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Bioreactors for Cardiac Tissue Engineering

Jesus Paez-Mayorga, Gustavo Hernández-Vargas, Guillermo U. Ruiz-Esparza, Hafiz M. N. Iqbal, Xichi Wang, Yu Shrike Zhang, Roberto Parra-Saldivar, and Ali Khademhosseini**

The advances in biotechnology, biomechanics, and biomaterials can be used to develop organ models that aim to accurately emulate their natural counterparts. Heart disease, one of the leading causes of death in modern society, has attracted particular attention in the field of tissue engineering. To avoid incorrect prognosis of patients suffering from heart disease, or from adverse consequences of classical therapeutic approaches, as well as to address the shortage of heart donors, new solutions are urgently needed. Biotechnological advances in cardiac tissue engineering from a bioreactor perspective, in which recapitulation of functional, biochemical, and physiological characteristics of the cardiac tissue can be used to recreate its natural microenvironment, are reviewed. Detailed examples of functional and preclinical applications of engineered cardiac constructs and the state-of-the-art systems from a bioreactor perspective are provided. Finally, the current trends and future directions of the field for its translation to clinical settings are discussed.

1. Introduction

Cardiovascular disease, a set of pathologies that include myocardial infarction (MI) and heart failure, is the leading cause of death around the globe.[1,2] The healthy tissue that remains after an ischemic event lacks the necessary mechanical support to properly maintain cardiac function, leading to stress and chronic inflammatory response that eventually cause heart failure.[1,2] While pharmacological approaches have been able to delay the progression toward this devastating disease, the only permanent treatment for end-stage heart failure is cardiac transplantation.[3,4] However, the limited availability of heart donors and the problem of immunosuppression limits the widespread use of this approach.[3,5]

J. Paez-Mayorga
Tecnologico de Monterrey
School of Medicine and Health Sciences
Ave. Eugenio Garza Sada 2501, Monterrey, N. L., CP 64849, Mexico

G. Hernández-Vargas, Prof. H. M. N. Iqbal, Prof. R. Parra-Saldivar
Tecnologico de Monterrey
School of Engineering and Sciences
Campus Monterrey
Ave. Eugenio Garza Sada 2501, Monterrey, N. L., CP 64849, Mexico
E-mail: r.parra@itesm.mx

Dr. G. U. Ruiz-Esparza, Dr. X. Wang, Dr. Y. S. Zhang, Prof. R. Parra-Saldivar, Prof. A. Khademhosseini
Division of Engineering in Medicine
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
Cambridge, MA 02139, USA
E-mail: khademh@ucla.edu

Dr. G. U. Ruiz-Esparza, Dr. X. Wang, Dr. Y. S. Zhang, Prof. R. Parra-Saldivar, Prof. A. Khademhosseini
Harvard-MIT Division of Health Sciences and Technology
Massachusetts Institute of Technology
Cambridge, MA 02139, USA

Prof. R. Parra-Saldivar
Microsystems Technologies Laboratories
MIT
Cambridge, MA 02139, USA

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Prof. A. Khademhosseini
Department of Bioengineering
Department of Chemical and Biomolecular Engineering
Henry Samueli School of Engineering and Applied Sciences
University of California-Los Angeles
Los Angeles, CA 90095, USA

Prof. A. Khademhosseini
Department of Radiology
David Geffen School of Medicine
University of California-Los Angeles
Los Angeles, CA 90095, USA

Prof. A. Khademhosseini
Center for Minimally Invasive Therapeutics (C-MIT)
University of California-Los Angeles
Los Angeles, CA 90095, USA

Prof. A. Khademhosseini
California NanoSystems Institute (CNSI)
University of California-Los Angeles
Los Angeles, CA 90095, USA

Prof. A. Khademhosseini
College of Animal Bioscience and Technology
Department of Bioindustrial Technologies
Konkuk University
Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea

Prof. A. Khademhosseini
Center for Nanotechnology
King Abdulaziz University
Jeddah 21569, Saudi Arabia

Tissue engineering (TE) with a focus on the heart has gained a particular interest among academic and research-based organizations as heart diseases are one the leading causes of death in modern society. Due to the inadequate understanding of the regulatory mechanisms of specific physicochemical parameters, new alternatives with the potential to regenerate/facilitate damaged heart tissues are in high demand. Thus, cardiac tissue engineering (CTE) has emerged as a promising field that offers noteworthy alternatives to develop novel clinical approaches to treat heart disease.

Over the past two decades, significant efforts have been made toward the development of functional and biomimetic cardiac constructs.[6,7] An array of heart valve constructs has shown promising results reaching clinical trials.[8] Also, small myocardial grafts have been successfully engineered using 3D bioreactors that enable the control of specific parameters such as mechanical and electrical stimulation.[9,10] However, the inability to produce scalable and functional 3D constructs remains a major difficulty in the field. Microchanneled scaffolds that allow intratissue perfusion, an improvement of constructs using the cell sheet technology, as well as recellularization and re-endothelialization of decellularized matrices have been proposed as plausible solutions.[11–13]

Based on literature data, several research efforts have been made to engineer novel constructs with CTE potentialities, though using different methods and materials. Among them, the development of bioreactor-based systems, with requisite features, has gained particular interest that produces clinically effective cardiovascular tissue-based constructs.[14] For a said purpose, a bioreactor-based system for TE applications should possess/offer the following criteria, i.e., 1) uniform cell distribution, 2) controlled maintenance of physicochemical requirements of the cell, e.g., i) O_2, ii) growth nutrients, and iii) growth factors, etc., 3) controlled mixing system for diffusion and convection purposes, 4) increased mass transport and proper distribution of culture medium, 5) cell exposure to physicochemical and electrical stimuli, 6) provide stability and maintain scaffold properties, 7) control monitoring of target phenotypes, 8) control functional maturation of 3D substitutes, 9) establish a substantial level of cellular distribution, 10) proper waste exchange and management, 11) reduce excessive turbulence in the fluid flow, 12) maintain a high degree of reproducibility, and 13) control monitoring and precise automation, etc.

The present review aims to scrutinize the development of novel types of bioreactors used for CTE. The first part gives a brief history of the evolution of traditional tissue engineering, through the development of functional tissues. Then, various bioreactors that have been used for the delivery of biochemical, mechanical, and electrical cues, are discussed. We conclude with a brief insight into future developments on the application of bioreactors for CTE.

Hafiz M. N. Iqbal is a full-time research professor at the School of Engineering and Sciences, Tecnologico de Monterrey, Mexico. He completed his Ph.D. in biomedical sciences with specialization in applied biotechnology and materials science at the University of Westminster, London, UK. He does research in bioengineering, biomedical engineering, and environmental engineering.

Roberto Parra Saldivar is an assistant professor and Sustainable & Applied Biotechnology Group Leader at Tecnologico de Monterrey, Mexico. He has over 15 years of experience and leads projects funded by Mexico's CONACyT programs, and the US Army among others. Currently, he is on sabbatical leave as a Harvard–MIT Visiting Professor under the framework agreement Tecnologico de Monterrey MIT funded Program.

Ali Khademhosseini is the professor of bioengineering, chemical and biomolecular engineering and radiology at the University of California-Los Angeles (UCLA). He is the founding director of the Center for Minimally Invasive Therapeutics at UCLA as well as an associate director of the California NanoSystems Institute. He joined UCLA in November 2017 from Harvard University where he was a professor of medicine at Harvard Medical School (HMS). At HMS, he directed the Biomaterials Innovation Research Center (BIRC), a leading initiative in making engineered biomedical materials. His laboratory has pioneered numerous technologies and materials for controlling the architecture and function of engineered vascularized tissues.

2. Overview

2.1. Tissue Engineering

TE uses a combination of the structural, biological, and physicochemical characteristics of cells, engineering, and materials science to create solutions for the replacement, repair, and regeneration of tissues and organs.[15] The ever-increasing demand for organs, limited donor availability, high cost in practicing donor to recipient transplantation procedures, and chronic immunosuppression, increases the clinical need for TE approaches,[15,16] by in vitro fabrication of functional 3D tissues suitable for subsequent transplantation without donor involvement.

TE technologies are divided into three types of approaches: 1) cells-alone, 2) regenerative materials, and 3) cells combined with materials.[17] The first approach consists of cell implantation to deliver the required functionality to a particular site. For example, cell transplantation of cardiac progenitor cells to infarcted tissue has limited scar formation.[16] However, the cells are dependent on the host organ stroma for anchorage and organization, which may lead to their loss of function as well as immune rejection.[16,18] In the second approach, tissue-inducing materials are used to promote tissue growth and regeneration,[18] and in the third approach, the systems can be operated in open and closed models. In contrast to open systems where cells are directly exposed, in closed systems, cells are encapsulated in a semipermeable membrane to avoid immune rejection while still allowing the interchange of nutrients and waste products. This approach has yielded promising results for the transplantation of hepatocytes and insulin-producing cells, as well as for engineering cartilage constructs.[19–21] A downside of the technique, however, is the growth of fibrotic tissue on the semipermeable membrane, causing a reduction of diffusion.[16] Open systems aim to create implantable grafts onto the preexisting tissue by using synthetic or natural matrices as substrates for cell adhesion. Skin, cartilage, and liver models were among the first to use this type of approach.[22–24]

2.2. Functional Tissue Engineering

The United States National Committee on Biomechanics formed a subcommittee in 1998 that introduced "functional tissue engineering" (FTE).[25] FTE focuses on the biomechanics and mechanobiology of load-bearing structures such as those comprised in the musculoskeletal system. The ''FTE Road Map'' describes the iterative process for development of FTE from tissue engineering in culture to engineered constructs after implantation.[15] In addition, the FTE also stresses on the structural and mechanical characterization of the native tissues and their correlation with the material properties to establish an appropriate reference for engineered constructs (**Table 1**). During the first few years of tissue engineering development, much of the research was conducted by the incorporation of known biocompatible materials with cells in the hope that they would self-organize in a biomimetic manner.

Over the past decades, some of the mechanisms that induce tissue formation have been deciphered including the importance of mechanical stimulation, and with the advent of micro and nanotechnology, new fabrication techniques have emerged.[26–29] For example, Khani et al. modified the secretion rate of transforming growth factor-ß (TGF-ß) from human mesenchymal stem cells (hMSCs) by exerting cyclic uniaxial strain.[30] The enhanced TGF-ß expression is important that activate the osteogenic differentiation pathways in hMSCs. Moreover, current endeavors to engineer vascular grafts recognize the beneficial effects of exerting mechanical stress on developing constructs for the proper matrix, smooth muscle, and endothelial cell organization.[31–34] Other studies have shown a closer phenotypic resemblance in vascular smooth muscle cells between native vessels, and mechanically stimulated constructs.[32,34,35]

Table 1. A comparative evaluation between TE and FTE.

Characteristics	TE	FTE
Seeks architectural mimicry	√	√
Seeks functional mimicry	X	√
Culture conditions		
Static versus Dynamic	Static	Mainly dynamic
2D	√	√
3D	X	√
Biochemical stimulation	√	√
Mechanical and/or electrical stimulation	X	√
Compare results to native parameters	X	√

The ability to mimic in vivo conditions on in vitro culture systems, promises better fabrication and functionalization of engineered tissues. Bioreactor systems facilitate the replication and control of such conditions, and they have been widely used in muscle, cartilage, and bone TE.[36,37] Over the past decades, bioreactors have been incorporated into CTE to deliver biochemical cues, exert mechanical and electrical stimulation, and promote vascularization of constructs. **Figure 1** illustrates a generalized scheme of a bioreactor with intermittent (upper) and continuous (lower) medium and gas exchange systems. The bioreactor illustration also shows the culture conditions in the presence of a TE construct for the evaluation of cardiac differentiation efficacy.

2.3. Cardiac Tissue Engineering

The main focus of CTE is to ameliorate the complications that arise following myocardial infarction (MI). The first endeavors regarding CTE were made using direct cell transplantation at the infarcted site. Several cell candidates have been studied for this purpose, and each of them has shown a particular set of advantages and disadvantages.[38] For example, skeletal myoblasts (SMs) and mesenchymal stem cells (MSCs) are the two most widely studied cell types.[38,39] Nevertheless, a suitable scaffolding that allows cells to attach and reside in the targeting tissue greatly limits the success of this approach.[38,39] This technique is probably best suited for simple tissues with small lesions.

It has been established that engineered constructs for CTE should in general possess, at least, the following essential features:[39–42]

1) Mechanical strength
2) Architectural anisotropy
3) Sufficient vascularization
4) Electrophysiological stability
5) Contractility at physiologically relevant rates
6) Excitation-contraction coupling (conversion of electrical stimulation to mechanical responses)

Other important criteria include easy harvest of cells, high proliferation, nonimmunogenicity, resistance to ischemia,

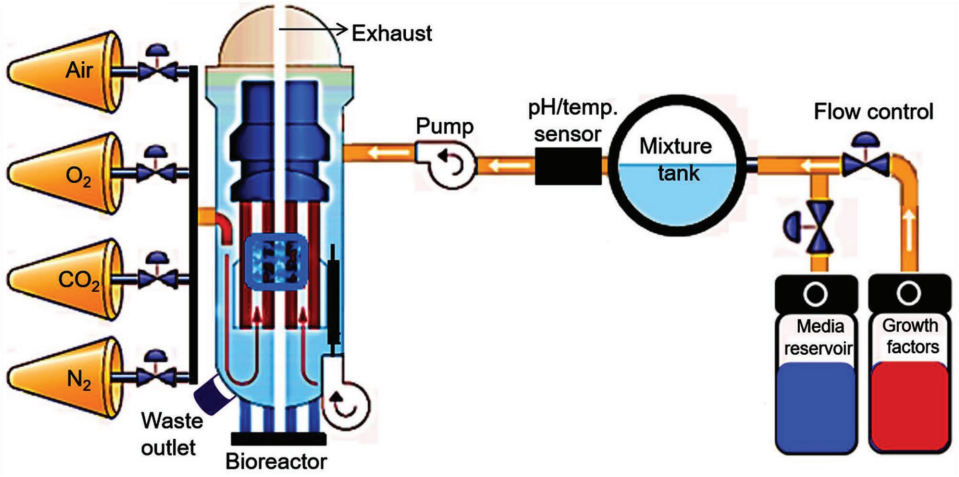

Figure 1. A generalized scheme of a bioreactor with continuous medium and gas exchange systems.

ability to differentiate into mature, and functional cardiomyocytes. Whereas, from the materials perspective, scaffolding cues must be nontoxic and nonimmunogenic, and should trigger functional cardiogenic differentiation.[43,44] **Figure 2** illustrates a schematic representation of CTE.

Seeded biodegradable scaffolds and polymer-cell molding techniques provide a unique platform to construct in vitro templates that can be remodeled and become functional upon implantation. In this approach, natural or synthetic polymers are used to create a matrix onto which cardiomyocyte progenitors are seeded. Upon implantation, the polymers degrade and are substituted by the extracellular matrix (ECM) produced by the transplanted cells.[16,45] Zimmermann et al. developed an "engineered heart tissue" (EHT) using an MI rat model.[46] The EHT showed mature cardiomyocyte differentiation, formed patent vasculature that anastomosed to the recipient's vessels, had strong electrical coupling to host tissue with conduction velocities comparable to the healthy myocardium, was nonarrhythmogenic, and improved the systolic and diastolic function of the left ventricle.[3] Further studies had been conducted to address vascularization in which endothelial cells (ECs) were cocultured on the same cell sheet as SMs or MSCs. After implantation, these constructs showed the formation of capillary networks congruent with the amount of ECs seeded.[45,47] Recently, Schürlein et al. developed a vascularized human cardiac patch based on a reendothelialized biological scaffold (BioVaSc) (**Figure 3**).[14] Various physiological cardiac functions and expression of cardiac-specific markers were detected after 2 weeks. The vessel patency and tissue viability were verified 1 week after BioVaSc-based autologous patch implantation. In an earlier study, Guyette et al. bioengineered functional myocardial tissue based on the combination of human cardiac matrix and human induced pluripotent stem cells (iPSCs)-derived cardiomyocytes.[48]

3. Bioreactors in CTE

3.1. Bioreactors with Biochemical Stimulation in CTE

Oxygen levels, pH, CO_2 concentration, and nutrient availability are important biochemical cues that need to be closely monitored for successful TE. Bioreactor systems are integrated with tools that continuously monitor such parameters and maintain them at physiological levels. Electrical and mechanical stimulation also need to be considered as they play an important role in cell maturation and tissue coordination.[49] Moreover, growth factors including vascular endothelial growth factor (VEGF),

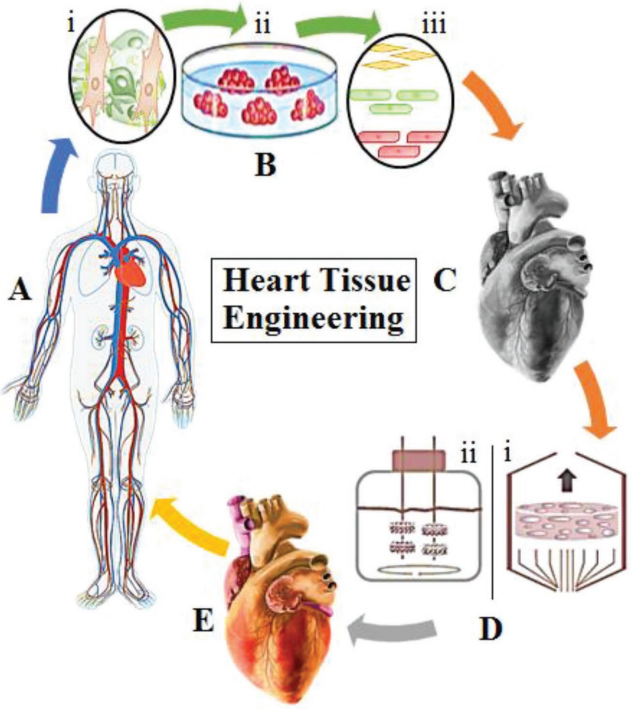

Figure 2. A generalized schematic representation of CTE. A) affected patient, B) specific cells: i) fibroblast and other cell lines, ii) iPSCs, and iii) cardiomyocytes and other necessary cells, C) 3D porous biomaterial-based heart scaffold of different nature and architecture, and cultured under dynamic conditions in D) bioreactor system, which nurture the development of heart tissue by supporting efficient nutrition of cultured cells and applying mechanical stimuli that are critical for functional regeneration and E) engineered heart as a potential alternative.

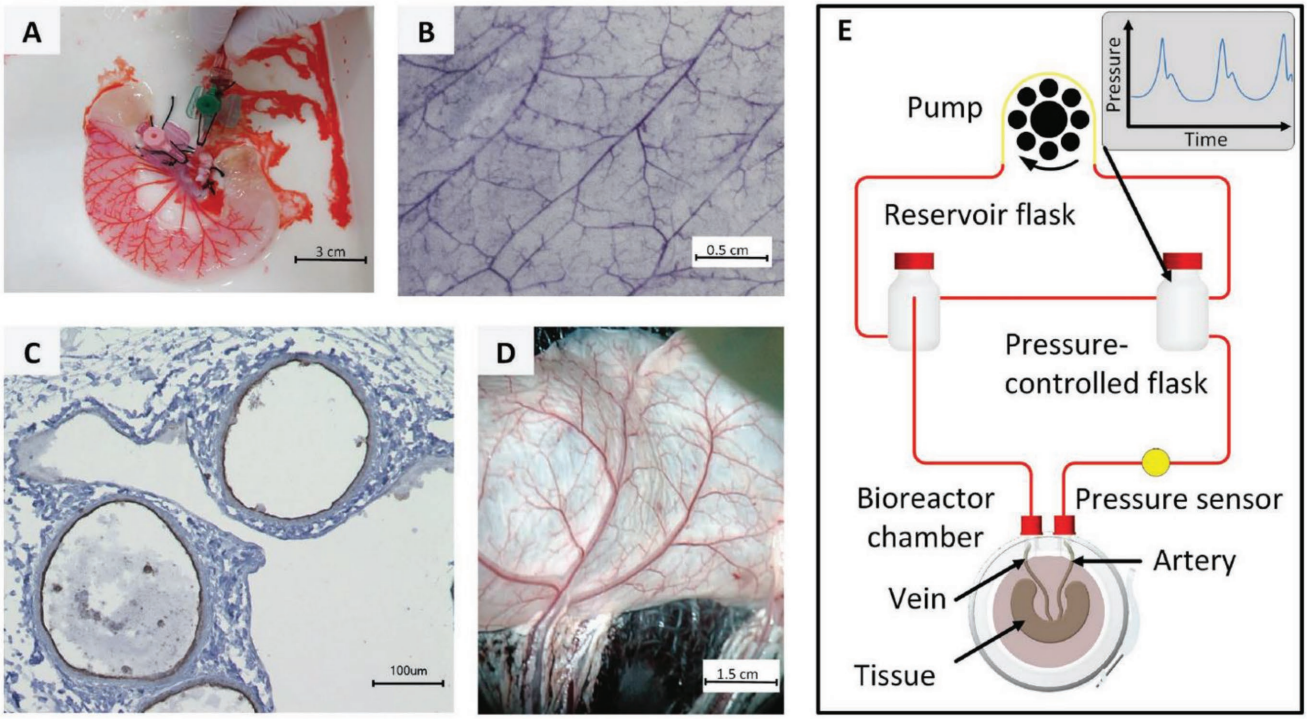

Figure 3. Generation of a vascularized cardiac patch. A) A decellularized biological vascularized scaffold (BioVaSc) derived from a porcine jejunum segment served as the basis for the generation of the 3D cardiac tissue. Flushing of the vasculature of the cell-free scaffold with phenol red displayed the complex vessel structure. A highly branched vessel network starts from a central artery and meets at a venous outlet. B) At 1 week after seeding endothelial cells into the vasculature, reendothelialization was confirmed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. C) Cell identity was demonstrated by staining against the endothelial-cell-specific marker CD31. D) When flushing the vasculature with human blood, vessel patency is maintained for one week. E) Bioreactor technology supported controlled process conditions up to four months of culture. A fluid system composed of a medium reservoir flask and a pressure flask in which pressure curves of 120 80 $mmHg^{-1}$ at 1 Hz were generated enabled a physiological perfusion of the vasculature. Reproduced with permission.[14] Copyright 2017, Wiley-VCH.

basic fibroblast growth factor (FGF2), hepatocyte growth factor, platelet-derived growth factor, and insulin-like growth factor 1 are essential for CTE, as they promote vascularization, proliferation, differentiation, survival, and phenotype maintenance of cardiac cells.[50]

Depending on the bioreactor design, the growth factors can be incorporated into the culture medium and delivered in a controlled manner. Bioreactors have also been used for escalation and differentiation of progenitor cells to the cardiomyocyte lineage. Embryonic stem cells (ESCs) are stem cells derived from the undifferentiated inner mass cells of a human embryo. Matsuura et al. used a batch bioreactor to expand (300-fold) and differentiate mouse ESCs to cardiomyocytes.[51] Concomitantly, they elucidated the beneficial effects of noggin and granulocyte colony stimulating factor on cardiomyocyte differentiation. After 10 d of culture in the enriched medium, the cells expressed α-actinin and cardiac troponin T, which indicated differentiation to mature cardiomyocytes. Additionally, cell sheets cultured using the cells expanded in the bioreactor showed spontaneous contractility. A perfused rotary bioreactor has been used to differentiate ESCs embedded in 2.5 mm spherical alginate hydrogel constructs to mature cardiomyocytes.[52] **Figure 4**A illustrates a simplified schematic representation of a perfused rotary bioreactor.[37] After 21 d of incubation, late cardiogenic markers (cardiac troponin T, α and β myosin heavy chains, α-actinin) expression were confirmed using flow cytometry and qPCR analyses. However, the cells were not further plated to assess cohesion, contractility, or stimulation-contraction coupling. Other research groups have used the cardiac-conditioned medium (medium collected from cardiac cell culture) to culture their constructs, as they are believed to contain an array of cues beneficial for cardiomyocyte growth.[53]

3.2. Bioreactors with Mechanical Stimulation in CTE

Bioreactors used for mechanical stimulation tend to have a simple setup composed of a culture chamber where the construct is placed, a device for stretching the construct that is simultaneously attached to a force-generating gear, and an electrical circuit that executes a programmed stimulation protocol.[9] These bioreactors are configured to fit inside a conventional incubator, maintain the temperature and gas exchange constant, and replenish the medium manually (**Table 2**). Salazar and colleagues developed a bioreactor that generated mechanical stimulation through a magnetic field using a linear voice coil actuator (VCA), which reduced vibration, compared to the traditionally used stepper motor.[54] The amount of stimulation was controlled based on the current applied to the VCA. Fibrin gels seeded with neonatal rat cardiomyocytes (NRCM)

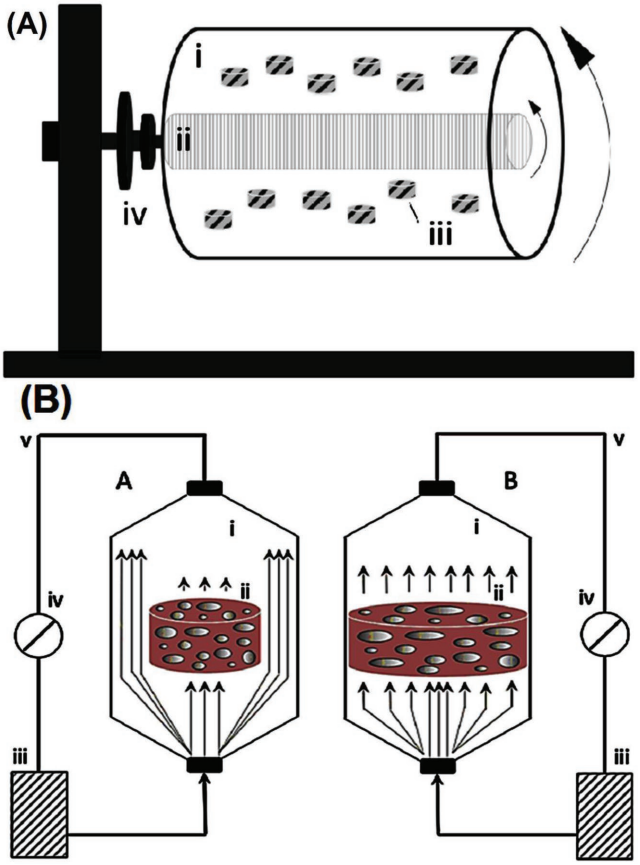

Figure 4. A) A perfused rotary bioreactor. Schematic representation of a rotating wall vessel showing the outer i) and inner ii) cylinders, the cell/scaffold constructs iii) and the rotator base iv). Rotating wall vessels are systems completely filled with culture medium (without a gas—liquid interface), where medium oxygenation is provided via a silicone-rubber gas-transfer membrane. Constructs are cultured in a "free-fall" state when the velocity of the rotating fluid is equal and opposite to the sedimentation rate of the constructs. B) Perfusion bioreactor. Schematic representation of an indirect (A) and a direct (B) perfusion bioreactor showing i) the culture chambers, ii) the cell/scaffold constructs, iii) the culture medium reservoirs, iv) the peristaltic pumps, and v) the tubing systems. In indirect perfusion bioreactors the culture medium follows the path of less resistance around the constructs. In direct perfusion bioreactors the cell/scaffold constructs are placed in a press-fitted fashion in the culture chamber and the medium is perfused throughout the constructs. Reproduced under the terms and conditions of the Creative Commons Attribution license 3.0.[37] Copyright 2014, the authors.

were stimulated at a frequency of 1 Hz, with 10% stretch, for a 4 h period. The stimulation protocol was applied once after spontaneous contraction of the construct was observed (around day 9) to assess the effect of mechanical stimulation on twitch force. A total of 10 constructs were mechanically stimulated, and an average increase of 1.5-fold in-twitch force was observed. Immunohistochemical analysis revealed enhanced alignment, orientation, and coupling of cardiomyocytes by expression of elevated levels of α-actinin, connexin-43 (Cx43), and collagen type I. Despite numerous advantages, one limitation of this system is that it renders asymmetrical stimulation because the constructs are fixed on one side and stretched by the other. To overcome this, one study used 2 mm thick arginine-glycine-aspartate (RGD)-conjugated alginate scaffolds seeded with NRCM (also containing a small population of cardiac fibroblasts) to assess the effects of compression on cardiac tissue development.[55] The constructs were mechanically compressed with pistons for 30 min d^{-1} or in a continuous fashion for 4 d at a frequency of 1 Hz and 15% strain. The bioreactor mimicked the state of the heart when ejecting blood through compression (a piston-compressed construct) and shear stress (achieved by the perfused medium). Intermittent compression resulted in cell elongation, organized myofibrils, and well-defined Z-lines. Moreover, FGF2 and TGF-ß secretion were upregulated in intermittently stimulated constructs. Electrical and metabolic coupling was enhanced with intermittent stimulation as made evident by elevated expression of Cx43. Thus, intermittent mechanical stimulation led to the organization of tissue and secretion of survival cues. It is interesting that continuous stimulation rendered inferior results, considering that the adult heart beats at a constant frequency of approximately 1 Hz. This raises the question of how much stimulation is appropriate for maturation of the construct. Another research group attempted to overcome asymmetrical elongation of constructs by fixing a seeded scaffold using four stainless steel pins.[56] A motor-controlled system subjected the construct to cyclic strain by moving the four pins back and forth for 6 d at an amplitude and frequency of 1 mm and 1 Hz, respectively. The construct was stimulated symmetrically, and the distribution of the mechanical force was asymmetrical with higher mechanical stress around the fixation points. Morphological analysis showed enhanced cell alignment in areas of high mechanical stress (around the fixation points). The center of the construct had a nondifferentiated architecture characterized by rounded and randomly oriented cells. By comparing the cellular morphology of the high and low-stress areas within the same construct, this experimental approach demonstrated that mechanical stimulation enhances cardiac cell orientation and coupling.

Under physiological conditions, the heart is mechanically strained eccentrically during diastole (ventricular filling) and then concentrically during systole (ventricular emptying). Hülsmann and colleagues developed a complex perfusion bioreactor, coupled with an inflatable latex balloon to simulate this biphasic stimulation closely.[57] Perfusion bioreactors use a pump mechanism to force medium directly through the construct (Figure 4B).[37] In this way, mass transfer is enhanced as it occurs both by convection and diffusion allowing O_2 to penetrate deeper into the tissue.[58,59] Additionally, the same group used a computational software, which allowed manipulation of balloon volume and frequency of stroke, medium pressure, perfusion rate, pH, O_2 concentration, CO_2 concentration, and temperature. As a proof of concept, they used reseeded decellularized rat hearts, placed the balloon inside the left ventricle, and perfused the medium through the coronaries. After 24 h of static incubation to allow seeding, mechanical stimulation was applied for 96 h. Cells showed anisotropic orientation following the matrix pattern poststimulation. However, cell maturation was not assessed. This bioreactor design had advantages over others reported because it allowed independent regulation

Table 2. Mechanical stimulation bioreactors.

Cell type + Scaffold	Bioreactor type	Construct size	Merits	Potential limitations	Reference
2.5 mm alginate hydrogel beads seeded with embryonic stem cells	Perfused rotating bioreactor	2.5 mm in diameter spherical beads	- High yield - High differentiation - Fast mass transfer.		[52]
Umbilical cord mesenchymal stem cells (UCMSCs) were seeded on clinically approved cardiovascular patch composed of expanded Polytetrafluoroethylene (ePTFE) coated with titanium	Perfusion bioreactor	0.4 mm in thickness	- Allows comparison of three samples, each with different media reservoir (3 different scaffolds, 3 different media, 1 stimulation) - Designed to fit inside a conventional incubator - Transparent for macroscopic visualization of the process - Manipulation of rate (bpm) and pressure (mmHg)	- Nonbiodegradable scaffold - Does not functionalize construct. - Limited contribution to cardiac tissue construction.	[62]
Arginine-Glycine-Aspartate (RGD)- conjugated alginate scaffolds seeded with neonatal rat cardiac cells (CM and CF)	Fed-batch bioreactor	5 mm in diameter; 2 mm in thickness. Only 100 µm-deep functionalization	- Allows stimulation of 48 constructs simultaneously - Can be adjusted for a variety of scaffolds - Medium perfusion due to compression - Simple set-up	- Production of TGF-ß should be monitored to prevent fibrosis- - Mechanical stress at a physiological frequency (1 Hz continuous) seemed detrimental.	[56]
Chitosan-collagen scaffolds coated with fibronectin seeded with NRCMs	Fed-batch bioreactor	14 mm in diameter × 2 mm in thickness	Channeled scaffold increases area of O_2 and nutrient diffusion. Scaffold architecture can be easily modified	- Cannot measure contractile force. Just qualitatively by observation - Not uniform mechanical stimulation throughout the construct that translates into a nonuniform maturation of the cells. - No control over biochemical parameters.	[56]
Decellularized rat hearts seeded with murine myoblasts	Perfusion bioreactor	Whole rat heart	- Fully automated - pH, PO_2, T° can be controlled independently - can design acidosis/alkalosis, hypoxia models. - Addition of an electrophysiological monitoring or stimulation modulus is feasible - Low cost solution - Whole heart model	- Cell maturation was not assessed: markers, contraction, relaxation, stimulation-contraction, - Mechanical stimulation yielded lower cell viability. - Re-endothelialization was not performed	[57]
NRCMs seeded onto a fibrin gel	Fed-batch bioreactor	20 mm × 20 mm patches. Monolayer of CM (about 24 µm in thickness)	- Reduced vibration by using VCA compared to the commonly used stepper motor. - Stimulation circuit can be connected to more than one bioreactor - No need of physically transferring the tissues to the bioreactor - All components can work inside a conventional incubator	- No control over any parameter other than mechanical stimulation. - Escalation (thicker constructs) seem unattainable using this approach as it bases nutrition wholly on diffusion. - Construct tearing under long stimulation periods - Stretch is applied unilaterally (asymmetric stimulation)	[54]
NRCMs seeded onto a fibrin gel embedded with Fe_3O_4 nanoparticles.	Fed-batch bioreactor Magnetic stretch	2 cm × 2 cm. Monolayer	- Noncontact stretch - Achieves 15% axial strain with low saturation of magnetic nanoparticles. - F_3O_4 is nontoxic - Magnetic stretch showed functional benefits on days 4 and 6 of culture.	- After 8 d of magnetic stretch there was no difference on twitch force between stimulated and static constructs. - No benefit on cell alignment by stretch protocol.	[77]

NRCM: Neonatal Rat Cardiomyocytes; CM: Cardiomyocytes; CF: Cardiac Fibroblasts.

of each parameter through its computation software. A wide variety of scenarios can be easily generated to gain insight into the effects of mechanical stimulation under pathological conditions such as hypoxia, acidosis, or alkalosis. Morgan and Black also proposed a bioreactor system able to modify the frequency of the mechanical stimulation.[60] They hypothesized that this could improve the mechanical response of cells due to adaptation to the stimuli. The bioreactor consisted of a distensible mandrel that expands when the air was injected.[61] The frequency was controlled through the action of solenoid valves via a custom-made LabVIEW program. Cardiomyocytes were seeded in a fibrin hydrogel and stimulated at 1 and 3 Hz. At the end of the study, no differences were observed in cell proliferation. However, important changes were detected in gap junctions that play an important role in intercellular communication.

To demonstrate that the adhered cells can withstand friction forces, mechanical stimulation bioreactors can be used to assess the integrity of seeded scaffolds as vehicles for cell delivery.[62] Since such bioreactors do not aim to functionalize cardiac constructs or precondition them for implantation, their contribution to CTE could be used as an initial phase of designing a construct to establish seeding parameters. Hollweck et al. designed a bioreactor that allowed simultaneous stimulation of three bioreactors, each with an independent medium reservoir.[62] The bioreactor stimulated the sample by generating pressure curves that exerted stress on the construct surface. It allowed manipulation on rate (bpm) and pressure (mmHg) and could be fitted inside a standard incubator.

3.3. Bioreactors with Electrical Stimulation in CTE

Excitation-contraction coupling is a fundamental characteristic of heart tissue. Electrical stimulation of cardiac cells leads to expression of genes implicated in the formation of the cardiac syncytium, which in turn allows the propagation and transduction of the action potential to synchronous muscular contraction.[49,63] In a recent study, carbon nanotubes (CNTs) and electrical stimulation were used to successfully differentiate MSCs toward a cardiomyocyte lineage that expressed progenitor (Nkx2.5), early (GATA-4), and mature (cardiac troponin T, Cx43, CMHC) cardiac markers. It was shown that the inclusion of electrical stimulation influenced cell morphology and alignment after 14 d (**Figure 5**A). Cardiomyocytes seeded on

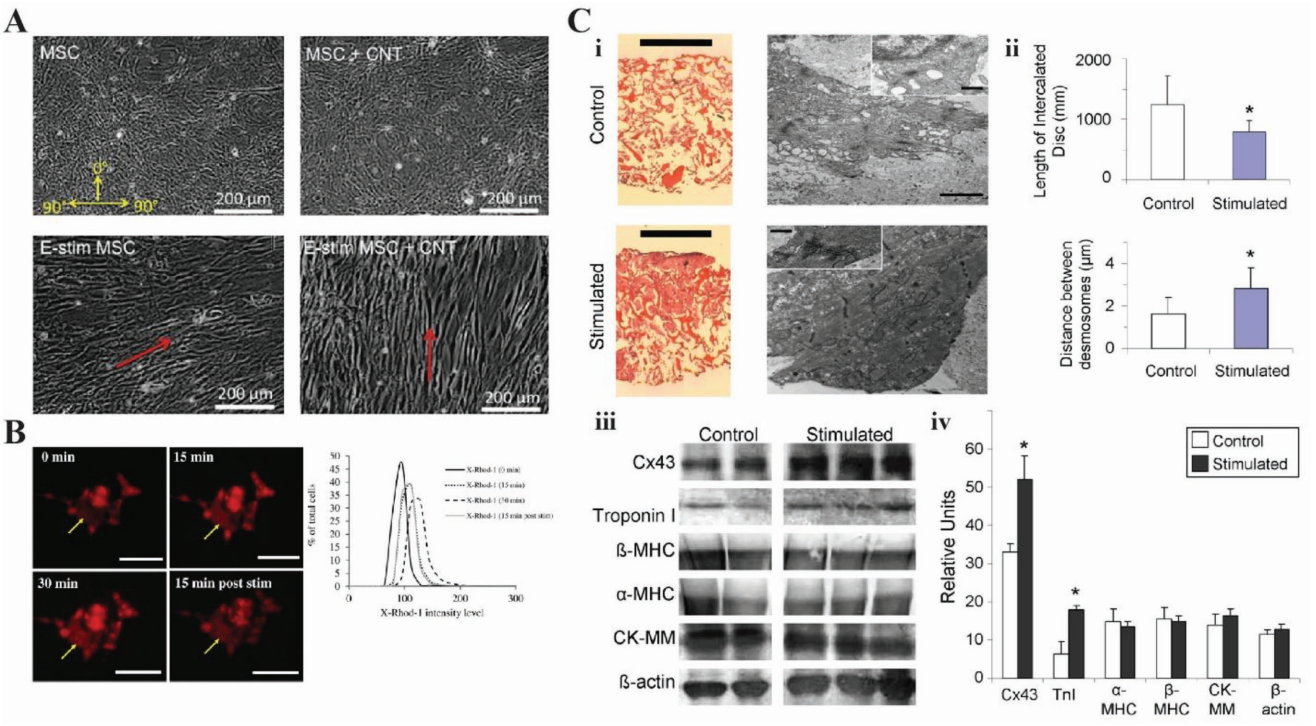

Figure 5. A) Effect of CNT and/or electrical stimulation on MSC morphology after 14 d. Stimulated MSC appeared elongated and aligned after 14 d compared to unstimulated control cultures. B) Levels of intracellular calcium were monitored in SH-SY5Y cells during stimulation with monophasic square waves. After 30 min of stimulation, calcium-binding dye X-Rhod-1 levels (yellow arrows) were increased, 15 min after stimulation was discontinued a decrease in X-Rhod-1 levels were observed. The corresponding intensity values of the field over 30 min stimulation were 5 V, 1 Hz square waves, and 15 min poststimulation as shown in the histogram. Scale bar = 100 µm. C) Cardiac constructs were stimulated with monophasic square wave pulses of 3 V amplitude, 3 Hz frequency, and 2 ms duration. i,ii) Hematoxylin and Eosin staining of the constructs show areas of compact tissue in the stimulated construct (Scale bar indicates 1 mm), transmission electron microscopy images with insets of sarcomeres, show better developed sarcomeres, longer intercalated discs and longer desmosome distance when stimulated (scale bar indicates 2 µm in main image, 500 nm in inset). iii, iv) Connexin-43 (C × 43) and Cardiac Troponin I were upregulated under stimulation, β myosin heavy chain (β-MHC), α myosin heavy chain (α-MHC), muscle-type creatine kinase (CK-MM), and β-actin did not have any significant changes as shown in the western blot protein analysis. Adapted under the terms and conditions of the Creative Commons CC-BY 4.0 license.[68] Copyright 2015, the authors. Adapted with permission.[63] Copyright 2009, Macmillan Publishers Limited. Adapted with permission.[69] Copyright 2011, John Wiley & Sons.

CNT-based scaffolds also showed more cardiomyocyte-like morphology.[49,64] Furthermore, electrical stimulation has been linked to appropriate cell migration and heart chamber development.[65] Dynamic bioreactor systems can aid in the functionalization of cardiac constructs by facilitating the delivery of controlled electrical cues to developing tissues (**Table 3**). An ideal electrical stimulation bioreactor should: (1) have a programmable stimulation protocol (duration and shape of wave, amplitude, frequency, time); (2) use biocompatible electrodes; (3) avoid nonreversible Faradaic reactions between the electrodes and medium; (4) have an integrated analytical measurement tool to assess the effects of electrical stimulation on stimulation threshold (ST), maximum capture rate (MCR), and contractile amplitude of the construct; and (5) a perfusion system to allow constant medium renewal and enhanced mass transfer.[63]

The ST is the minimum voltage required to produce sustained synchronous contractions,[66] the MCR is the maximum frequency of sustained synchronous contraction obtained by stimulation at 150% of ST,[66] and the contractile amplitude of the construct is the percentage change in the area of the construct per contraction.[66] In short, the ideal conditions of electrical stimulation would be those that yield low ST, high MCR, and high contractile amplitude in addition to galvanotropism (electric-field-induced morphological change) toward mature cardiac tissue and expression of terminal molecular cardiac markers (cardiac troponin T, Cx43, CMHC). The formation of nonreversible Faradaic reactions between the electrodes and medium should be avoided because of net charge changes in the medium, which cause electrode degradation and promote the formation of harmful cellular byproducts.[67] Application of biphasic pulses reduce the formation of such reactions; however, the second impulse may cause tissue hyperpolarization and inhibit the formation of a subsequent action potential.[63] Stated differently, the biphasic impulses circumvent the formation of nonreversible Faradaic reactions at the expense of effective electrical stimulation. Monophasic square waves minimize the formation of such reactions at the electrode-medium interphase. Thus, their use is preferred, as in some cell types membrane depolarization increases intracellular calcium levels, resulting in gene expression and activation of certain physiological functions (Figure 5B).[68]

Over the past decade, several optimal parameters for electrical stimulation of cardiac constructs have been elucidated. Tandon and colleagues compared the efficiency of electrodes made of carbon, titanium, or titanium nitride based on their capability to produce the lowest ST and best-improved excitability under dynamic culture conditions.[69] Titanium and titanium nitride electrodes showed no statistically significant difference to control groups and, thus, were labeled as nonideal. On the other hand, carbon electrodes were rendered the most suitable due to their biocompatibility, resistance to corrosion, and improved excitability.[63] Moreover, a study conducted using NRCM seeded onto Ultrafoam collagen scaffolds found the optimum stimulation amplitude, frequency, and duration of the impulse to be 3 V cm^{-1}, 3 Hz, and 2 ms pulses, respectively. The combination of these parameters achieved a twofold improvement in the amplitude of contraction (Figure 5C).[69] Another study determined that a 3 d incubation period before initiating electrical stimulation is optimal since premature stimulation hinders cardiac protein expression, while late stimulation has no beneficial effect on the formation of the cardiac

Table 3. Electrical stimulation bioreactors.

Cell type + Scaffold	Bioreactor type	Construct size	Merits	Potential limitations	Reference
Alginate scaffold seeded with neonatal rat cardiac cells	Perfusion bioreactor	5 mm in diameter; 2 mm in thickness	- Control over the stimulation waveform - Modular cultivation of several constructs	- The study showed higher levels of a-actinin quantitatively but no change in cell morphology. - No cell viability results to assess the beneficial effects of the reactor on cell nutrition. - External fixation of the construct is necessary	[59]
Poly(glycerol sebacate) (PGS) scaffold seeded with neonatal rat cardiac cells	Perfusion bioreactor	8 mm in diameter; 1 mm in thickness	- Cell seeding procedure ensures a homogeneous distribution of cells without blockage of channels. - No fixation of the construct that allows free contraction - Channeled scaffold permits medium flow through the construct rather than merely around it. - Low flow rate prevent tissue damage caused by sheer stress. - Linear electric field	- Although results showed marked trends, they were not statistically significant. - The cells formed clusters throughout the construct but did not form a single interconnected tissue probably because of lack of a gel carrier and poor adhesiveness to the PGS scaffold. - Patent channels at the expense of cell adhesion (no gel carrier)	[71]

syncytium.[70] Furthermore, by day 8 of culture, the formation of gap junctions and assembly of striated myofibrils should be prominent.

Coupling perfusion bioreactors with electric field stimulation have an additional beneficial effect on cardiac construct engineering. Recently, a perfusion bioreactor that allowed independent control over the stimulation waveform, stimulation frequency, and perfusion rate as well as control over pH, CO_2 concentration, and O_2 concentration in consort was designed.[59] The system was tested using a 5 mm in diameter × 2 mm in thickness alginate scaffold seeded with neonatal rat cardiomyocytes. The cells were subjected to electrical stimulation and medium perfusion, resulting in high α-actinin and Cx43 expression that denotes cardiac maturation. A similar study also implemented a perfusion bioreactor to assess the effect of perfusion and electrical stimulation on cardiac tissue development.[71] An 8 mm in diameter × 1 mm in thickness porous poly(glycerol sebacate) (PGS) scaffold with parallel cubical channels (to enhance mass transfer) was fabricated, and cells were seeded dynamically through perfusion that allowed their homogeneous distribution without channel blockage. The constructs were held in place by the vertical flow of medium, circumventing the need for external fixation. At 3 d postseeding, the constructs were either constantly stimulated (3 V cm^{-1}, 1 Hz, monophasic square wave, 2 ms duration) or cultured statically for additional 5 d. The perfused and stimulated constructs had the lowest ET, and highest MCR and contractile amplitude. Moreover, the constructs maintained a homogenous distribution of cells throughout the construct thickness in comparison with static cultures where more cells were localized on the surface (closer to the medium), emphasizing the benefit of perfusion in mass transfer. Overall, this study accentuates the beneficial effects of integrating electrical stimulation to perfusion bioreactors for CTE.[71]

3.4. Bioreactors for the Formation of Vascularized Constructs

A major drawback in CTE has been the inability to create thick, viable constructs that surpass the diffusion barrier of 100 μm,[72] as native cardiac cells are extremely metabolically active and require constant nutrients. As a result, heart tissue must be thoroughly perfused within an intercapillary distance as small as 25 μm.[72] Novel CTE approaches have integrated the use of perfusion bioreactors to promote functional vascularization of constructs through the delivery of proangiogenic cues such as VEGF, coculture of angiogenic cells with cardiomyocytes, and re-endothelialization of decellularized matrices (**Table 4**).

Sekine and colleagues recently developed a vascularized 3D cardiac construct using a cell sheet technique in combination with a perfusion bioreactor.[12] First, triple-layered sheets were prepared using NRCMs cocultured with neonatal rat endothelial cells (NRECs). Then, a vascular bed was resected from the femoral vascular network of mice, placed on a bioreactor, and perfused with culture medium enriched with FGF2 using the artery as an inlet and the vein as an outlet. The triple-layered sheet was placed over the vascular bed and incubated for 3 d. Histological analysis showed the formation of tubular and patent chimeric capillaries (newly formed capillaries anastomosed with preexisting ones from the vascular bed). Furthermore, escalation of the construct was attempted using a typical three-cell-layer overlapping approach followed by a 3 d incubation period in between the addition of each new sheet. Medium perfusion enhanced the integration of cell sheets with the vascular bed and formation of chimeric capillaries by creating a diffusion gradient of FGF2. Moreover, the integration and survival potential of the construct was assessed through implantation in a murine model by anastomosing the artery and vein of the construct to the carotid artery and the jugular vein, respectively. The vascularized graft with blood vessel anastomoses remained viable and beating for 2 weeks after the implantation procedure.[12]

In a similar study, Sakaguchi et al. used a perfusion bioreactor to promote angiogenic cell migration and anastomosis to preformed microchannels.[11] Triple-layer sheets of NRCMs+NRECs were placed over a microchannel (300 μm in diameter), and collagen hydrogel was perfused with VEGF+/FGF2- medium using a syringe pump. After a 5 d cultivation period, 24 μm thick constructs were viable and formed a patent capillary network that penetrated the collagen hydrogel and anastomosed with the microchannel. Escalation to a twelve-cell-layer construct was achieved by overlapping triple layer constructs at 5 d intervals. Integrated patent capillaries expanded the construct and anastomosed with the microchannel. This construct was 110 μm in thickness, just above the diffusion threshold for O_2 (100 μm). Therefore, from three- to six- cell layers the constructs could have remained viable due to the delivery of O_2 through convection by the constant perfusion of media rather than by perfusion through the capillary network. Attention has shifted toward enhancing the delivery of O_2 and nutrients to cardiac constructs. Failure to provide sufficient nutrients and O_2 to thicker constructs results in the formation of necrotic cores, rendering the tissues nonviable.[3,7] Increasing the perfusion rate in parallel to construct escalation could improve O_2 diffusion and reduce necrosis.

Another promising scaffold for CTE is the porcine cardiac ECM (pcECM) that can be re-seeded and re-endothelialized.[73] One study characterized pcECM as a viable matrix for CTE.[74] The group established optimal culture and seeding parameters and evaluated the effect of dynamic culture conditions and use of functional vascularization on construct seeding and cell viability. They achieved a higher cell penetration under dynamic conditions (perfusion) than under static conditions (400 vs 100 μm), probably attributable to higher nutrient diffusion by convection. Furthermore, they assessed re-endothelialization of the coronary tree using HUVECs in dynamic perfusion conditions with the VEGF-enriched medium. Moreover, they assessed the relevance of pcECM matrix for CTE by seeding the scaffold with human ESC-derived cardiomyocytes (hESC-CMs) under static conditions. Synchronously beating constructs were formed at 3 d postseeding with a penetration of 100 μm (equal to hMSC test in static conditions). The reactor was equipped with a balloon and electrodes for mechanical and electrical stimulation; however, the effects of these types of stimulation were not assessed in the study. In short, they proved pcECM as a viable scaffold for CTE (good seeding capacity), defined the optimal culture parameters, developed a dynamic bioreactor

Table 4. Bioreactors for the formation of vascularized constructs.

Cell type + Scaffold	Bioreactor type	Construct size	Merits	Potential limitations	Reference
Bioreactors for the formation of vascularized constructs					
NRCM + NREC cell sheets placed over a microchannel collagen gel	Perfusion bioreactor	12 cell layer (about 110 µm in thickness)	- Simple bioreactor set-up - Medium perfusion creates a VEGF-FGF2 gradient that promotes EC migration and organization (angiogenesis)	Thicker constructs developed discrete necrosis	[11]
NRCM + NREC cell sheets mounted on a vascular bed	Perfusion bioreactor	12 layers (about 110 µm in thickness using Sakaguchi et al. as a parameter)	- Formation of chimeric capillaries - Easily implantable - Vascularization allows escalation of the construct - Fast gap junction formation in cell sheets = electrical communication. - Media perfusion triggered formation of tubular EC network. - Remained viable after implantation.	- Several cycles of sheet overlapping are required for escalation, giving rise to multiple opportunities of failure/contamination. - A preexisting vascular bed is required - When scaling-up, uniform perfusion may be insufficient for tissue survival, limiting the final thickness of the construct.	[12]
pcECM seeded with NRCM and HUVECs for re-endothelialization	Perfusion bioreactor	Whole porcine heart	- Whole-heart model - Human size heart model - Porcine hearts have high similarity in architecture and physiology to human heart. - Vascular integrity maintained - Valvular integrity maintained that is important for mechanical profile.	- High cost - Large amount of cells required for re-cellularization. - No platform for mechanical stimulation. - Electrical stimulation yielded low electrical activity	[76]
pcECM seeded with hMSC and HUVECs for re-endothelialization	Perfusion bioreactor	1.7 mm in thickness	- Has inlets for a balloon and electrodes for future mechanical and electrical stimulation. - Allows parallel assessment of two constructs for statistical repetition - Has sampling port that allows media sampling without disturbing culture conditions. - ECM cell holding capacity closely resembles human parameters. - Pre-vascularized matrix	- No mechanical or electrical profile of constructs - Construction using CM was not assessed thoroughly.	[74]
HUVECs embedded in a 3D bioprinted alginate and GelMA scaffold coated with NRCM.	Perfusion bioreactor	5.5 $mm^2 \times 3.5$ mm in thickness	- Synthesis of anisotropic scaffolds. - Allows control of flow rate and O_2 distribution. - Formation of 3D endothelialized networks - Cell alignment followed anisotropic scaffold. - Spontaneous and uniform contraction rate (55–75 bpm). - Platform for cardiovascular drug assays.	- Endothelialized networks were not patent. - Crossing fibers counteracted overall construct anisotropy. - Contraction rate decreased with time. - Bioreactor only allows loading of 1 mm thick constructs	[78]

NRCM: neonatal rat cardiomyocytes; NREC: neonatal rat endothelial cells; pcECM: porcine cardiac extracellular matrix; HUVEC: human umbilical vein endothelial cell; hMSC: human mesenchymal stem cells.

that yielded higher cell penetration through perfusion of the pre-vascularized tree, re-endothelialized that tree, and made 1.7 mm thick viable constructs. Although the dimensions were inferior to the human ventricular wall thickness,[75] this effort showed the importance of fabricating a functional vascular tree within the scaffold for the construction of thicker tissues.

Weymann et al. used a similar approach to successfully re-seed and re-endothelialize a human-sized heart model using a complete decellularized pcECM (in contrast to the aforementioned study were pcECM was cut into slabs).[76] Characterization of the pcECM showed the conserved architecture, patent coronaries up to the fourth level of ramification, preservation of elastin in large vessels, and similar biomechanical profiles between native and decellularized hearts (LV pressure and loading volume). For re-endothelialization, HUVECs were delivered through coronary perfusion using a bioreactor

system, while NRCMs were delivered through five intramural injections in the left ventricle (LV). PECAM1 staining showed incomplete re-endothelialization. Nevertheless, ventricular recellularization was higher at sites of injection. Nonetheless, cells preserved viability, and the heart had discrete foci of electrical activity after stimulation with pacemakers (indicating the formation of an embryonic syncytium). Although the functionality of the bioartificial hearts is limited, this study generated a whole heart scaffold with perfusable coronary vasculature, patent cardiac valves, and intact 3D architecture. Enrichment of culture medium with cardiomyogenic and angiogenic cues could increase cellularity and homogeneity.

4. Current Trends

The engineering of multifunctional cardiovascular constructs has become one of the main focuses in biotechnology and TE (**Figure 6**). Several techniques and approaches have been described in this review, and most studies have highlighted the importance of selecting the proper combination of cells and materials to create the optimum microenvironment for cell growth. The successful fabrication of a biomimetic scaffold will result in cellular homeostasis, where cardiomyocytes can perform the mechanical function of the heart, ECs are critical for vascularization, and cardiac fibroblasts for ECM synthesis.

For the generation of large constructs, cell sheet technologies and decellularized matrices are being explored. However, scale-up issues on cell sheet technologies limit their use for future applications unless multiple surgical interventions can be circumvented. Even though improvement toward the development of thicker tissues has been made, no construct comes close to the native left ventricular myocardial thickness. A recent study measured this thickness on 300 subjects using chest magnetic resonance. They reported an average thickness of 6–8 and 5–7 mm in men and women, depending on the region of the heart cavity.[75] No engineered tissue reviewed herein came close to those figures,[74] and until such thickness is achieved, the application of cardiac constructs in clinical scenarios will continue to be out of reach. Proper electrical and mechanical integration of constructs within the native tissues are imperative to prevent secondary complications such as arrhythmias, or cardiac ruptures.[48] Moreover, efforts have to be made to replicate complex physiological conditions such as the Frank-Starling response within a construct.

Comparatively, decellularized hearts have structural anisotropy, and the framework of the vascular tree is required for appropriate perfusion. The use of decellularized whole hearts as scaffolds seeded with all the different cardiovascular lineages incorporated with biochemical, mechanical, and electrical stimulation in a perfusion bioreactor holds great promise for the development of new tissues. However, the generation of all functional components such as heart valves using the aforementioned 3D construct of clinically relevant dimensions remains a serious challenge. In this context, promising results have been obtained using bioreactor system in combination with scaffold and fully differentiated vascular-derived cells. Even though specific parameters for stimulation are still under investigation, the combination of the aforementioned cues has shown potential to create physiological mimicry effectively. TE using bioreactor and decellularized scaffolds, either with or without incorporating the aforementioned biological cues along with freshly isolated autologous cells independently or in combination, may represent the CTE paradigm of the future.

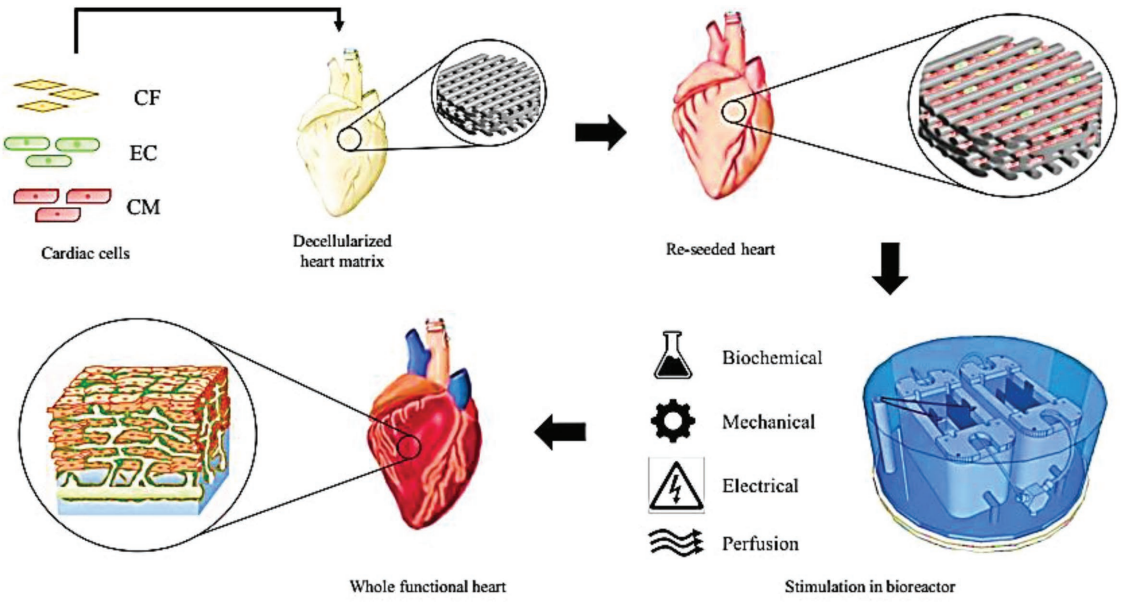

Figure 6. Current trend for CTE. Cardiac cells are seeded on a heart matrix and functionalized in a bioreactor to yield a vascularized heart with biomimetic activity. CF: Cardiac fibroblasts; EC: Endothelial cells; CM: Cardiomyocytes. Adapted under the terms and conditions of the Creative Commons Attribution license 3.0.[11] Copyright 2011, the authors. Adapted under the terms and conditions of the Creative Commons Attribution 4.0 (CC BY) license.[74] Copyright 2015, Mary Ann Liebert, Inc.

5. Concluding Remarks and Future Perspectives

Considerable advances have been made over the past decade in the field of CTE, due to the integration of bioreactor systems as tools to create tightly controlled culture conditions that can be tailored to deliver biochemical, mechanical, and electrical stimulation. A variety of cardiac and vascular growth factors have been incorporated into culture systems and efficiently delivered to developing constructs using perfusion bioreactors. Furthermore, mechanical bioreactors initially used for cartilage and bone TE have served as a stepping stone for the development of suitable tissue models in cardiac applications. However, the widespread setups for these types of bioreactors remain rudimental and do not readily permit the replication of hereditary conditions in vitro.

Though a plethora of information is available in the literature about different bioreactors, only a few bioreactors made their way to the market with several concerns, i.e., whether the proposed configurations are optimal for translation in specific clinical situations. From the regulatory administration and legalization authorities' perspective, the development of customized bioreactors that meet specific clinical requirements is expensive, time-consuming, and often do not comply with international regulatory needs for translation into the clinics. Besides the above-mentioned needs, other critical requirements that can impact the design of a bioreactor includes (1) dependent/independent role of the bioreactor environment, (2) scaffold properties, (3) cell origin and phenotype, (4) control functional maturation of 3D substitutes, (5) standardized protocols for CTE, among others. Over the next few years, efforts to build more complex bioreactors should be pursued. As an example, considerable efforts toward the definition of optimal electrical stimulation parameters have been made; however, an electrically functional construct has yet to be developed. Perhaps the greatest accomplishments have been in the construction of vascularized cardiac tissues, as perfusion bioreactors have permitted the effective re-endothelialization and repopulation of decellularized scaffolds; in the near future, a human size heart might be successfully reseeded. In the case of functional myocardium, a transition toward the use of human cells instead of the commonly used neonatal rat cells could render a higher level of mimicry with possible translation to clinical trials. Overall, the use of bioreactors in CTE has proven to be beneficial for differentiation, acquisition of a mature tissue phenotype, and formation of the cardiac syncytium. Nevertheless, the field is still in its infancy but holds great promise for the development of more sophisticated systems that mimic the human heart.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

bioreactors, cardiac diseases, cardiac tissue engineering, electrical stimulation, mechanical stimulation, tissue engineering, vascularization

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[1] World Health Organization, "WHO | Cardiovascular diseases (CVDs) :Fact sheet", http://www.who.int/mediacentre/factsheets/fs317/en/, (accessed: February 2018).
[2] WHO, *World Health* **2014**, 176.
[3] E. C. Martinez, T. Kofidis, *Expert Rev. Cardiovasc. Ther.* **2009**, *7*, 921.
[4] L. Lilly, *Pathophysiology of Heart Disease. A Collaborative Project of Medical Students and Faculty*, Wolters Kluwer/Lippincott Williams & Wilkins, New York **2010**.
[5] P. Barnett, M. J. B. Van Den Hoff, *Med. Biol. Eng. Comput.* **2011**, *49*, 723.
[6] V. Barron, E. Lyons, C. Stenson-Cox, P. E. McHugh, A. Pandit, *Ann. Biomed. Eng.* **2003**, *31*, 1017.
[7] J. Liu, H. Zheng, P. S. P. Poh, H. G. Machens, A. F. Schilling, *Int. J. Mol. Sci.* **2015**, *16*, 15997.
[8] L. Iop, G. Gerosa, *Biomed Res. Int.* **2015**, *2015*, 1.
[9] R. K. Birla, Y. C. Huang, R. G. Dennis, *Tissue Eng.* **2007**, *13*, 2239.
[10] G. Jin, G.-H. Yang, G. Kim, *J. Biomed. Mater. Res., Part B* **2015**, *103*, 935.
[11] K. Sakaguchi, T. Shimizu, S. Horaguchi, H. Sekine, M. Yamato, M. Umezu, T. Okano, *Sci. Rep.* **2013**, *3*, 1.
[12] H. Sekine, T. Shimizu, K. Sakaguchi, I. Dobashi, M. Wada, M. Yamato, E. Kobayashi, M. Umezu, T. Okano, *Nat. Commun.* **2013**, *4*, 1399.
[13] L. Ren, D. Ma, B. Liu, J. Li, J. Chen, D. Yang, P. Gao, *Biomed Res. Int.* **2014**, *2014*, 301279.
[14] S. Schürlein, R. Al Hijailan, T. Weigel, A. Kadari, C. Rücker, F. Edenhofer, H. Walles, J. Hansmann, *Adv. Biosys.* **2017**, *1*, 1600005.
[15] D. E. Ingber, V. C. Mow, D. Butler, L. Niklason, J. Huard, J. Mao, I. Yannas, D. Kaplan, G. Vunjak-Novakovic, *Tissue Eng.* **2006**, *12*, 3265.
[16] S. Lalan, I. Pomerantseva, J. P. Vacanti, *World J. Surg.* **2001**, *25*, 1458.
[17] R. Langer, J. P. Vacanti, *Science* **1993**, *260*, 920.
[18] A. H. Reddi, N. S. Cunningham, *J. Biomech. Eng.* **1991**, *113*, 189.
[19] R. M. Nerem, *Med. Biol. Eng. Comput.* **1992**, *30*, CE8.
[20] W. C. Puelacher, S. W. Kim, J. P. Vacanti, B. Schloo, D. Mooney, C. A. Vacanti, *Int. J. Oral Maxillofac. Surg.* **1994**, *23*, 49.
[21] A. Sun, W. Parisius, H. Macmorine, M. Sefton, R. Stone, *Artif. Organs* **1980**, *4*, 275.
[22] K. Asonuma, J. P. Vacanti, *Crit. Care Nurs. Clin. North Am.* **1992**, *4*, 249.
[23] C. A. Vacanti, J. P. Vacanti, R. Langer, **1994**, pp. 16–34.
[24] I. V. Yannas, J. F. Burke, *J. Biomed. Mater. Res. Part A* **1980**, *14*, 65.

[25] F. Guilak, D. L. Butler, S. A. Goldstein, F. P. T. Baaijens, *J. Biomech.* **2014**, *47*, 1933.
[26] D. L. Butler, S. A. Hunter, K. Chokalingam, M. J. Cordray, J. Shearn, N. Juncosa-Melvin, S. Nirmalanandhan, A. Jain, *Tissue Eng., Part A* **2009**, *15*, 741.
[27] F. T. Moutos, L. E. Freed, F. Guilak, *Nat. Mater.* **2007**, *6*, 162.
[28] S. Pal, *Design of Artificial Human Joints & Organs*, Springer US, Boston, MA **2014**.
[29] T. A. L. Wren, S. A. Yerby, G. S. Beaupr??, D. R. Carter, *Clin. Biomech.* **2001**, *16*, 245.
[30] M.-M. Khani, M. Tafazzoli-Shadpour, Z. Goli-Malekabadi, N. Haghighipour, *J. Mech. Behav. Biomed. Mater.* **2015**, *43*, 18.
[31] S. Dermenoudis, Y. Missirlis, *J. Biomech.* **2010**, *43*, 1426.
[32] E. J. Lee, L. E. Niklason, *Tissue Eng., Part C* **2010**, *16*, 1191.
[33] Y. Narita, K.-I. Hata, H. Kagami, A. Usui, M. Ueda, Y. Ueda, *Tissue Eng.* **2004**, *10*, 1224.
[34] L. Song, Q. Zhou, P. Duan, P. Guo, D. Li, Y. Xu, S. Li, F. Luo, Z. Zhang, *PLoS One* **2012**, *7*, e42569.
[35] A. H. Huang, Y.-U. Lee, E. A. Calle, M. Boyle, B. C. Starcher, J. D. Humphrey, L. E. Niklason, *Tissue Eng., Part C* **2015**, *21*, 841.
[36] N. Mabvuure, S. Hindocha, W. S. Khan, *Curr. Stem Cell Res. Ther.* **2012**, *7*, 287.
[37] M. Sladkova, G. de Peppo, *Processes* **2014**, *2*, 494.
[38] R. Sui, X. Liao, X. Zhou, Q. Tan, *Stem Cell Rev. Rep.* **2011**, *7*, 172.
[39] J. Leor, N. Landa, S. Cohen, *Expert Rev. Cardiovasc. Ther.* **2006**, *4*, 239.
[40] W. H. Zimmermann, T. Eschenhagen, *Trends Cardiovasc. Med.* **2007**, *17*, 134.
[41] C. Muscari, E. Giordano, F. Bonafè, M. Govoni, C. Guarnieri, *Stem Cells Int.* **2014**, *2014*, 434169.
[42] H. Parsa, K. Ronaldson, G. Vunjak-Novakovic, *Adv. Drug Delivery Rev.* **2016**, *96*, 195.
[43] P. Christalla, J. E. Hudson, W. H. Zimmermann, *Cells Tissues Organs* **2011**, *195*, 82.
[44] K. Y. Lee, M. C. Peters, K. W. Anderson, D. J. Mooney, *Nature* **2000**, *408*, 998.
[45] H. Sekine, T. Shimizu, T. Okano, *Cell Tissue Res.* **2012**, *347*, 775.
[46] W.-H. Zimmermann, I. Melnychenko, G. Wasmeier, M. Didié, H. Naito, U. Nixdorff, A. Hess, L. Budinsky, K. Brune, B. Michaelis, S. Dhein, A. Schwoerer, H. Ehmke, T. Eschenhagen, *Nat. Med.* **2006**, *12*, 452.
[47] S. Miyagawa, *Jpn. J. Anesthesiol.* **2014**, *63*, S82.
[48] J. P. Guyette, J. M. Charest, R. W. JMills, B. J. Ank, P. T. Moser, S. E. Gilpin, G. R. Gaudette, *Circ. Res.* **2016**, *118*, 56.
[49] W. L. Stoppel, D. L. Kaplan, L. D. Black, *Adv. Drug Delivery Rev.* **2016**, *96*, 135.
[50] M. Tallawi, E. Rosellini, N. Barbani, M. G. Cascone, R. Rai, G. Saint-Pierre, A. R. Boccaccini, *J. R. Soc., Interface* **2015**, *12*, 20150254.
[51] K. Matsuura, M. Wada, K. Konishi, M. Sato, U. Iwamoto, Y. Sato, A. Tachibana, T. Kikuchi, T. Iwamiya, T. Shimizu, J. K. Yamashita, M. Yamato, N. Hagiwara, T. Okano, *PLoS One* **2012**, *7*, e52176.
[52] A. Teo, A. Mantalaris, K. Song, M. Lim, *Biotechnol. Lett.* **2014**, *36*, 947.
[53] K. Ojima, M. Oe, I. Nakajima, M. Shibata, K. Chikuni, S. Muroya, T. Nishimura, *EuPA Open Proteomics* **2014**, *5*, 1.
[54] B. H. Salazar, A. T. Cashion, R. G. Dennis, R. K. Birla, *Cardiovasc. Eng. Technol.* **2015**, *6*, 533.
[55] M. Shachar, N. Benishti, S. Cohen, *Biotechnol. Prog.* **2012**, *28*, 1551.
[56] T. Zhang, L. Q. Wan, Z. Xiong, A. Marsano, R. Maidhof, M. Park, Y. Yan, G. Vunjak-Novakovic, *J. Tissue Eng. Regener. Med.* **2012**, *6*, 748.
[57] J. Hülsmann, H. Aubin, A. Kranz, E. Godehardt, H. Munakata, H. Kamiya, M. Barth, A. Lichtenberg, P. Akhyari, *J. Artif. Organs* **2013**, *16*, 294.
[58] A. B. Yeatts, J. P. Fisher, *Bone* **2011**, *48*, 171.
[59] Y. Barash, T. Dvir, P. Tandeitnik, E. Ruvinov, H. Guterman, S. Cohen, *Tissue Eng., Part C* **2010**, *16*, 1417.
[60] K. Y. Morgan, L. D. Black, *J. Tissue Eng. Regener. Med.* **2017**, *11*, 342.
[61] B. C. Isenberg, R. T. Tranquillo, *Ann. Biomed. Eng.* **2003**, *31*, 937.
[62] T. Hollweck, B. Akra, S. Häussler, P. Überfuhr, C. Schmitz, S. Pfeifer, M. Eblenkamp, E. Wintermantel, G. Eissner, *J. Funct. Biomater.* **2011**, *2*, 107.
[63] N. Tandon, C. Cannizzaro, P.-H. G. Chao, R. Maidhof, A. Marsano, H. T. H. Au, M. Radisic, G. Vunjak-Novakovic, *Nat. Protoc.* **2009**, *4*, 155.
[64] E. Mooney, J. N. Mackle, D. J. P. Blond, E. O'Cearbhaill, G. Shaw, W. J. Blau, F. P. Barry, V. Barron, J. M. Murphy, *Biomaterials* **2012**, *33*, 6132.
[65] J. Zhao, M. Griffin, J. Cai, S. Li, P. E. M. Bulter, D. M. Kalaskar, *Biochem. Eng. J.* **2016**, *109*, 268.
[66] Y. Barash, T. Dvir, P. Tandeitnik, E. Ruvinov, H. Guterman, S. Cohen, *Tissue Eng., Part C* **2010**, *16*, 1417.
[67] D. R. Merrill, M. Bikson, J. G. R. Jefferys, *J. Neurosci. Methods* **2005**, *141*, 171.
[68] G. M. Xiong, A. T. Do, J. K. Wang, C. L. Yeoh, K. S. Yeo, C. Choong, *J. Biol. Eng.* **2015**, *9*, 14.
[69] N. Tandon, A. Marsano, R. Maidhof, L. Wan, H. Park, G. Vunjak-Novakovic, *J. Tissue Eng. Regener. Med.* **2011**, *5*, e115.
[70] M. Radisic, H. Park, H. Shing, T. Consi, F. J. Schoen, R. Langer, L. E. Freed, G. Vunjak-Novakovic, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18129.
[71] R. Maidhof, N. Tandon, E. J. Lee, J. Luo, Y. Duan, K. Yeager, E. Konofagou, G. Vunjak-Novakovic, *J. Tissue Eng. Regener. Med.* **2012**, *6*, e12.
[72] S. Sekiya, T. Shimizu, T. Okano, *Regener. Med.* **2013**, *8*, 371.
[73] B. Wang, M. E. Tedder, C. E. Perez, G. Wang, A. L. De Jongh Curry, F. To, S. H. Elder, L. N. Williams, D. T. Simionescu, J. Liao, *J. Mater. Sci.: Mater. Med.* **2012**, *23*, 1835.
[74] U. Sarig, E. B.-V. Nguyen, Y. Wang, S. Ting, T. Bronshtein, H. Sarig, N. Dahan, M. Gvirtz, S. Reuveny, S. K. W. Oh, T. Scheper, Y. C. F. Boey, S. S. Venkatraman, M. Machluf, *Tissue Eng., Part A* **2015**, *21*, 1507.
[75] N. Kawel, E. B. Turkbey, J. J. Carr, J. Eng, A. S. Gomes, W. G. Hundley, C. Johnson, S. C. Masri, M. R. Prince, R. J. van der Geest, J. A. C. Lima, D. A. Bluemke, *Circ. Cardiovasc. Imaging* **2012**, *5*, 500.
[76] A. Weymann, N. P. Patil, A. Sabashnikov, P. Jungebluth, S. Korkmaz, S. Li, G. Veres, P. Soos, R. Ishtok, N. Chaimow, I. Pätzold, N. Czerny, C. Schies, B. Schmack, A. F. Popov, A. R. Simon, M. Karck, G. Szabo, *PLoS One* **2014**, *9*, e111591.
[77] M. Hogan, Y.-T. Chen, A. G. Kolhatkar, C. J. Candelari, S. Madala, T. R. Lee, R. Birla, *ACS Biomater. Sci. Eng.* **2016**, *2*, 1619.
[78] Y. S. Zhang, A. Arneri, S. Bersini, S. R. Shin, K. Zhu, Z. Goli-Malekabadi, J. Aleman, C. Colosi, F. Busignani, V. Dell'Erba, C. Bishop, T. Shupe, D. Demarchi, M. Moretti, M. Rasponi, M. R. Dokmeci, A. Atala, A. Khademhosseini, *Biomaterials* **2016**, *110*, 45.