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ABSTRACT

In the modern world, myocardial infarction is one of the most common cardiovascular diseases, which are responsible for around 18 million deaths every year or almost 32% of all deaths. Due to the detrimental effects of COVID-19 on the cardiovascular system, this rate is expected to increase in the coming years. Although there has been some progress in myocardial infarction treatment, translating pre-clinical findings to the clinic remains a major challenge. One reason for this is the lack of reliable and human representative healthy and fibrotic cardiac tissue models that can be used to understand the fundamentals of ischemic/reperfusion injury caused by myocardial infarction and to test new drugs and therapeutic strategies. In this review, we first present an overview of the anatomy of the heart and the pathophysiology of myocardial infarction, and then discuss the recent developments on pre-clinical infarct models, focusing mainly on the engineered three-dimensional cardiac ischemic/reperfusion injury and fibrosis models developed using different engineering methods such as organoids, microfluidic devices, and bioprinted constructs. We also present the benefits and limitations of emerging and promising regenerative therapy treatments for myocardial infarction such as cell therapies, extracellular vesicles, and cardiac patches. This review aims to overview recent advances in three-dimensional engineered infarct models and current regenerative therapeutic options, which can be used as a guide for developing new models and treatment strategies.

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I. INTRODUCTION

Cardiovascular diseases (CVD) have been the leading cause of death in the developed world for over 100 years, responsible for more deaths than all forms of cancer combined.[1] In the United States alone, CVDs are responsible for over 650 000 deaths yearly.[2] In addition to the burden on human health, CVDs cost the United States more than $350 billion every year, causing a massive economic burden.[2] Moreover, with the onset of the COVID-19 pandemic, cardiovascular complications arising from COVID-19 have added an extra burden to the healthcare systems.[3] Furthermore, with the average age of the population steadily increasing, the risk and costs of CVD related diseases have risen dramatically over the last decades, and will continue to rise in the future.[4]

Of the deaths caused by CVDs, myocardial infarction (MI) is responsible for over 50%.[1,2] The main mechanism of damage during MI is ischemic/reperfusion injury (I/R).[5] During MI, ischemia, or the lack of oxygen to the affected myocardium, leads to necrosis and cell death. This damage is further exacerbated following reperfusion of the damaged tissue, as the sudden influx of oxygenated blood leads to the development of reactive oxygen species (ROS) causing oxidative stress and additional cell death.[6–8] The limited healing capacity of cardiomyocytes (CMs) and the formation of fibrotic scar tissue produced by the activated cardiac fibroblasts (CFs) to repair damaged tissue leads to the loss of heart function and eventually heart failure. Accordingly, there is a significant amount of ongoing research across various disciplines to prevent and treat MI.[9–11] Although many approaches have been used to treat MI, limited success has been achieved due to the limited self-repair ability and regenerative capacity of native heart cells.[12]

Currently, heart transplantation is the only long-term therapeutic option for patients once end stage heart failure has been reached.[1,2,9,10] Unfortunately, the current demand for donor hearts significantly exceeds the amount available, leading to the death of many patients before this therapeutic option becomes available.[13,14] Additionally, many potential hearts are deemed unusable for transplant, with the major reasons including age and geographical distance from donor to recipient.[15] Therefore, it is not ethical or possible to experiment on a great number of these donor organs as they are vital for transplant patients.

With the inability to use human hearts at a rate required for experimental research, the majority of our current knowledge on how the constituents of the cardiovascular system (CVS) function has been obtained through the use of animal models.[5] Similarly, much of our understanding of heart disease comes from carefully manipulated animal models that possess a desired phenotype, which is not always attained in a physiologically realistic manner. Even though these experiments include carefully selected control groups, it is impossible to consider all potential variables, which may lead to contradictory outcomes.[16–19] Furthermore, due to the differences in human and animal physiology and pathology, there are often drastic differences between animal studies and human trials leading to poor success in clinical translation.[20]

Over the past few decades, tissue engineering has proven itself as a useful *in vitro* option for the study of heart disease under controlled conditions using human cells.[21–25] Such systems allow for the study of cellular responses to various stressors without the disadvantages of traditional *in vitro*, *ex vivo*, and *in vivo* models.[26] Furthermore, these engineered models facilitate direct testing on human tissue-like structures, which are invaluable for discovering preventive approaches and treatments while avoiding possible discordances seen when using non-human models. Additionally, tissue engineered models allow for the high-throughput investigation of physiological and pathological phenomena on local levels, while systemic influences seen in *in vivo* models could be antagonistic.[17,18,27–30] Additionally, the use of model tissues allows for selective inclusion, exclusion, or direct manipulation of individual cell types comprising the tissue.

Many current *in vitro* tissue models utilize biological scaffolds, hydrogels, or decellularized matrices to provide a 3D environment for cardiovascular cells that mimic their native environment.[17,24,25,27,29–36] The application of these novel engineering methods has led to the creation of heart tissue models that have enabled an understanding of MI that was not previously possible with the traditional methods.[17,18] Specifically, many of these models have been utilized to study the diagnosis, pathogenesis, and recovery before, during, and after I/R, as well as potential novel therapeutics for this pathology.[17,25,27,28,37]

Current therapeutics to treat the MI-induced damaged cardiac tissue include cellular therapies,[38–40] biomaterial-based therapies[41–43] or cell incorporated biomaterials, and direct reprogramming of fibroblasts into CMs.[44] Although cellular therapies have shown some success, recently the focus has been shifted to the paracrine signaling in the infarct tissue and therapies using cell secreted factors such as cytokines, growth factors, and microRNAs (miRNAs) packaged in extracellular vesicles (EVs) and exosomes.[45,46] Integration of exosomes with biological scaffolds such as cardiac patches has proven effective in preclinical trials and is under investigation in multiple ongoing clinical trials.[47]

In this review, we compile studies that engineered cardiac tissue models as platforms to study MI and fibrotic tissue models to investigate the mechanisms of MI and review the available regenerative therapeutic options for treating the infarct tissue. We briefly discuss the current understanding of MI pathogenesis as well as the subsequent endogenous response, both with and without clinical intervention. We then summarize the current limitations of *in vivo* and *ex vivo* models to better understand the need for tissue engineered models. Next, we review numerous 3D cardiac tissue models to study MI, as well as the infarct tissue models, based on fabrication method and discuss how they provide new insights into MI compared with current pre-clinical models. Then, we look at the current state of regenerative therapies applied for healing the infarct tissue. Finally, we discuss the current limitations of these tissue engineered models and therapies and how they can be addressed to further advance the field and subsequently patient care.

II. PHYSIOLOGICAL CHANGES IN THE HEART DURING AND AFTER MI

The main role of the heart is to provide blood to the entire body, including itself, via coronary arteries.[6] Through both genetic background and lifestyle choices, excess plaque can build up in these arteries leading to atherosclerosis.[48] This can in turn lead to complications in blood delivery to the myocardium.[6] If an extensive blockage occurs, oxygenated blood can no longer be provided to the tissue, leading to MI and I/R.

MI is the process of heart tissue necrosis from either a complete or a partial coronary blockage of downstream CMs. Without oxygen, CMs halt oxidative phosphorylation which in turn leads to adenosine triphosphate (ATP) depletion, the breakdown of mitochondrial membrane depolarization, and compromised contraction.[5] Cellular metabolism switches to glycolysis, creating an acidic (low pH) environment.[6] With the decreased pH, the Na^+–H^+ ion exchanger is activated causing an increase in intracellular Na^+. However, due to a lack of ATP during ischemia, the cell is unable to remove excess sodium through the Na^+–K^+ pump and is thus forced to run the Na^+–Ca^{2+} ion exchanger in reverse causing a cellular overload of Ca^{2+}.[6] If this cascade is allowed to continue unhindered for longer than 20 min, a wave of cell death begins at the infarct site traveling out.[5,6]

In order to prevent MI complications such as heart failure or patient death, the obstructed coronaries must be opened via either thrombolytic therapy or percutaneous coronary intervention (PCI).[49] However, the reperfusion of ischemic myocardium can itself induce CM death, typically increasing the final infarct size by 50%.[6–8] In the first few minutes following reperfusion, a large production of ROS occurs from a variety of sources.[5–8] This induces opening of the mitochondrial permeability transition pore (MPTP),[6,7] which contributes to the Ca^{2+} overload, damaging the cell membrane via lipid peroxidation.[5–8] Finally, the increased ROS levels cause enzyme denaturation and DNA damage[6] (Fig. 1).

Following the initial onset of MI, a systemic response and remodeling process begins. Immune cells play a significant role in the initial systemic response.[50–53] Neutrophils and macrophages infiltrate the wounded area within several hours of the infarct and remain there for several days to digest necrotic tissue. Afterward, macrophage-mediated cytokine release initiates remodeling, apoptosis, necroptosis, and neovascularization.[50,51] In addition to the immune response following MI, scar formation plays a significant role in cardiovascular remodeling. As CMs are incapable of proliferating, once the necrotic tissue has been successfully digested, it is replaced by activated CFs, which immediately begin to close the resulting wound.[50,51,54] This new fibrotic tissue contains high levels of collagen, which leads to an

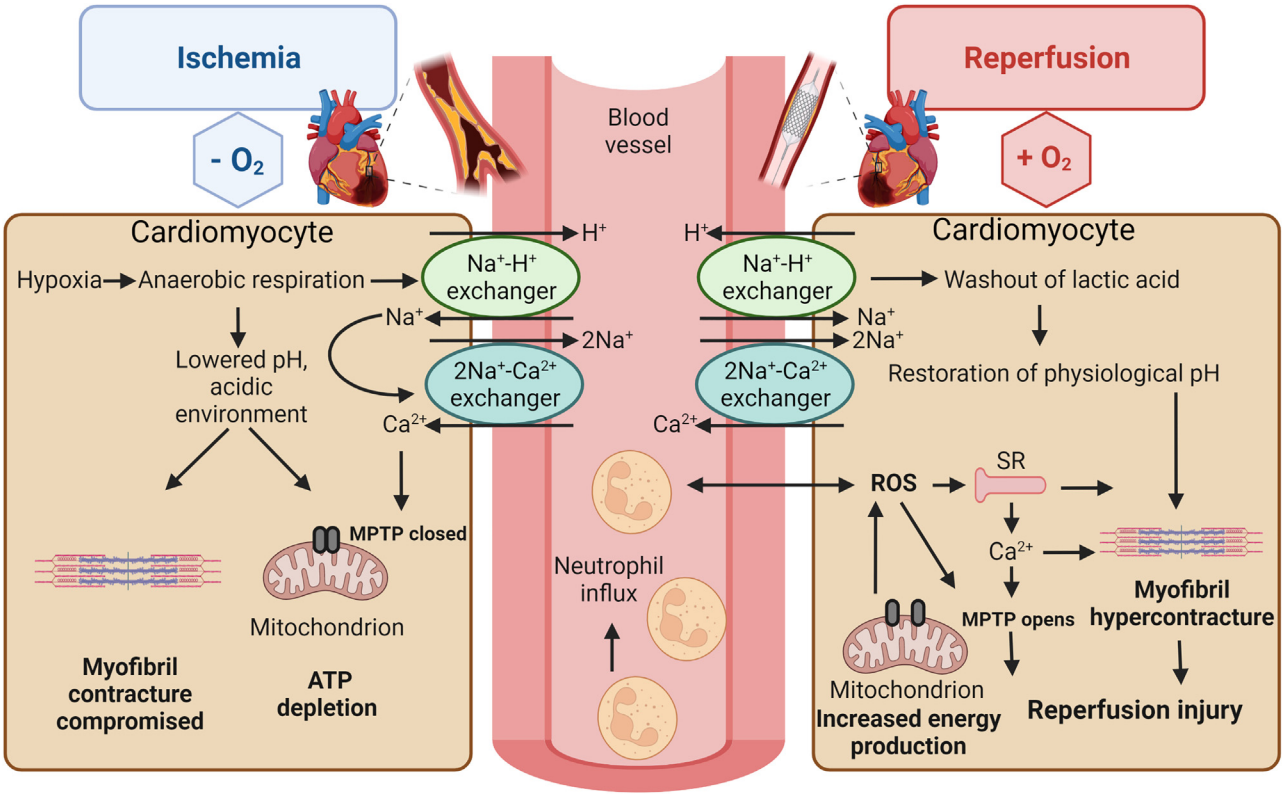

FIG. 1. Governing intracellular, extracellular, and molecular mechanisms in myocardial ischemia/reperfusion injury. During ischemia (on the left), lactate production results in reduction in intracellular pH. Na^+–H^+ and Na^+–Ca^{2+} exchangers are activated causing the overload in intracellular Ca^{2+}, restricting the MPTP opening on the mitochondrial membrane and cardiomyocyte contractility. During reperfusion (on the right), physiological intracellular pH is quickly restored, MPTP reopens, and cardiomyocytes contract abnormally due to increased ROS production and ion influx.

increase in heart stiffness at the injury site.[50,51,54] The increased stiffness, along with the inability of CFs to beat, results in decreased cardiac function and output, significantly increasing the risk of death from subsequent MIs.[6] Since MI contributes to heart disease progression and probable patient death, there has been tremendous research across many fields aimed at treating, mitigating, as well as modeling the pathophysiology of MI both *in vivo* and *in vitro*.

III. MI MODELS

Due to the limited regenerative capacity of the human heart, few therapeutic options are available that can be used to treat MI. Therefore, preclinical models used to mimic MI conditions are extremely beneficial for studying the biology of MI and testing new drugs and therapeutic options to treat the post-MI damaged tissue. This section is dedicated to reviewing the *in vivo*, *ex vivo*, and *in vitro* models that have been developed to better understand the outcomes of MI and I/R and on some occasions, used as platforms to test new drugs and therapies.

A. *In vivo* models

In vivo models are one of the most commonly used models to study MI and are greatly valuable for testing drug efficacy and safety, as well as the systemic response of the body under physiological conditions, which is not possible to do *in vitro* or *ex vivo*. With the help of *in vivo* MI models, researchers can create clinically relevant infarct sites, assess infarct size and microvascular damage, and evaluate the neovascularization, scar formation, inflammation or immune response, and changes in physiology and blood biomarker levels in the body in response to MI and treatments.[55] Methods to create MI models in animals are primarily surgical ligation (of left coronary artery), occlusion/constriction of arteries, intubation, chemical exposure (isoproterenol), cryoinjury, and genetic modification.[55,56]

Typically, MI is studied in small animal models like mice[56–59] and rats,[60–63] but larger animals such as rabbits,[64–67] dogs,[68–70] sheep,[71–73] pigs,[74–77] and minipigs[78,79] have also been used. Although small animal models are logistically convenient and easier to handle, they are limited by their different anatomy, physiology, and histology compared with humans. On the other hand, although larger animal models better mimic the anatomy and physiology of humans, they are difficult to work with due to their high cost, risk of infection, problems with arrhythmia and inconsistent perfusion, and ethical concerns.[55,57] Finally, due to the complexity of the *in vivo* environment, it is not possible to do mechanistic studies or dissect the effect of a treatment on a specific pathway.[80,81]

B. *Ex vivo* models

Ex vivo models involve the use of animal or human hearts outside the body or in culture conditions to evaluate the heart physiology in response to treatment. Several approaches have been used to create *ex vivo* models. In one approach, the whole heart is harvested and subjected to normal (working heart) or retrograde (Langendorff) perfusion through a pulmonary vein or coronary vasculature, respectively, using a special buffer/medium.[82–84] To create MI conditions, the perfusion is blocked for up to 3 h (ischemia) and restored (reperfusion), and the changes in heart physiology are recorded.[82–84] In another approach, the living heart tissue is cut into thin slices using a high-precision vibratome and maintained in culture.[85–90] The heart slices retain their viability and beat for up to 28 days in culture. *Ex vivo* models are beneficial because they allow for the accurate measurement of the infarct size, easy and reproducible assessment of left ventricular (LV) function and troponin levels, and high-throughput analysis.[55,91,92] However, these models eliminate the effect of systemic components like immune response, circulation, and interaction with other tissues in the body, which can make interpretation of the findings more difficult. Though less complex than *in vivo*, *ex vivo* models are still not simple enough for parametric studies; it is impossible to dissect the effect of one individual parameter or know the reasons for the observed outcome. Moreover, the risk of edema, the limited source of energy in culture conditions, and the limited stability of the tissues outside the body prevent these models from being preferable models to study MI.[55]

C. *In vitro* models

In vitro cardiac models involve the use of primary cells and/or cell lines, in the presence or absence of cytokines and biomaterials, in a controlled environment. The cells are usually obtained by isolating CMs from animal or human hearts or by differentiating stem cells. Although there is an ongoing discussion on the benefits and limitations of using cells isolated from neonatal or adult donors, primary CMs are the main cells used to study cardiac cell physiology;[93,94] however, their usage is restricted due to their limited proliferative capacity.[95,96] Moreover, adult donor cells require a technically challenging isolation process, transfection with viral vectors, a short-term culture-period after isolation, and are unable to spontaneously beat in culture.[97] Therefore, as an alternative, mesenchymal stem cells (MSCs),[98–100] embryonic stem cells (ESCs),[101] or induced pluripotent stem cells (iPSCs)[24,31,102] can be differentiated into CMs after amplification. These cells are especially valuable when creating MI models for high throughput assays or for engineering 3D models, particularly if they are from human-origin, allowing for patient-specific models.

Two-dimensional (2D) *in vitro* models are simple models obtained by culturing cells on a flat surface such as the bottom of culture plates or petri dishes. 2D culture is cost-efficient, easy to use and manipulate, and can be high throughput. It allows for better analysis of the cells and dissection of the factors that play roles in a particular mechanism.[103] Therefore, many studies choose to evaluate the effect of ischemic and reperfusion mimicking conditions, using 2D cardiac models.[104–106] However, 2D models overlook cell–matrix interactions and fail to account for the effects of other tissues or other systemic effects (circulatory, immune, and endocrine systems). Moreover, cell morphology in 2D is different than *in vivo*, leading to differences in the cell physiology and function compared to native tissue.[107] For instance, primary CMs change their phenotype when cultured in 2D, while stem cell (usually iPSC)-derived CMs (iCMs) are not as mature compared with 3D cultured cells.[108] Furthermore, 2D models usually include CMs alone; therefore, they lack the interaction between different cell types that are prominent in the heart. Additionally, 2D cultures are more sensitive to changes in oxygen levels compared with 3D models, which is an important parameter to study in MI models. These limitations prevent 2D models from closely recapitulating *in vivo* conditions, making them inadequate platforms to study I/R and result in poor clinical translation.[109]

The heart microenvironment is a dynamic 3D network, where the CMs and the stromal, endothelial (EC), and immune cells interact with each other, as well as with the extracellular matrix (ECM). Engineered models aim to recapitulate this 3D microenvironment as closely as possible. Since 3D constructs support the cell–cell and cell–ECM interactions, they yield more physiologically relevant results than 2D models. As the characteristics of each component can be controlled, the effect of each factor in these components on both normal heart physiology and function, and MI progression can easily be studied. Recent advances in tissue engineering, microfluidics, and stem cell technologies have made it possible to obtain human-representative and reliable results from engineered 3D MI models.[24,31,94,102] On the other hand, the inability to account for the *in vivo* systemic changes that occur after MI, as well as the immaturity of iCMs, the main cell type used in engineered cardiac models, compared with native CMs (which will be discussed in detail in Secs. III C 1 and III C 2), are limiting factors for 3D *in vitro* models. Another challenge in 3D engineered models is the restricted nutrient and gas transfer within these constructs, which limits the size and thickness of the construct that can be achieved. To address this problem, constructs that allow controlled ingrowth of blood vessels within the engineered tissue should be designed.[110,111] Hence, scaffolds should be made with large pores and contain growth factors to induce angiogenesis. Blood vessel formation is essential for the success of these constructs as these vessels help maintain cell viability and are critical for integration of the construct with the host tissue, and thus allow for the treatment of larger defects in the heart.

Regardless of these limitations, these models are becoming the platform of choice to study MI. However, it should be noted that 3D *in vitro* models aim to be complementary to *in vivo* models, as *in vivo* models are crucial to evaluate the safety, biodistribution, and efficacy of a drug/treatment, and the systemic response of the body against it.

1. Engineered 3D models to study I/R

To engineer more biomimetic 3D *in vitro* models that recapitulate cell–cell and cell–ECM interactions, various methods have been utilized including cell sheets, spheroids/organoids, microfluidic devices, and bioprinting (Fig. 2). Section III C 1 brings together recent studies on 3D cardiac tissue models using these techniques, specifically focusing on the effect of I/R using these models.

a. Cell sheets, spheroids and organoids. Cell sheets are usually engineered by seeding cells on a temperature-sensitive polymer such as poly (N-isopropylacrylamide) (PIPAAm), which changes its adhesive properties in response to temperature.[112–114] At normal culture conditions (37 °C), the surface is slightly hydrophobic and cell-adhesive, and at lower temperatures (<32 °C), the surface is highly hydrophilic and non-adhesive. CMs are seeded on the polymer at normal culture temperature when the substrate is adhesive, allowing for cell attachment. After the cells form a monolayer, the temperature is lowered to make the surface non-adhesive. As a result, the cells detach from the surface as a layer to form the cell sheet [Fig. 2(a)]. Alternatively, a soluble polymer can be used to form the cell sheets before the polymer is dissolved away. Cell sheets are widely used in cardiac tissue engineering applications,[115,116] specifically as cardiac patches;[112,117,118] however, due to the lack of 3D structure and limited cell types within the sheets, their use for I/R modeling is limited. In one such study, a human iCM-based cell sheet platform was developed to model I/R.[104] iCMs were first seeded on gelatin, and following cell-sheet formation, the gelatin was dissolved. Cell sheets were exposed to anoxia or normoxia for up to 24 h, to create I/R conditions. The researchers showed that the beating rate decreased under anoxia and reperfusion, but the cells adapted to the anoxic conditions in time and started to beat at a higher rate after 15 h in anoxia. This indicates that

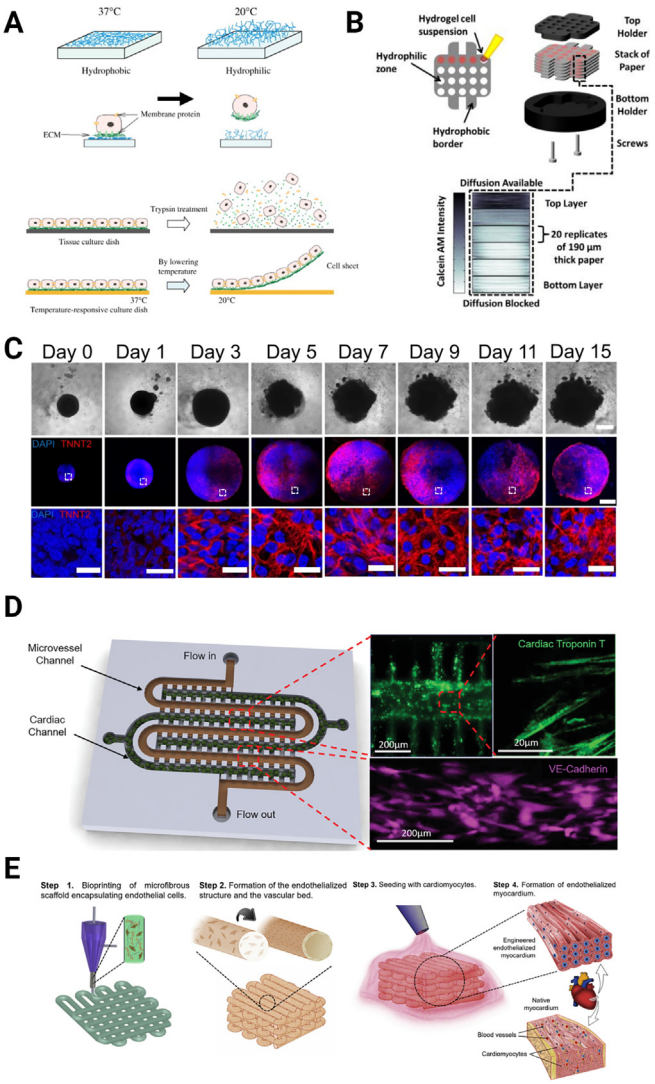

FIG. 2. Various methods used to create cardiac tissue models *in vitro*. (a) Cell sheet [Reproduced with permission from Masuda *et al.*, Adv. Drug Delivery Rev. **60**, 277–285 (2008). Copyright 2008 Elsevier], (b) paper-based model of cardiac ischemia [Reproduced with permission from Mosadegh *et al.*, Adv. Healthcare Mater. **3**, 1036–1043 (2014). Copyright 2014, Wiley], (c) organoids [Reproduced with permission from Lewis-Israeli *et al.*, Nat. Commun. **12**, 5142 (2021). Copyright 2021 Author(s), licensed under a Creative Commons Attribution (CC BY) License], (d) microfluidic devices [Reproduced with permission from Ellis *et al.*, Small **18**, 2201330 (2022). Copyright 2022 Wiley], and (e) bioprinting [Reproduced with permission from Zhang *et al.*, Biomaterials **110**, 45 (2016). Copyright 2016 Elsevier].

the CM physiology might have changed in CVD patients with coronary artery occlusion. Similarly, Yamasaki *et al.* engineered human cardiac cell sheets using human iCMs and evaluated contractile force under either constant hypoxia for long-period (8 days), or short-period hypoxia (4 days) followed by normoxia (8 days) to mimic I/R conditions.[119] They reported decreased contractile force, contraction velocity, Ca^{2+} transient kinetics, and ATP levels, yet unchanged sarcomere structure and cell number following hypoxia.[119]

While cell sheets support cell–cell interactions and improve the beating of the cells, they do not take into account the effect of the microenvironment, which limits their use alone as I/R models. Spheroids are 3D cell aggregates, usually created in a scaffold-free environment, that may consist of single or multiple cell types with strong cell–cell interactions.[120] Similarly, organoids are three-dimensional (3D) organ-like multicellular models that are derived from stem cells[121,122] [Fig. 2(c)]. The intrinsic ability of stem cells to self-assemble allows researchers to study natural development, structural organization, regeneration, and disease progression *in vitro* using spheroids and organoids.[123]

To date, successful organoid models of many organ systems including brain, kidney, liver, and heart have been reported.[124–128] However, the use of organoids in cardiac tissue engineering is relatively new,[123,129,130] and only a few of them have been dedicated to studying disease models,[131] such as congenital heart disease,[132] arrythmia,[133] short QT syndrome (SQTS),[133] familial cardiomyopathy,[134] and I/R in MI.[135] In the study of interest, Voges *et al.* combined collagen and iCMs to create human cardiac organoids (hCOs) and studied the regenerative potential of the hCOs following cryoinjury.[135] They reported for the first time that hCOs were recapitulating fetal cardiac tissue characteristics, and that increased CM proliferation led to regeneration and functional recovery of MI-mimicking local cryoinjury within 2 weeks. Similarly, in a recent study, Sharma *et al.* engineered mouse and human cardiac spheroids and exposed them to I/R mimicking conditions *in vitro*.[136] Under these conditions, they observed that a high percentage of the cardiomyocytes died whereas only some portion of the fibroblasts and endothelial cells died. Moreover, they evaluated the cardiac damage-related gene expressions for *in vivo*, *in vitro* human cardiac spheroids (hCSs), and *in vitro* mouse cardiac spheroids I/R models, and reported that the gene expression of hCSs was more similar to *in vivo*.[136]

Additionally, hCOs have also been used to model contractile pathophysiology, especially with patient-specific iPSCs that might carry genetic defects such as short QT syndrome (SQTS) and familial cardiomyopathy.[133,134] Researchers used CRISPR-Cas9 to correct the disease-related mutations to obtain isogenic control and compared disease vs healthy phenotype *in vitro*. Such patient-specific organoids hold great potential to provide new mechanistic insights to explain comorbidities in heart-related pathologies.

Despite the difficulty of controlling organoid size, the lack of support in the form of vasculature, and the difficulty of integrating various cells into the organoids, these studies showed that organoids can be a promising tool for developing preclinical treatment and disease profiling.[122,123]

b. Scaffold-based 3D models. In order to most closely recapitulate the native cardiac tissue in the 3D *in vitro* models, researchers took into consideration different fundamental properties of the heart, such as stiffness, age, multiple cell types, and CM alignment. In that regard, the most commonly used biomaterials to construct the microporous and hydrogel-based 3D MI models are collagen,[137,138] collagen–fibrinogen,[139] Matrigel,[140,141] gelatin methacryloyl (GelMA),[142,143] poly(ethylene glycol)–(arginine–glycine–aspartic acid) (PEG–RGD),[142,144] and chitosan.[145]

Acun *et al.* created young and aged tissue models by encapsulating young (day 35–55) and aged (days 100–120) iCMs in GelMA-PEG hydrogels (with three different stiffnesses representing fetal, adult, and aged heart tissue).[142] They evaluated the stress response of each model when exposed to MI mimicking conditions.[142] They reported a significant decrease in cell viability for aged cardiac tissue constructs compared with young tissue constructs after going through conditions representing ischemia and reperfusion.[142] Similarly, Chen and Vunjak-Novakovic also utilized iCMs to engineer 3D tissue models via encapsulation in collagen–fibrinogen hydrogels.[139] Similar to the previous study, they showed that the 3D model recapitulated the *in vivo* conditions; more cell death was observed through apoptosis in the reperfusion phase than in the ischemia phase, and the addition of cardioprotective therapeutics (cyclosporine A and N-acetyl-l-cysteine) or lowering the pH, reduced the detrimental effects of I/R. Interestingly, lowering the pH (pH 6.4) showed the most effective protection against the injury caused by the rapid normalization of intercellular pH that occurs during reperfusion.[139] In one of the early studies, Liu *et al.* engineered a 3D tissue model by embedding adipose derived stem cells (ADSCs) in chitosan hydrogel, and mimicked I/R by inducing ROS production through hydrogen peroxide treatment.[145] They showed that the chitosan components, (1→4)-2-acetamido-2-deoxy-β-d-glucan (N-AC-Glu) and (1→4)-2-amino-2-deoxy-b-d-glucan (d-Glu), reduced the effect of I/R and verified this *in vivo*, where ADSCs engraftment, survival, and endogenous stem cell homing in the ischemic heart were enhanced in the presence of chitosan.

In another study utilizing rhesus monkey-derived iCMs in collagen gels to study I/R, researchers found that the 3D model was more sensitive to ischemic conditions than the 2D model.[138] Transcriptomic and pathway analyses revealed that pathways related to cell–cell and cell–ECM interactions, energy metabolism, and paracrine signaling were similar in the engineered 3D model compared to the native rhesus monkey myocardium, showing that these models can recreate the *in vivo* conditions.

In their study, Katare *et al.* preferred to use primary CMs, and they engineered a 3D model by seeding neonatal rat CMs onto a ring-shaped collagen scaffold and cultured the construct under hypoxic conditions (1% O_2) for 6 h.[137] The engineered constructs showed conduction defects, dephosphorylation of connexin-43, and down-regulation of cell survival proteins similar to the infarcted adult heart. When they treated the 3D MI model with cell protective agents, cyclosporine A and acetylcholine, they showed reduction in the ischemia-induced effects. Recently, Funcke *et al.* engineered 3D MI models by mixing all isolated rat heart cells with fibrinogen and thrombin and placing them in agarose molds and in between two silicone posts and analyzed the effect of two different hypoxic conditions on the contraction force and release of cardiac troponin I.[146] Then, they created an engineered human heart model by using iCMs instead of rat CMs and exposed them to the same hypoxic treatment conditions and used them to assess the cardioprotective property of DOR agonist [d-Ala2, d-Leu5]-enkephalin (DADLE); for which they observed a positive

effect for engineered rat hearts, and not with engineered human heart models.

Hidalgo *et al.* engineered tissue models incorporating CM alignment in the model by constructing Matrigel-coated microgroove patterns to align immature and metabolically matured human iCMs, and tested their response to I/R conditions.[141] They showed that mature iCMs were more sensitive to I/R than immature cells, with a 5%–30% increase in cell death in response to I/R after the cells were metabolically matured. Apoptosis was reduced under ischemic/reperfusion conditions after the addition of cyclosporine A into the media.

To engineer more physiologically relevant and mature cardiac tissue models, some studies incorporated other relevant cells including CFs,[140,147] endothelial cells[144] or a combination of these cells[148] with CMs.[149] Mosadegh *et al.* engineered multiple layers of paper coated with Matrigel containing rat neonatal CMs, CFs, or 3T3 fibroblasts and stacked these paper layers such that the CMs were in the bottom layer where oxygen diffusion was blocked, mimicking the ischemic conditions that occur as a result of MI [Fig. 2(b)].[140] To reduce oxygen diffusion from the top, more paper layers with 3T3 cells were added to the 3D model. They showed that the more hypoxic the CMs became, the more fibroblasts migrated toward the bottom layers containing the CMs, mimicking the native tissue environment.[140] Recently, del Campo *et al.* engineered human myocardium by combining CMs and human foreskin fibroblasts (7:3 ratio) with collagen, and performed cryoinjury on this model to mimic the heart attack.[150] After identifying the effect of the cryoinjury on the tissue model, they used it as a platform to analyze the efficacy of an extracellular vesicle (EV)-based treatment.[150] In another study, Yue *et al.* created 3D co-cultured platforms of rat CMs and iECs by encapsulating them in PEG-RGD hydrogels and investigated the effect of CM-EC crosstalk on the viability of CMs when exposed to MI mimicking conditions.[144] They observed improved CM viability in the presence of iECs as well as significantly different gene expression under oxidative stress indicating that iECs protected CMs from oxidative stress.[144] In a previous study, they also investigated the ischemia induced deterioration of endothelium by encapsulating CRISPR/Cas9 edited iECs in GelMA hydrogels and maintaining the 3D constructs under hypoxic conditions.[143]

c. Microfluidic devices. A different approach to modeling cardiac systems is using microfluidic systems. Recent advances in microfluidic systems and lab-on-a-chip models have allowed for the development of biomimetic organ-on-a-chip models, which are powerful tools for addressing limitations in typical 2D cell cultures, such as 3D cell–cell interactions, fluid flow, and ECM/environmental interactions.[151–153] Organ-on-a-chip models enable high control over cell placement, perfusion parameters, and vascularization to mimic a wide variety of biological systems such as the heart, lungs, and kidneys, for purposes such as drug development, disease modeling, and even general physiology studies.[154]

For heart models in particular, the degree of control over the 3D environment, tissue perfusion, and cell placement allows for greatly enhanced modeling of cardiac injuries and CVDs[21,155] as well as drug testing,[156] mechanistic studies of regenerative therapies such as EVs,[157] and general cardio physiological studies,[158–161] given the complexity and importance of vascularization of cardiac tissue in many healthy and diseased states.[17,162]

Heart-on-a-chip models have become a preferred tool to model disease conditions, such as I/R during MI. Martewicz *et al.* engineered a microfluidic system using neonatal rat CMs to observe the effect of hypoxic conditions on the intercellular Ca^{+2} handling properties.[163] Ellis *et al.* developed and fully characterized a myocardium-on-chip model by encapsulating iCMs in GelMA hydrogel in the middle channel, representing the cardiac muscle, and seeding iECs on the side channels, representing the microvasculature, which can be used as a platform to study I/R *in vitro*.[24] In a recent study, Ellis *et al.* improved this myocardium-on-chip model and showed that it can mimic physiological miRNA expression during both the ischemic and reperfusion phases of MI *in vitro* by controlling cell placement, perfusion, and oxygen availability, showing similar results to time matched clinical plasma samples [Fig. 2(d)].[164] In a similar manner, Veldhuizen *et al.* engineered a heart-on-a-chip model using stem cell-derived CMs and CFs encapsulated in collagen hydrogels and used it as a platform to observe the effect of hypoxic culture conditions.[165] Recently in a similar study, Liu *et al.* developed a heart-on-a-chip model integrated with bioelectronic devices to measure the intra- or extracellular electrophysiological readouts under ischemic conditions using immortalized mouse atrial HL-1 cells.[166]

While microfluidic devices are a powerful tool for modeling tissues, there are also inherent limitations to this methodology that should be considered. First, organ-on-a-chip microfluidic devices still utilize cultured cells and thus inherit many of the limitations of cell culture.[155] These include control of cell maturity, stress due to culturing, variances due to cell passaging, and, in the case of stem cell-derived cells, sometimes poorly defined properties and mechanisms of physiological activity. Second, by modeling a single tissue, many microfluidic devices also fail to account for systemic factors in disease and drug response, as well as in normal tissue homeostasis.[155,167] This can result in non-physiological responses to certain treatments or disease states. Finally, specifically with regard to printed microfluidic devices, resolution can be a significant limiting factor, especially when attempting to model microvasculature effects.[167] While the resolution has drastically improved over the years, the highest resolution obtained under ideal conditions is approximately 20 μm, as opposed to the maximum 10 μm diameter of a typical capillary.[168] These limitations currently control the degree to which *in vivo* systems can be accurately modeled in microfluidic systems.

d. Three-dimensional (3D) bioprinting. Three-dimensional (3D) bioprinting is a layer-by-layer additive manufacturing technology allowing accurate spatial deposition of the biological materials and active cells in a pre-designed pattern and is thus considered a promising technique for fabricating biomimetic cardiac tissue that can be used to study I/R *in vitro*. In order to have functional bioprinted cardiac tissue constructs, it is crucial to mimic the organized structure of cardiomyocytes in the native heart.[169,170] Resolution is very important for the precise control of the location and organization of the printed tissue construct, which is critical for the functionality of the printed cardiac tissue model. Unfortunately, one of the limitations of the extrusion based bioprinting is the relatively low resolution (around couple hundred micrometers[171]) which is a function of various parameters such as the nozzle diameter, the distance between nozzle and the stage, printing speed, bioprinted material, and its flow rate.[172] Although the resolution for the extrusion based printing does not

allow for cell-scale fabrication, recent studies have shown that CM alignment can be achieved by utilizing conductive nanoparticles (NPs) with an electric field,[173] and modified nozzles.[174] Even though there are many studies that focus on engineering cardiac tissue constructs using 3D bioprinting [Fig. 2(e)] (reviewed in detail by Sharma *et al.*,[109] Liu *et al.*,[175] and Wang *et al.*[176]) up until today, none of them used the bioprinted constructs to study I/R.[109,158,177]

Using bioprinting is beneficial for creating complex tissue constructs with different cell types. However, for the long-term viability of the printed constructs, it is necessary to include a functional vasculature network. Although some studies have included the vasculature network,[178–180] it is difficult to print the vasculature system with the required dimensions due to the relatively low resolution of 3D bioprinting, especially for extrusion-based 3D printers. Another limitation is introducing flow through the vasculature. It is crucial to design the vasculature network to have one inlet and one outlet in order to easily combine it with a pump to constantly supply the media through the constructs. Moreover, how to combine the tubing to the construct is another point to take into consideration. Another difficulty that comes with a relatively larger scale of 3D printed models is the challenges with imaging. Due to the thickness of the 3D-printed constructs, it can be difficult to use the current imaging tools efficiently while analyzing the condition of the construct. Given these limitations, a 3D printed construct has never been used to study I/R before. Microfluidics devices were preferred over 3D printed constructs due to their benefits mentioned previously, even though they lack the scale of the 3D printed constructs.

2. Engineered 3D post-MI tissue models

Although many animal models have been established to study the changes in post-MI tissue when treated using different therapies, few studies have focused on *in vitro* models. A few studies included the scar tissue formed after MI while engineering the 3D models and used them either to evaluate the efficacy of therapeutics or observe the effect of the scar (fibrotic) tissue on the beating function of the heart. Numerous methods have been used to create such *in vitro* models, including organoids, bioprinting, and microfluidics (Fig. 3). This section is dedicated to reviewing the recent developments in materials and methods used for creating 3D infarct tissue models *in vitro*.

a. Spheroids and organoids. As mentioned earlier, cardiac spheroids and organoids have been shown to be promising tools to create *in vitro* models to study the human heart microenvironment and drug cytotoxicity.[181] Recently organoids have been employed to investigate fibrosis development and as a platform for the assessment of therapeutic treatments.[182,183] Richards *et al.* developed a 3D *in vitro* post-MI myocardial tissue model by mixing human iCMs and non-CMs (4:2:1 ratio of cardiac ventricular fibroblasts: umbilical vein endothelial cells (HUVECs): adipose-derived stem cells (hADSC), without the use of any ECM proteins, and placed this cell mixture in agarose molds to form hCOs. By leveraging nutrient transport principles (e.g., oxygen diffusion) through the organoid, they created a gradient throughout the spheroid which included an apoptotic center resembling the infarct region, a boundary region, and a healthy region.[182] Hallmarks of MI such as metabolic changes, impaired calcium handling, and increased

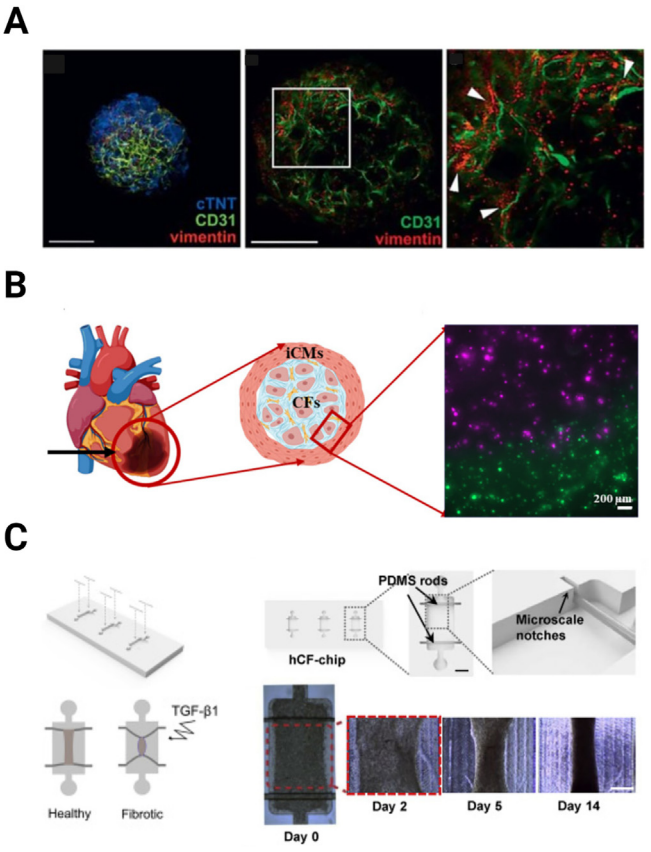

FIG. 3. Different methods used to create the fibrosis models. (a) Organoids. Laser scanning confocal microscopy collapsed Z-stack of vascularized cardiac spheroids stained with antibodies against markers of cardiac fibroblasts (CFs), endothelial cells (ECs), and cardiomyocytes (CMs) (vimentin, CD31, and cTNT, respectively) (left). A single laser scanning confocal microscopy image showing vimentin-positive CFs are positioned immediately next to CD31-positive ECs (middle and right). Reproduced with permission from Figtree *et al.*, Cells Tissues Organs **204**, 191 (2017). Copyright 2017 Karger Publishers. (b) 3D bioprinting. Schematic showing the infarct region model and corresponding cell types. Inset picture showing the successful infarct region printing using stained iCMs (green) and hCFs (magenta) [Reproduced from Basara *et al.*, Gels **7**, 70 (2021). Copyright 2021, MDPI], (c) microfluidic devices. A schematic showing the healthy and TGF-β treated fibrotic tissue-on-chip (left). Display of the hCF-chip containing three microwells (top-middle). Top view image showing a well with two elastomeric rods (arrows) suspended on each end (inset-top-middle). (Scale bar: 1 mm). Schematic of the microscale notches on each end of the well, help hold the elastomeric rod in place (top-right). Representative images for control tissues at days 0, 2, 5, and 14 post-seeding (bottom right). (Scale bar: 500 μm) [Reproduced with permission from Mastikhina *et al.*, Biomaterials **233**, 119741 (2020). Copyright 2020 Elsevier].

fibrosis was detected at the transcriptomic, structural, and functional levels. Moreover, they reported an upregulation in the fibrosis-related genes and an increase in elastic modulus similar to the fibrosis development *in vivo*.[182] In another study, Figtree *et al.* developed cardiac spheroids using hanging drop cultures of freshly isolated rat primary ventricular cardiac cells and used TGFβ1 treatment to create the fibrotic tissue [Fig. 3(a)].[183] As expected, TGFβ1 treatment increased cardiac fibrotic markers and ECM deposition in the spheroids, making

them adequate post-MI *in vitro* fibrosis models. Using this model, they evaluated the cytotoxic effect of doxorubicin (DOX) treatment and observed that DOX treatment increased the apoptosis marker expression, as well as the spheroid volume.[183]

b. Scaffold based 3D models and 3D bioprinting. Direct encapsulation of the cells in hydrogels along with micro molding techniques have been widely used in cardiac tissue engineering applications.[184] For post-MI cardiac fibrosis models, however, it is not a preferred method as it is more challenging to spatially pattern the different cell types and/or materials compared to 3D bioprinting or using microfluidic devices. Sadeghi *et al.* developed a 3D hydrogel-platform consisting of primary rat CMs and CFs and tuned the mechanical properties of the GelMA hydrogel to match the native cardiac tissue.[185] To create the fibrotic tissue microenvironment, they stimulated this construct with TGFβ1 to activate the CFs.[185]

As mentioned in Sec. III C 1 d, 3D bioprinting has become a powerful tool for cardiac tissue engineering applications, because it enables accurate spatial control of different materials and cell types, and thus is a promising method to create a 3D cardiac fibrosis model. Basara *et al.* and Koti *et al.* utilized multi-material printing to create the infarct region using CMs and fibroblasts in different printheads [Fig. 3(b)].[31,186] Both studies showed that 3D bioprinting can be used to engineer viable infarct region models, by characterizing the mechanical properties and printability of the used bioink, evaluating their cytocompatibility, and printing the scar tissue and healthy tissue together by utilizing two printheads.[31,186]

c. Microfluidic devices. Microfluidic devices have become a preferred tool to model post-MI infarct region *in vitro* and the subsequent cardiac fibrosis[187–189] because they not only allow for spatially patterning of different cell types in the channels, but also enable observation of changes in biochemical and biomechanical properties over time. Modeling cardiac fibrosis, in particular, has gained increased interest recently, due to the increasing awareness of the role that cardiac fibrosis plays in post-MI remodeling [Fig. 3(c)].[188] As such, developing complex biomimetic models has become imperative for both understanding and developing effective treatments for MI.[157] The application of modern bioprinting techniques has allowed for the development of some models with sophisticated cell positioning or vasculature that would be difficult to achieve otherwise,[190,191] making it possible to engineer heart-on-a-chip models with complex microvasculature.[192] While this remains an existing challenge, the use of novel techniques such as multi-nozzle printing makes it possible to create complex *ex vivo* models of post-MI or fibrotic tissue to study the progression of fibrosis and various intervention strategies.[193] These models can be used to identify contributing pathways and biomarkers of a pro-fibrotic state through various techniques, including clinical imaging techniques, effluent biomarker analysis, and effluent EV analysis, as well as investigating the mechanics behind these changes.[154,155] Furthermore, bioprinted microfluidic organ-on-a-chip models are gaining recognition as alternative pre-clinical drug testing platforms in terms of predicting drug toxicity.[194] Many drugs fail Phase 1 clinical trials due to the inability of 2D cell culture and existing models to accurately recapitulate a drug's full effect on an organ, and bioprinted organ-on-a-chip models provide an attractive and cost-effective alternative to increase the success rate of drug trials. The ability to generate such a wide breadth of data for both preclinical studies and physiological understanding of relatively understudied aspects of MI, such as cardiac fibrosis and microenvironment alterations, demonstrates the versatility and utility of these models.

IV. REGENERATIVE THERAPIES FOR TREATING POST-MI TISSUE

The development of improved pharmaceutical and therapeutic intervention methods for MI-related damage has become a major motivator for the development of many biomimetic cardiac models, in large part due to the prevalence of MI in worldwide deaths and medical costs. With the aforementioned advances in modeling cardiac behavior in both healthy and disease states, recent novel interventions have gained traction after success in these models. These novel therapies have been developed to treat damaged tissue following MI, including stem cell (SC) therapies,[195,196] extracellular vesicle (EV) or exosome treatments,[45,46] direct reprogramming of fibroblasts into CMs,[44] biomaterial-based therapies,[197,198] and cardiac patches.[118,199] In this section, recent developments for each of these therapeutics used for treating post-MI tissue will be reviewed (Table I).

A. Cell therapies

There has been extensive effort to develop cell therapies to treat MI patients. Two decades ago, the first generation of cardiac cell therapies started with adult cell types such as bone marrow-derived mesenchymal stem cells (MSCs), skeletal myoblasts, and cardiac progenitor cells (CPCs).[200] With the advancements in iPSCs in the 2000s, the second generation of cardiac cell therapies arose including iPSCs, ESCs, and iPSC-derived CPCs and CMs.[201] Most recently, because the cardiac cell makeup consists of more than one cell type and MI is molecularly complex, the third generation of cardiac cell therapies has emerged with combinational cell therapies using more than one cell type with complementary roles.[202] Overall encouraging results regarding efficacy, safety, and functional and structural improvements were reported upon cell therapy.[200] However, the mechanism of action of these cell therapies and whether the cells or the secreted paracrine factors facilitate the therapeutic effects is still subject to debate.[203] Here, we focus on recent advancements in stem cell therapies as they are the most favored post-MI cell therapies.

1. Mesenchymal stem cells (MSCs)

MSCs are multipotent adult stem cells that can differentiate into multiple lineages, including endothelial and CM-like cells and can be derived from a variety of tissues including bone marrow and adipose tissue, expanded extensively *in vitro*, and exhibit so low immunogenicity that even allogeneic MSCs would not cause any immune reaction. Most of the MSC studies date back to the early 2000s, yet MSC therapy is still one of the most popular post-MI treatment methods.[200,204,205] In preclinical animal studies, MSCs have been shown to integrate into the host tissue, support cardiac repair and function, and reduce infarct size, while also enhancing vascular density.[200,206,207] Especially regarding vascular density, the mode of action of MSCs appeared to be primarily paracrine.[208–210] MSCs are known to secrete a variety of regulatory and trophic factors such as growth factors (i.e., SDF-1α, HGF-1, ILGF-1, VEGF, FGF, and PGF) and cytokines (i.e., ANG-1, MMPs,

TABLE I. Comparison of different techniques used for treating MI considering the selected cell type, biomaterials, methods for evaluation, and limitations.

Treatment	Cell source	Biomaterials	Assessment Method *in vivo*	Limitations
Cell therapies	MSCs,[205,207,210,213,216,217,233] iPSCs,[221–224] iCMs[205,233–235]	–	LVEF, infarct size, electric activity, angiogenesis	Tumor formation, low retention, low cell survival, low cell maturity
Biomaterials based	MSCs,[270,294] endothelial progenitor cells (EPCs),[293,334] iPS-cardiac progenitor cells (iPS-CPCs),[295] iCMs[296]	dECM,[197,269–271,292] adamantane-modified HA (Ad-HA) and β-cyclodextrin-modified HA (CD-HA),[293,334] sodium alginate,[294] MeHA,[295] gold nanoparticle (AuNP)-hyaluronic acid (HA),[296] elastin-like recombinamers (ELRs)[297]	Walk distance test (clinical),[197] LVEF, fractional shortening, cardiac muscle area angiogenesis, infarct size	Variations in biological response depending on the post-MI delivery time point, immune response
EVs	MSCs,[294,295] cardiac stromal cells (CSCs),[253] iPSCs,[250] iCMs,[250] EPCs,[293,334] epicardial cells[150]	MeHA,[295] dECM,[253] collagen,[250] Ad-HA and CD-HA,[293,334] sodium alginate[294]	Cardiac function assessment, LVEF, fractional shortening, angiogenesis	Low retention, not yet mechanistically well-understood
Cardiac patches	Primary rat CMs,[298,309,311,312,316,317,324,331–333] iCMs,[36,41,60,177,299,300,306,320,321] iECs,[300,306,320,321] FBs,[41,60,300,306] human coronary artery endothelial cells,[305,317,333] H9C2 cardiomyoblasts,[307,319] rat bone marrow-derived stem cells (r-BMSCs),[310] ESCs,[313] CPCs,[318] smooth muscle cells,[320] CSCs,[253,322] human dermal fibroblasts[333]	Fibrinogen–thrombin–matrigel,[298] decellularized placenta,[299] fibrin–matrigel–thrombin,[300] collagen,[41,304] $Ti_3C_2T_x$ MXene-PEG,[36] decellularized porcine myocardium slide,[303] carbon nanotubes (CNTs) incorporated methacrylated collagen (MeCol)–alginate,[305] nanocellulose-PGS-PPy,[307] PU-PANI-SiO_2,[308] nCe-PCL-gelatin,[309] decellularized bovine pericardium,[310] oxidized alginate–gelatin–polyacrylic acid,[311] cellulose,[312] fibrin,[313,332] spider silk reinforced fibrin,[314] PCL-both ends capped with nitrates,[315] CNT-reinforced non-mulberry silk,[316] GelMA-colagen,[60] alginate–gelatin,[317] gelatin-HA,[318] PCL-PGS,[319] GelMA,[320] decellularized human omental tissue,[321] fibrinogen–thrombin–aprotinin,[322] PPy-silk fibroin,[324] methacrylated elastin-gelatin-CNTs,[331] decellularized porcine myocardium[253]	Infarct size, cardiac functionality LV wall thickness, LVEF, angiogenesis	Arrhythmias, endurance integration, immune response

IL-1, IL-6, and PLAT), especially when subjected to hypoxic conditions.[211–214] Considering that post-MI cell death results from a deficiency of oxygenation, MSCs are valuable cells to promote angiogenesis and vasculogenesis to oxygenate the infarct zone. To show the efficacy of the MSCs for treating post-MI tissue, Guo *et al.* recently formed a human umbilical cord mesenchymal stem cell (hUCMSC) sheet and applied it to *in vivo* post-MI mouse model.[215] They reported that applying the hUCMSC sheet reduced fibrosis in the border zone and increased the LV wall thickness compared to untreated and cell suspension-treated controls.[215]

Chen *et al.* conducted the first clinical trial with 69 post-MI patients, in which intracoronary injection of autologous bone marrow-derived MSCs significantly improved cardiac function and reduced scar size, showing their regenerative and restorative potential.[216] Recently, Florea *et al.* isolated human MSCs (hMSCs) from four male donors (median age: 24-year-old) and delivered either low or high numbers (20×10^6 or 100×10^6) of hMSCs to the border zones of the chronically infarcted myocardial territory of 15 patients (median age: 66-year-old).[217] They reported reduced scar size with both doses, while increased ejection fraction (EF) was reported only in the high cell dose group, highlighting the importance of using high cell doses and concentrations in cell therapies.[217] Although MSC therapy has been proven to improve cardiac repair, there remains challenges with its use due to low retention rate, slow migration to the infarct zone, and low survival rate.[218,219]

2. Induced pluripotent stem cells (iPSCs)

iPSCs are produced by reprogramming somatic cells, allowing the use of the patient's own cells and reducing the rejection risk.[220] Similar to MSCs, iPSCs can also be expanded extensively for large-scale cardiac repair purposes. Nelson *et al.* were the first to intramyocardially deliver iPSCs to immunodeficient mice,[221] reporting significant improvement in ventricular function and reduced post-MI adverse remodeling, such as fibrosis, and confirmed the cardiac differentiation of the injected iPSCs *in vivo*. However, the injected iPSCs formed tumors in the immunodeficient mice, ultimately leading to reduced cardiac function due to the stress created by the tumor. Many other studies also verified the tumorigenic potential of the transplanted iPSCs in post-MI murine hearts.[222,223] On the other hand, Templin *et al.* assessed the therapeutic potential of iPSCs in a large animal MI model for the first time and reported vascular differentiation and durability of iPSCs engraftment.[224] Even though the tumor formation is a risk with iPSC treatments, there are a few ongoing clinical trials investigating the safety and efficacy of iPSCs.[38]

3. Stem cell-derived cardiomyocytes (ESC-CMs, iPSC-CMs, or iCMs)

Early studies regarding stem cell-based MI treatment strategies heavily relied on the use of ESC-derived CMs. ESC-CMs significantly improved electromechanical properties with regular calcium transient and regenerated the infarcted hearts in both small and large animal studies including nonhuman primates.[225,226] However, ESCs and ESC-derived cells are ethically controversial and have the risk of rejection as well as tumorigenesis or teratoma formation once transplanted.[227–230] Therefore, iCMs emerged as an attractive therapeutic option to replace the lost CMs without having the risks of rejection or tumorigenesis and have been the focus of many studies in recent years.[39,231,232] Citro *et al.* were the first to compare the therapeutic efficacy of iCMs to the "gold standard" MSCs in 2014.[233] Their results revealed that iCMs were equipotent with MSCs in improving cardiac function, while iCM therapy also attenuated cardiac fibrosis in rat hearts. Similarly, in large animal studies, iCMs significantly improved post-MI contractile function and cardiac bioenergetic efficiency.[234] However, few iCMs were reported to survive in the long term (8 weeks post-transplantation).[201,234] Recently, Shiba *et al.* delivered allogeneic iCMs into the infarct and border zones of non-human primates (cynomolgus monkey) and reported improved contractility for at least 12 weeks, but also an increased risk of non-lethal tachycardia.[235]

Overall, preclinical iCM therapies have proven to be effective in post-MI hearts, but challenges such as low retention rate, cardiac immaturity, population heterogeneity, and possible arrhythmia and tumorigenicity risks remain unsolved.[39,220] Several strategies have been offered to mature iCMs, including biochemical manipulations,[236–238] mechanical,[239–241] and electrical stimuli.[242–244] Even though structural maturity is often achieved, the contractile properties of iCMs remain at suboptimal levels, which limits the translation of iCMs to the clinic.[231]

4. Combinational cell therapies

Novel combinational cell therapies aim to leverage the synergistic effects of more than one cell type in repairing both the myocardium and the vasculature of the post-MI hearts. Park *et al.* reported that human iCMs combined with human MSC-loaded porcine heart ECM patches amplified cardiac repair in the rat MI model.[40] MSC patches not only enhanced the retention and the engraftment of the iCMs but also constituted pleiotropic effects to promote iCM maturity. Moreover, researchers identified the key secreted factor(s) from hMSCs to be pro-angiogenetic (VEGFa, IGF-1, FGF2), anti-inflammatory (TGFβ1, IL-10), and anti-fibrotic (TIMP-2). Similarly, Natsumeda *et al.* reported that allogeneic MSCs and cardiac stem cells (CSCs) synergistically reduced scar size and improved cardiac function.[245] Göttingen swine (minipig) were subjected to MI and administered either MSCs (200m/injection), CSCs (1m/injection), or the combination of these two cell types (200m MSCs:1m CSCs). This allogeneic cell combination therapy (ACCT) was reported to be safe and regenerative with no arrhythmogenicity and immune response up to 3 months post-therapy. In terms of cardiac function improvement, ACCT increased the perfusion in the infarct region, prevented post-MI adverse remodeling and enhanced cardiac contractility compared with either cell type alone. Additionally, the combination of MSCs and their derived exosomes have been reported to improve heart performance.[246] In both *in vitro* and rat models, sequential delivery of exosomes, followed by MSCs (2×10^6 MSCs) three days later, improved the MSC retention and survival at the infarct region, reduced the scar size, promoted neovascularization, and improved cardiac function (i.e., LVEF). Overall, combinational cell therapies hold great potential as they benefit from the pleiotropic effects of the additional cells and/or their secretome.

5. Mechanisms of action

There are two proposed mechanisms of action for the cell therapies: First is that the transplanted cells will proliferate and repopulate the damaged myocardium. This proposed mechanism is, however, seriously challenged by studies, showing that transplanted cell survival and retention is extremely low and the surviving cells cannot be the source of cardiac recovery.[247] The second, and currently prevailing, mechanism of action is that secreted biomolecules from transplanted cells promote endogenous repair through stimulating pro-angiogenic and anti-inflammatory pathways and recruiting immune cells to the myocardium.[247,248] In a pilot study, immune modulation therapy (i.e., altering immune response) was shown to significantly reduce the risk of death and hospitalization following MI.[249] Moreover, a recent study

revealed the underlying reason to be the regional accumulation of CCR2+ and CX3CR1+ macrophages.[248] This once again highlights the effect of the paracrine factors secreted by the transplanted cells in modulating the post-MI recovery.

B. Extracellular vesicles (EVs)

While the exact mechanisms by which stem cells promote cardiac repair and immunomodulation *in vivo* are not well understood, recent studies suggest that paracrine signaling pathways stimulated by molecules such as cytokines, growth factors, and microRNAs (miRNAs) are major effectors of both pro- and anti-fibrotic signaling pathways post-MI.[208–210] Cytokines and miRNAs are attractive targets for intervention because these signaling molecules are often packaged in EVs in a highly controlled way, enabling efficient targeting of these biomolecules to designated cell types. Additionally, EVs can be isolated from blood plasma and other biofluids, from individual patients, or from cell effluent, using stem cells or *in vitro* models, with relative ease compared to the complex processes required for other methods.[250] Exosomes, an EV subgroup with diameters typically between 30 and 200 nm, are commonly released from most cell types and are major vehicles for cytokines, chemokines, miRNA, and other miscellaneous signaling molecules that can be internalized by and affect function in recipient cells (Fig. 4). These contents are known to influence many diverse and pathologically relevant biological processes, including angiogenesis, immune cell phenotype, immunomodulation, endothelial, and epithelial to mesenchymal transition, and cell differentiation, and as such, exosomes are both packaged and released from cells in a highly controlled manner, often as a response to detected stimuli.[251]

The usage of exosomes secreted from stem cells as a cell-free therapy has recently come under investigation, both as an independent treatment and in conjunction with injectable biomaterials. Stem cell exosomes have been shown to improve cardiac recovery during both ischemia and reperfusion phases in MI for CMs, endothelial cells, and CFs *in vitro*, and reducing infarct size and adverse inflammation and improving functional recovery post-MI in *in vivo* studies, with more *in vivo* and human clinical studies under way.[252] While this effect appears to be independent of the stem cell type, MSCs, particularly bone-marrow-derived MSCs (BMMSCs) have been most widely investigated.[197,252] Integration of BMMSC exosomes with existing cardiac

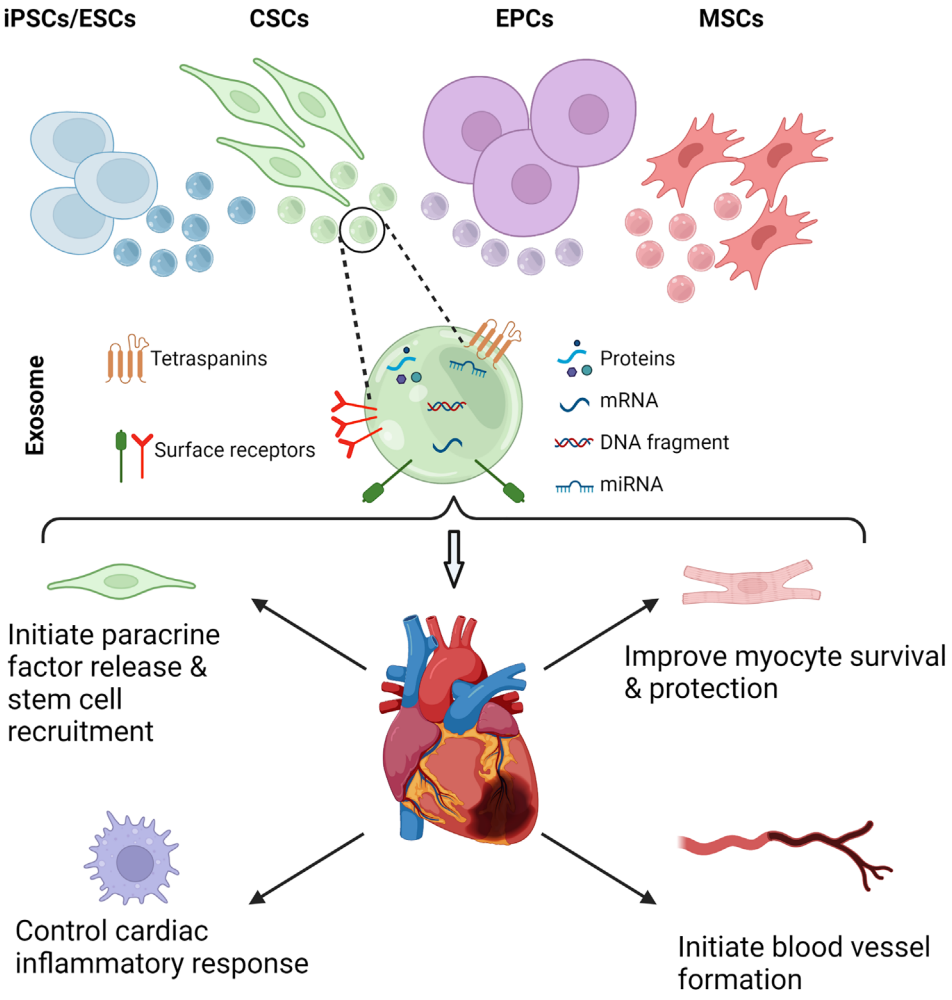

FIG. 4. Exosomes are excreted from numerous stem cells such as induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), cardiac stem cells (CSCs), endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs) (top). Exosomes consist of proteins, mRNA, miRNA, and DNA fragments (middle). When applied on the damaged heart tissue, exosomes initiate paracrine factor release leading to stem cell recruitment, modulate immune response, improve myocyte survival and protection, and initiate new blood vessel formation.

patch strategies has proven effective in a host of preclinical trials and is under investigation in a number of ongoing clinical investigations, as demonstrated by Fields *et al.*[47] Completed preclinical studies by Huang *et al.* suggest that this integrated, cell-free cardiac patch can provide similar or improved cardioprotective effects compared with existing treatments and that the use of this cell-free type of patch reduces the risk of autoimmune response to the patch, increases viable storage time, and is easily produced, combining the benefits of exosomal treatment with the utility of a cardiac patch.[253]

C. Direct reprogramming of cardiac fibroblasts into cardiomyocytes

With the discovery of the prospect of direct differentiation of CFs to CMs, a novel method for treating the infarct region has emerged.[44] This reprogramming is attainable through introducing three cardiogenic transcription factors, namely, Gata4, Mef2c, and Tbx5 (GMT).[254] Over the last decade, studies were dedicated to investigating the effect and role of using different miRNAs,[255,256] culture conditions,[257,258] epigenetic factors,[259,260] transcription factors[257,261,262] and mechanobiology in direct reprogramming.[263,264]

Although direct reprogramming has a great potential for treating the fibrotic tissue formed after MI, there are some limitations toward translation to clinical trials. First, the reprogramming efficiency was reported to be insufficient in humans, compared to small animal *in vivo* models, and it must be enhanced. Second, the safety and efficacy of this treatment should be verified with large animal *in vivo* models. Finally, the underlying molecular mechanism of the direct reprogramming has not been fully understood yet, and therefore, more studies should be dedicated to investigating it.[44]

D. Biomaterials-based therapies

Biomaterials-based tissue engineering approaches combine natural or synthetic polymers, bioactive molecules, and/or cells for post-MI recovery purposes. Previously, natural polymers such as collagen, alginate, hyaluronic acid (HA), and decellularized ECM showed high biocompatibility and improved therapeutic function.[197,265,266] Specifically, decellularized ECM and collagen scaffolds have been shown to enhance endogenous cardiac repair while also providing mechanical and electrical support for the damaged cardiac tissue.[267–271] Similarly, in their study Yokoyama *et al.* used fibronectin, which is an ECM protein, to create 3D iCM cardiac tissues through a special layer-by-layer fabrication method and reported improved cell viability under *in vitro* hypoxic conditions, as well-improved LVEF when implanted in a rat MI model.[272] Another protein used to induce CM regeneration is agrin. Agrin was shown to induce CM proliferation *in vitro*,[273] and promote regeneration of infarcted heart in mice[273] and pigs.[274] It was also shown that periostin-loaded gelatin foams induced cardiac regeneration with improved myocardial function in a rat model by reintroducing mononucleated CMs to the cell cycle without inducing fibrosis.[275] Alternatively, synthetic polymers such as poly(lactic-co-glycolic) acid (PLGA) and poly-(L-lactic) acid (PLLA) show controllable and strong mechanical and physical properties.[43] To benefit the robust biocompatibility of the natural polymers along with the strength and consistency of the synthetic materials, researchers have been using hybrid biomaterials with or without cells.[276,277] On the other hand, the use of non-conductive biomaterials may hinder electrical signal propagation in scar tissue, resulting in non-synchronous contraction of CMs.[278] Therefore, employing electroconductive materials that complement the conductive nature of heart tissue have been shown to be promising in improving the electrophysiological properties after MI.[278,279] Additionally, composites of non-conductive polymers with conductive nanoparticles such as carbon nanotubes (CNTs),[280,281] graphene,[282,283] gold nanoparticles,[284,285] or MXene[286] have been shown to improve the electroconductive behavior of the polymers they were blended in, and enhanced cell–cell communication and functional properties.[284]

The mode of delivery is usually injection to the infarct zone to attenuate the adverse cardiac remodeling and increase cell retention in the damaged heart.[287] Alternatively, engineered 3D constructs (i.e., cardiac patches) can be delivered through a catheter for minimally invasive percutaneous therapies, which is explained in more detail in Secs. IV D 1 and IV E.

1. Injectable hydrogels

Hydrogels are crosslinked 3D polymer networks that can absorb a large amount of water. The polymer can be locally injected and then crosslinked *in situ* to form a stable hydrogel via physical and chemical stimuli, without the need for any major surgery.[268,288]

ECM has been an attractive biomaterial for regeneration and functional recovery and is shown to be more efficacious when derived from younger tissues.[265,289,290] Preclinical studies showed the feasibility of ECM use to mediate post-MI ventricular remodeling, improve function, and increase the number of endogenous CMs.[42,291,292] In 2019, Traverse *et al.* conducted a first-in-man study and transendocardially injected decellularized porcine cardiac ECM-derived hydrogel (VentriGel) into 15 post-MI patients (57–62 years old). Through LV volumes, EF, and scar size evaluation by cardiac magnetic resonance (CMR) imaging, serum B-type natriuretic peptide (BNP) level, the 6-min walk test distance, and quality of life using New York Heart Association (NYHA) functional classification assessment at 3- and 6-month post-treatment, researchers verified the safety, feasibility, and effectiveness of injecting ECM hydrogels in the clinic.[197] A recent approach for post-MI recovery is local and sustained delivery of ECM subsets such as EVs and nucleic acids via injected hydrogels. Methacrylated-HA (MeHA) hydrogel-based miRNA delivery showed improved outcomes in a mouse model, ensuring sustained CM proliferation for two weeks and functional improvements for four weeks with a single injection.[293] Similarly, in both small and large animal models, natural hydrogels improved the therapeutic effect of EVs due to precise localization and increased retention.[293–295]

Additionally, researchers have engineered hydrogels for enhanced mechanical and/or electrical performance. Li *et al.* combined gold nanoparticles (AuNP) with RGD functionalized MeHA hydrogel (AuNP-HA) and encapsulated iCMs for post-MI cardiac recovery.[296] They reported enhanced angiogenic effects exerted by encapsulated iCMs. Additionally, due to the conductive nature of AuNP-HA, it increased gap junction expression and resynchronized electrical conduction in the mouse heart. In a sheep model, Contessotto *et al.* developed a degradable elastin-like recombinamer or peptide (ELR or ELP) hydrogel with mechanical and bioactive customization. Intramyocardial injection of ELR hydrogels resulted in a less fibrotic and more angiogenic ischemic core, as well as CM functional improvement.[297]

The efficacy of injectable biomaterials has spurred interest in the clinical applications of individual bio-components while improving the storage capability and production efficiency of these biomaterials. In particular, the combination of stem cells and collagen-based hydrogels has reliably improved cardiac function post-MI in porcine models without any adverse inflammatory effect, showing promising potential for translation to clinical trials.[295] Additionally, this methodology is further refined to develop a cell-free patch that recapitulates this success, in order to provide demonstrated clinical benefits while minimizing the risk of an immune response or other negative effects associated with cell-dependent therapies.[253]

E. Cardiac patches

Cardiac patches are porous matrices that aim to physically support cardiac function, and repair damaged cardiac tissue.[298] Although the main purpose of placing a cardiac patch on the scar tissue is to promote functional CM migration toward the damaged tissue and repair it, the patch can also be used as a delivery vehicle to protect the therapeutic cargo molecules and improve their survival and stability, as explained in Secs. IV B and IV D.[299] In both regards, therapeutic cardiac patches have shown great potential in improving cardiac function.[41,298,300]

Cellular cardiac patches have been engineered by combining different cell types such as stem cells[299,301] and iCMs[41,299] with natural and synthetic biomaterials as well as conductive hydrogels or conductive nanoparticle-hydrogel composites.[302] Recently, EVs, growth factors, and miRNAs have become the preferred factors encapsulated in biomaterials, replacing cells.[45,46] The common approach to determining the effectiveness of the therapeutic patches is evaluating angiogenesis, infarct region size reduction, LV wall thickness, and cardiac contractility properties in animal models.[298,299,303] As mentioned previously, rat models have been the platform of choice for such evaluations, yet there are some studies that are done with other rodents and macaques, while some have recently transitioned to clinical trials.[199] Various techniques have been used to fabricate the cardiac patches such as direct encapsulation,[41,304] 3D bioprinting,[305–307] and electrospinning.[308,309]

Tens of papers have been published on therapeutic cardiac patches in 2021 alone (reviewed in Ref. 43). Here, we will focus on the most recent studies (published in 2021 and 2022) that have developed cardiac patches using various methods and evaluated their potential as a cardiac patch by assessing their mechanical and physical properties, stability, and cytotoxicity using *in vitro* models and/or their therapeutic potential using *in vivo* models.

1. Direct seeding or encapsulation of the cells

Numerous studies have developed cardiac patches by directly seeding the cells on biomaterials. Ozturk *et al.* seeded rat bone marrow-derived stem cells on the decellularized bovine pericardium, evaluated the effect of the electromechanically stimulated patch on the infarct region, and showed the patch was successfully integrated with the infarct region and that cell migration took place.[310] Similarly, Jiang *et al.* seeded iCMs on decellularized rat placenta and showed that compared to an acellular patch and iCMs alone, the cellularized patch was more effective in reducing the infarct region and improving neovascularization in a post-MI rat model.[299] Pretorius *et al.* engineered thick (~2.12 mm), viable, and functional cardiac patches using a layer-by-layer assembly of the iEC, iCM, and CF-seeded fibrin layers.[300] Through the 4 weeks of culture, they observed that the iCMs were synchronized with a beating frequency comparable to the human heart (55 beats per minute) and cardiac-specific marker expression increased. On the other hand, they also reported an increase in apoptosis/necrosis markers throughout the culture period, but the change was not significant.[300] Song *et al.* developed conductive cardiac patches by incorporating polyacrylic acid into alginate/gelatin hydrogel and seeding rat CMs on top.[311] They reported improved CM sarcomere alignment, improved cardiac function restoration, and new vessel formation in an *in vivo* rat MI model.[311] Distinctively, He *et al.* engineered cardiac patches by seeding rat CMs on cellulose obtained from sea squirts, which are responsible for biofouling in marine aquaculture, with the goal of converting "trash" to "treasure." They showed that the patches obtained were functional *in vitro* and improved cardiac function of MI rats *in vivo*.[312]

Menasche *et al.* conducted the first in human study using fibrin scaffold-based cardiac patches with embedded hESC-derived cardiac progenitor cells and showed restored heart function after implantation of the patches on to the fibrotic heart tissue.[313] Bobylev *et al.* suggested improving the poor mechanical properties of fibrin by reinforcing it with spider silk and reported that the obtained material is mechanically stable enough to be used as a cardiac patch.[314]

As briefly discussed in Sec. IV B, acellular cardiac patches have been used as a tool to deliver therapeutic factors to the infarct region. Liu *et al.* encapsulated an extracellular protein, reelin, in collagen patches and showed that it improves heart function, protects CMs from apoptosis, and reduces the infarct area in adult mice after MI.[304] In an interesting study, Zhu *et al.* designed a nitrate-functionalized patch by covalently binding nitrate groups to the biodegradable poly-(ε-caprolactone) (PCL) polymer so that small molecule drugs could be loaded onto the patch. As it is applied to the myocardium, nitric oxide (NO) is released from the patch, which provided effective cardioprotection, improved heart function, and decreased adverse remodeling.[315]

Most recently, Tiburcy *et al.* started clinical trials with 53 patients for the cardiac patch that they have developed over the last 20 years, in which they focused on creating ESC- and iPSC-engineered human myocardium using a repeatable manufacturing protocol.[41] They were able to create mature patches by co-culturing ESC-CMs or iCMs and hCFs (70% to 30% ratio), develop a special media to enhance iCM viability, establish a serum-free protocol, and scale up cardiac patch production.

2. 3D bioprinting

3D bioprinting is one of the most preferred tools to create cardiac patches (reviewed in Refs. 301 and 176). Here, we will focus on recent developments in bioprinted cardiac patches, which utilized different biomaterials and methods.

Hybrid biomaterials have been created by mixing various hydrogels and sometimes conductive materials to fabricate patches that are strong, conductive, and printable, to improve cellular response.[302] 3D bioprinted CNT-reinforced alginate and methacrylated collagen were combined with human coronary artery endothelial cells (HCAEC) to improve electrical conductivity, compressive modulus, and cell proliferation and migration, showing potential for future *in vivo* studies.[305]

Similarly, Mehrotra *et al.* created a hybrid conductive bioink by mixing non-mulberry-silk, GelMA, PEGDA, and CNTs and bioprinted anisotropic vascularized tissue constructs by mixing this bioink with HUVECs prior to printing. They kept this construct in culture in a perfusion-based reactor for 14 days to ensure vasculature formation, and then seeded it with neonatal rat CMs and incorporated the construct with calcium peroxide (CPO) and IL-10-based GelMA microspheres, hypothesizing that the release of oxygen from CPO microspheres would support CM and HUVEC viability after implantation at the MI region.[316] Ten days after implantation to a rat MI model, they observed neovascularization near the patch.[316] Basara *et al.* created conductive hybrid cardiac patches by printing $Ti_3C_2T_x$ MXene on PEG constructs using an aerosol jet printer and seeding iCMs on them, and reported that viable, patternable, functional tissue constructs can be developed using this method with improved contraction kinetics and cardiac related protein and gene expression.[36] In a similar manner, hybrid bioinks were created and characterized by different groups using GelMA and collagen with ventricle-type human CMs and CFs,[60] alginate and gelatin with endothelial cells,[317] HA and gelatin with human cardiac-derived progenitor cells (hCMPCs).[318] On the other hand, instead of utilizing hydrogels, Yang *et al.* created a different type of cardiac scaffold composing of poly-(glycerol sebacate) (PGS) (a thermoset polymer) and PCL (a thermoplastic polymer).[319] By utilizing fused deposition modeling 3D printing technology, they printed this construct, and seeded it with H9C2 rat CMs. In their *in vivo* rat MI experiments, they showed that application of this cardiac patch increased LV wall thickness, reduced infarct region size, improved vascularization, and promoted tissue repair.[319]

A number of studies have focused on the 3D bioprinting of scaffold-free cardiac patches by printing cardiac spheroids on needle arrays according to the desired 3D pattern.[177,306] Both studies reported enhanced vascularization, smaller scar tissue area and improved cardiac function when these patches were tested in rat MI models, indicating the potential of cardiac spheroids formed patches as a therapeutic treatment for MI.[177,306]

Gao *et al.* utilized a different method called multiphoton-excited 3D printing (MPE-3DP), to create a micropatterned scaffold with biomimetic cardiac ECM structure using a photoactive gelatin polymer solution and seeded this construct with iCMs, iECs and iPSCs-derived smooth muscle cells. The therapeutic effect of this cardiac patch was then evaluated in an *in vivo* MI murine model, which revealed elevated cardiac function, increase in wall thickness, better vascularization, and fewer apoptotic cells.[320]

In their study, Noor *et al.* created vascularized cardiac patches by mixing iCMs with decellularized omental tissue to create the cardiac tissue construct, and by mixing iECs with sacrificial bioink (gelatin).[321] They confirmed the functionality of the printed cardiac patches by evaluating the calcium transient properties and performing immunostaining. One main advantage of this method is the ability to create patient-specific cardiac patches with a thickness of a couple millimeters. Distinctively, Sue *et al.* utilized hydrodynamic focusing to create the cardiac patches.[322] They printed free-standing biomimetic microvessels using hydrodynamic focusing of HUVECs with gelatin, and Tisseel® fibrin gel with human cardiosphere-derived stromal cells (hCSCs) cast on top.[322] *In vivo* experiments with rat and pig MI models revealed that the patch promoted CM proliferation, new blood vessel formation, and cardiac function recovery.[322]

3. Electrospinning

In addition to 3D bioprinting, electrospinning has been widely utilized to engineer cardiac patches.[323] To create conductive cardiac patches, Feng *et al.* electrospun polyurethane/polyaniline/silicon oxide polymers and showed improved electrical signal transduction.[308] They performed an adhesion experiment using the patch and a porcine heart and reported adequate self-adhesion that can endure multiple diastolic and systolic movements of the heart.[308] Similarly, Liang *et al.* blended silk fibroin with conductive polypyrrole prior to electrospinning to create conductive biocompatible cardiac patches and reported improved Ca^{+2} kinetics, sarcomere structure, and cardiac-specific protein expression of the seeded neonatal rat CMs, showing a potential for future *in vivo* studies.[324] In another study, Jain *et al.* created a cell-free patch by electrospinning PCL and PCL blended with gelatin (PCLG) and coating it with cerium oxide nanoparticles (nCe). They showed that nCe/PCLG nanofibers reduced ROS production and prevented agonist-induced cardiac hypertrophy in neonatal rat CMs.[309]

4. Injectable cardiac patches

As discussed in Sec. IV E 3, various techniques have been used to create biomimetic 3D patches with fixed geometry and size.[325–327] Although these patches can range from 100 μm to a couple millimeters thickness with aligned and spontaneously beating CMs, traditional cardiac patch placement on the heart usually needs an open-chest surgery and suturing to the epicardial surface of the heart, and hence is traumatizing for the patient.[328,329] Recent studies aim for minimally invasive procedures that improve the translational capacity of the cardiac patches. Soft and flexible cardiac patches can be injected rather than implanted. Recently developed cardiac patches have shape memory due to their microfabricated lattice design that allow them to be folded into a needle as small as 1 mm to be delivered by injection. Montgomery *et al.* reported minimally invasive delivery of 1 cm x 1 cm cell-laden cardiac patch made of ultraviolet (UV)-crosslinkable and elastomeric polymer poly[octamethylene maleate (anhydride) citrate] (POMAC) on the porcine heart without open-chest surgery.[330] The effective elasticity of the POMAC patches allowed them to maintain size and shape as well as to integrate into the host heart with high cellular viability of the delivered CMs in the patch. Although patch delivery was not inferior to the surgical placed patches, the small size of the patch would necessitate delivery of multiple patches in the case of a large injury. Wang *et al.* used methacrylated elastin, gelatin, and carbon nanotubes to develop injectable and conductive cardiac patches.[331] In both rat and minipig MI models, both acellular and CM-loaded patches have maintained their shape and led to functional repair for up to 4 weeks. Focusing more on commercialization, Huang *et al.* developed cell-free, off-the-shelf patches by cryopreservation.[253] They combined the benefits of the native ultrastructural properties of the decellularized porcine myocardium and bioactivity of human cardiac stromal cell secretome for post-MI cardiac repair in both rodent and porcine models. As a novel approach, Tang *et al.* developed a regenerative fibrin patch by injecting *in situ* polymerizable biomaterials directly on the damaged tissue using a double-lumen syringe with the help of pressurized carbon dioxide, referred to as a spray painting device by the authors.[332] The platelet fibrin patch which is created by injecting platelet-rich plasma and media solution with calcium attenuated adverse remodeling, reduced the scar size, and preserved post-MI

cardiac function in a mouse MI model via the release of regenerative growth factors.

Cardiac patches are one of the most preferred tools to treat the damaged myocardium after MI, with some studies going through clinical trials.[41] Recent developments allow researchers to create complex cardiac patches including cell types that are primary for heart tissue and biomimetic materials, as well as vasculature network and biochemical factors. On the other hand, smooth integration of the cardiac patch with the heart tissue in terms of beating synchronously, withstanding the cyclic motion, and not creating arrhythmias still remains as limitations, and developing cardiac patches that meet these imperative requirements remains a challenge.[333]

V. CURRENT LIMITATIONS AND PROSPECTS

In this review, we looked at MI from a tissue engineering and regenerative medicine point of view and put together recent developments on different methods to engineer I/R models and post-MI tissue models as well as the regenerative therapies available to treat the long-term outcomes of MI. We highlighted the materials and methods used for each section and talked about their advantages and limitations. This review aims to provide the big picture of what has been studied and discovered regarding MI from modeling to treating, with a goal of guiding the present researchers through the materials and methods that can be used.

Despite the advancements in the tissue-engineering field, to date, some challenges remain to be resolved to create biomimetic *in vitro* cardiac tissue models. These challenges result from the human heart being a very complex organ.[109] In order to properly create the heart tissue *in vitro*, the model should have the aligned myofibril structure of the heart with longitudinal shape of ventricular myocytes, and should allow for the cells to communicate for authentic contraction.[335] Although some studies have been able to develop models addressing these considerations, creating functional models on a larger scale still remains a challenge due to difficulty in incorporating vasculature together with fabricated cardiac tissue.[336] Larger constructs engineered using mature cardiac cells and quick fabrication methods integrated with the vasculature and blood mimetic flow would be the next step of *in vitro* cardiac tissue models to study I/R.

Aside from modeling the complex heart tissue, it is near impossible to completely mimic the cascade of mechanisms that lead to scar tissue formation following MI. On the other hand, engineering fibrotic tissue models *in vitro* is less challenging than creating animal models, and it is important to create platforms for testing potential therapies in place of or before *in vivo* studies. Creating the fibrotic tissue models shares similar limitations as mentioned previously for engineering the cardiac tissue models. Additionally, taking into account the effect of the immune system as well as patient-specific variances, such as age, gender, and genetic makeup, remain a challenge.[337,338]

To date, not many studies have utilized *in vitro* models as a platform to evaluate the efficacy of the therapeutic options. As researchers develop more biomimetic, reliable, and clinically relevant models, it will become preferable to use these tools to test new therapies. Developing novel therapies for injured heart tissue has been the focus of numerous studies. However, due to the limited regenerative capacity of the human heart, it is challenging to find methods to fully repair and regenerate the infarct region post-MI. Cell therapies alone are limited due to their low retention and very short cell survival period after injection. For the biomaterials-based methods and cardiac patches, their biocompatibility as well as integration with host myocardium must be further improved. Additionally, the implementation of most cardiac patches is invasive and would require open heart surgery, which might pose a risk for patients.[43] In terms of therapeutics, future studies will most likely shift their focus from cell therapies to cell-secreted factor therapies (EVs, cytokines), which eliminate the risk of an inflammatory reaction of the host and are less invasive compared to placing cardiac patches. These factors may also be combined with injectable biomaterials to enhance the local retention and the effect of the factors, while keeping the procedure minimally invasive.

VI. CONCLUSIONS

Cardiac tissue engineering has developed profoundly in recent years, and with the currently available tools, it is possible to engineer more representative cardiac models to reliably study I/R as well as to create the fibrotic tissue after MI. However, researchers are still far from fully recapitulating the response of the human body and the human heart microenvironment *in vitro*. Engineering *in vitro* models is quite crucial for developing and testing new therapeutic options before animal studies. In that regard, understanding the benefits and drawbacks of the materials and methods used by seeing the broad view is essential to develop better models and therapies. An ideal model should recapitulate the native cardiac tissue as comprehensively as possible both in healthy and diseased states, and it should incorporate all the cell types in the heart at the correct ratios, densities, and organization, as well as the appropriate biomaterials with the necessary biochemical, biophysical, and biomechanical cues, so that functional tissues with functional vasculature are formed. With the developments in the tissue engineering field, researchers are getting closer to creating more biomimetic healthy cardiac tissue and infarct tissue models. From the regenerative therapy point of view, researchers are utilizing different tools to create the best treatment to recover the heart from the effects of MI. For the therapy to be successful, it should initiate regeneration, recover cardiac functionality, and prevent arrhythmias without triggering an immune response. Although some therapies are still due for primary results, there are potential therapies that have been translated into clinical trials reporting hopeful results.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Gozde Basara: Conceptualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **Gokhan Bahcecioglu:** Conceptualization (equal); Supervision (lead); Writing – original draft (equal); Writing – review & editing (equal). **S. Gulberk Ozcebe:** Writing – original draft (equal); Writing – review & editing (equal). **Bradley W Ellis:** Writing – original draft (equal); Writing – review &

editing (equal). **George Ronan:** Writing – original draft (supporting); Writing – review & editing (equal). **Pinar Zorlutuna:** Conceptualization (equal); Funding acquisition (lead); Project administration (lead); Resources (lead); Supervision (lead); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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