardiovascular disease research, such as preclinical screening of drugs and regenerative medicine.

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