

# Cardiac Tissue Engineering

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## INTRODUCTION

In the developed world, cardiovascular disease is the leading cause of death. Due to the minimal intrinsic ability of adult heart to regenerate itself following injury [1], myocardial infarction (MI) results in the rapid death of hundreds of millions of cardiomyocytes (CMs), and a vigorous inflammatory response. Over subsequent weeks to months, fibroblasts (FBs) and endothelial cells (ECs) form granulation tissue and a dense collagenous scar, which reduces the contractile function of the heart and leads to a pathological remodeling and, in many cases, heart failure. Adult CMs are terminally differentiated, thus it is not possible to expand them to sufficient numbers starting from small cardiac biopsies. Current clinical trials focus on cell replacement through the application of bone marrow mesenchymal stem cells, peripheral blood mononuclear cells, or resident cardiac cells. Most of these cell types have no intrinsic ability to give rise to a large number of CMs; instead they improve function through paracrine effects. Additionally, cells can be applied alone or in combination with different types and forms of biomaterials (e.g., hydrogels, scaffolds). An appropriate combination of a biomaterial, cell type, delivery method and tissue culture parameters can develop unique tissue constructs for multiple types of heart disease. Here we provide design criteria for generation of functional cardiac patches and discuss different biomaterials and cell types used during the tissue-engineering process.

## CLINICAL PROBLEM

Cardiovascular disease is the leading cause of death worldwide. In the United States cardiovascular diseases account for approximately 30% of all deaths, with the cardiovascular disease population constantly increasing [1,2]. In 2008 alone, 86.2 million Americans were living with some form of cardiac disease, and this number is projected to grow to 40.5% of the American population by the year 2030 [3]. Concomitantly, the financial burden of disease is also projected to rise, from present estimates of \$300 billion to \$800 billion [3]. On a global scale, cardiovascular disease is responsible for 60% of deaths, and will become increasingly important as global obesity continue to rise. Importantly, cardiovascular disease is responsible for 80% of non-communicable diseases in low and middle income countries [4], with grave social and economic consequences.

One of the key limitations for treating cardiovascular disease is the lack of regeneration after myocardial injury. The majority of existing therapies aim to mitigate the progression of heart failure, intervening in the cyclic progression of the neurohormonal cascade, but the options for

improvement or regeneration of diseased tissue are limited. The most common pathogenesis is ischemic heart disease, which occurs when a portion of the heart does not receive a supply of oxygen from the blood. Coronary artery disease, or a narrowing of the lumen in the coronary arteries, most often by atherosclerotic change, limits perfusion of certain sections of the heart. Limiting oxygen delivery below a certain threshold leads to angina, characterized by reversible discomfort or heaviness to the chest. Complete occlusion of an artery, or MI, is associated with a typical pathological progression. Depletion of adenosine triphosphate (ATP) occurs within seconds, leading to irreversible cell damage by 20–40 minutes [5]. Coagulative necrosis begins ~30 minutes after coronary occlusion, followed by a robust inflammatory response that begins with the release of reactive oxygen species and neutrophil invasion ~24 hours post-infarction, and continues for the next 2–3 days, in parallel to the continued necrosis. Macrophages ultimately dominate the infarcted zone by 5–7 days post-infarction, and are responsible for removing dead cells and creating granulation tissue. Weeks to months after infarction, collagen deposition dominates, and a fibrous scar is formed [5].

Heart failure, which is the inability of the heart to adequately pump blood, ensues most commonly after ischemic injury, but may also have other etiologies, including valvular disease, hypertension, or genetic cardiomyopathies [6]. An increase in cardiac work requirements leads to one of two patterns of dysfunction in the heart; one where pressure overload dominates, leading to concentric hypertrophy of cardiomyocytes and a thickening of the myocardial wall, or a second one where volume overload dominates, leading to eccentric hypertrophy of cardiomyocytes and a thinning of the myocardial wall [5]. In either case, increased fibrosis, abnormal gene expression, and insufficient vascular function lead to pump dysfunction and an activation of the neurohormonal system, in which the sympathetic nervous tone, renin secretion, and arginine vasopressin secretion all increase. Together, these cues lead to myocardial remodeling at the cellular level that contributes to the progression of heart failure, by mechanisms that are not well understood.

Presently, heart transplant and Ventricular Assist Devices (VAD) can improve cardiac function, but the numbers of available donor hearts are limited, and the VAD is only a temporary solution. Therefore, biological treatment strategies that can enhance cardiac function are especially attractive for countering the pathophysiological progression of heart failure. With the advent of induced pluripotent stem cells (iPS), there is newfound promise for cardiac regeneration using patient-specific cells, since cardiomyocytes were previously unattainable by any other means. Present approaches involve direct cell injection or the creation of a cardiac patch. Cell injections are attractive due to relative simplicity, though poor cell retention is a recurring obstacle [7,8]. A cardiac patch approach would be aimed at replacing or repairing the specific lesion created by a MI, using a lab-grown piece of contractile cardiac tissue. Continuing challenges are the vascularization and electromechanical integration of such a construct, and these are among the key areas of active research.

## ENGINEERING CARDIAC TISSUE: DESIGN PRINCIPLES AND KEY COMPONENTS

The heart functions as a highly organized physiological pump. The cardiomyocytes, comprising 80–90% of the heart volume, are not only elongated and hypertrophied, but are also aligned and electrically coupled to surrounding cardiomyocytes. They are constantly active, stimulated to beat, and therefore have a high metabolic demand for oxygen. Supporting cell types – endothelial cells and smooth muscle cells – organize themselves into a dense vascular network supplying nutrients to the cardiomyocytes. Fibroblasts support the cardiomyocytes and generate a collagen-dense matrix. On an organ level, pacemaker cells spontaneously generate action potentials that propagate the volume of the heart, generating a synchronous contraction. The flow of blood through the heart necessitates mechanical stress on it, as a preload that stretches the myocardium and an afterload to push against.

Fabrication of a functional cardiac patch depends on a multitude of parameters that collectively recapitulate some aspects of the complexity and function of the heart. Since cardiomyocytes are terminally differentiated, current studies are focusing on deriving a renewable source of them from embryonic and induced pluripotent stem cells. Other studies have aimed at recapitulating one or more physiologic aspects of cardiac tissue (e.g., the incorporation of multiple cell types, aligning cardiomyocytes, or electrical stimulation of cardiac tissue). Table 38.1 contains a collection of studies that aim to mimic aspects of the native myocardium. The three classical tenets of the tissue-engineering paradigm have been used for cardiac constructs: cell source, scaffold materials, and biophysical stimulation (Fig. 38.1).

## Cell source

The limited ability of human cardiomyocytes to divide and expand has restricted the scope and therapeutic potential of cardiac tissue engineering. The first evidence that the application of cells may be a viable therapeutic approach for MI came from animal studies which used injection of fetal or neonatal CMs, and found that CM injection improved left ventricular function and thickness, thus attenuating pathological remodeling upon MI [1a–4a]. Injected CMs integrated through gap junctions and intercalated disks with the host CMs [5a]. However, these findings have limited clinical relevance, and human fetal and neonatal CMs cannot be readily obtained for transplantation due to obvious ethical issues.

The search for a clinically relevant cell source has led to the transplantation of skeletal myoblasts [6a], embryonic stem cell derived cardiomyocytes (ESC-CMs) [7a–9a], bone marrow derived mesenchymal stem cells (MSCs) [10a–11a] and hematopoietic stem (HS) cells [12a–14a] into animal models of MI (reviewed in references [15a] and [16a]). Among these cell sources, skeletal myoblasts and MSCs were pursued into clinical trials. A meta-analysis of recent clinical trials with injection of bone marrow and peripheral blood mononuclear cells demonstrated a significant, albeit low (3%), increase in left ventricular ejection fraction (LVEF) as well as a significant reduction in infarct size (–5.6%) and end systolic volume (–7.4mL) in patients treated by intracoronary cell injection after acute MI [17a]. A dose-response effect of the injected cell volume on LVEF change was also reported [17a]. During the last year, resident cardiac stem cells, either c-kit+ (SCIPIO) or those derived from cardiospheres (CADUCEUS) demonstrated promising functional improvements in Phase I clinical studies and restoration of viable tissue per MRI imaging, presumably due to the new CMs in addition to vascular cells [18a,19a].

Although these studies are encouraging, modest long-term improvements in function upon cell injection have motivated the investigation of new cell sources and methods that increase survival and retention of injected cells. Pluripotent stem cells such as hESCs or iPSCs can both give rise to bona fide CMs and be expanded to sufficient numbers (millions/patient) using existing technologies. The discovery of human iPSCs [20a] and the ability to generate CMs from them [21a] could provide effectively unlimited numbers of autologous CMs for cell therapy without the ethical concerns raised by the use of hESCs has enormous implications. Studies from a number of groups have shown that it is possible to generate CMs from mouse [58] and human ESCs [53] and iPSCs [21a]. The most efficient protocols to date are those that replicate the signaling pathways regulating lineage commitment in the early embryo [53]. These protocols are now being adapted to defined culture conditions, with the use of small molecules such as glycogen synthase kinase 3 inhibitors and chemical inhibitors of wingless-int (WNT) signaling [22a].

While in many ways iPSC-derived cardiomyocytes are ideal, the exact approach for their utilization is not entirely clear. Though the differentiation process has become more specific than previous work relying on stochastic cell differentiation, further refinements are possible and necessary. In particular, there are multiple phenotypes of cardiomyocytes, each with a different set of functions [10]. Certain cardiomyocytes are pacemakers or chamber-specific myocytes, and fine control over the generation of these phenotypes remains elusive. Laflamme

**TABLE 38.1** Design parameters for cardiac tissue engineering

Native cardiac attribute	Engineering method	Results	References
Cell Source	Sequence of Activin and BMP-4 cytokines induces cardiac cell differentiation in human embryonic and iPS cells.	Flow cytometry at multiple timepoints revealed high sensitivity of multiple ES and iPS cell lines to concentrations of induction factors. However, optimization of the times and concentrations lead to improved differentiation yields.	Kattman et al., Cell Stem Cell 2011 [51]
	Lentiviral iPSC technology using epigenetically specific cell sources, ventricular cells vs. fibroblasts.	Ventricular cells as material for iPSCs improved cardiac differentiation potential when compared to using fibroblast cells, suggesting epigenetic memory for iPSC cell lines.	Xu et al. Cell Research 2012 [52]
Patient cell source	Induced pluripotent stem cells were derived from fibroblasts of patients with Long QT syndrome type I using infection with retrovirus encoded with Oct3/4, Sox2, KLF4, and c-MYC. They were subsequently differentiated into cardiomyocytes.	Generation of induced pluripotent stem cells was confirmed with Nanog staining, and generated cardiomyocytes showed striations when stained with cardiac troponin T. LQT1 disease phenotype of lengthened ADP 50 and ADP 90 was confirmed by single cell patch clamp. Myocytes generated from LQT1 patients showed KCNQ1 channel localization to the endoplasmic reticulum, suggesting a possible mechanism for the disease.	Moretti et al. NEJM 2010 [11]
	Induced pluripotent stem cells derived from patients with familial dilated cardiomyopathy were generated using Oct4, Sox2, Klf4, and c-MYC. They were subsequently differentiated into cardiomyocytes using the protocol developed by Yang et al. [53]	Immunofluorescence images of Oct4, Nanog, TRA-1–81 and SSEA-4, as well as hypomethylation as seen by quantitative bisulfite sequencing confirmed the generation of iPS cells. Multielectrode array analysis of dilated cardiomyopathy derived cardiomyocytes displayed similar beat frequency, interspike intervals, and field potential durations as the control group. Immunohistochemistry and transmission electron microscopy (TEM) displayed similar cell size, but dilated cardiomyopathy (DCM) derived cells had a higher relative percent of disorganized cells based on $\alpha$ -actinin staining. Overexpression of Serca2a resulted in restoration of contraction force as measured by atomic force microscopy (AFM). Treatment of cells with $\beta$ -blockers decreased the percentage of disorganized cells.	Sun et al. Science Translational Medicine 2012 [14]
Multiple Cell Types	Porous PGS scaffolds were pre-seeded with cardiac fibroblasts encapsulated in Matrigel for five days and were subsequently seeded with cardiomyocytes for another five days. This was compared to constructs of cardiac fibroblasts co-seeded with cardiomyocytes. Endothelial cells were co-cultured in cell sheets with cardiomyocytes at different ratios.	Immunofluorescent analysis of constructs pre-seeded with fibroblasts showed a greater percentage of actin positive cells compared with vimentin-positive cells, and compared with actin positive cells in the co-seeded group. The pre-seeded group further showed lower excitation threshold, higher fractional area change, and increased fluorometrically measured DNA and protein content. Higher seeding ratios of endothelial cells resulted in higher density of vascular networks as appreciated by fluorescent images. Enzyme-linked immunosorbent assay (ELISA) for	Radisic et al. J Biomed Mater Res Pt A 2007 [21]  Sekine et al. Circulation 2008 [22]

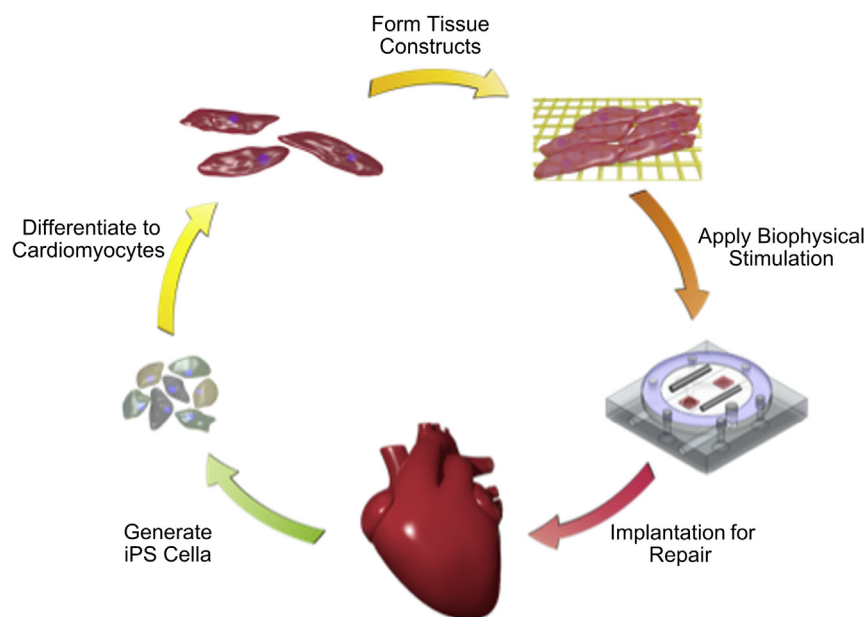
	External endothelial cell addition to cardiac tissue constructs.	secreted factors showed higher levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF) in endothelial co-culture groups compared to cardiomyocytes alone. Fluorescent views of implanted co-cultured cell sheets showed neovascularization into the myocardium. Increased cardiomyocyte DNA content demonstrated a 35% increase in cardiomyocyte proliferation due to addition of endothelial cells, validating co-culture effects of endothelial cells. Additionally vascular engraftment was shown when implanted with patent blood vessels.	Tulloch et al. Circ Res 2011 [23]
High Cell Density	Cell sheet method, where confluent layers of cells are detached from a temperature-dependent poly(N-isopropylacrylamide) substrate and stacked on top of other cell sheets using a cell sheet manipulator. Cell sheets were compared to cell injection in an infarct model.	There was a greater <i>in vivo</i> bioluminescence of implanted GFP-expressing cell sheets compared to injected GFP-cells. Macroscopic fluorescent views of cell sheets versus injected cells showed dense localization 4 weeks post transplantation. Immunohistochemistry showed a greater density of cells. TUNEL staining showed a significantly lower level of TUNEL positive nuclei compared to cell injection.	Sekine et al. Tissue Eng Pt A. 2011 [8] Haraguchi et al. Nat Protoc. 2012 [42]
Ultrastructure	Microcontact printing of fibronectin into rectangles of various aspect ratios and seeded with cardiomyocyte pairs.	Connexin-43 immunosignal and conductance as measured with dual-voltage clamp was greater in the 5.2 length:width ratio as compared to 3.5.	McCain et al. Am J Physiol-Heart C 2012 [54]
Alignment/ Anisotropy	Soft lithography of poly(glycerol) sebacate into accordion-like honeycombs of overlapping diamonds.  PDMS microcontact printing of fibronectin into shapes of various aspect ratios.	Differential long and short axis elastic moduli and excitation threshold that mimic the anisotropy of the heart. Immunofluorescent actin images demonstrating elongation in the preferred direction Image analysis of immunofluorescence actin images demonstrated greater anisotropy in shapes with > 2:1 aspect ratio as compared to the 1:1 group	Engelmayr et al. Nature Mater 2008 [31]  Bray et al Cell Motil Cytoskel 2008 [34]
Extracellular matrix	Neonatal hearts were decellularized with antegrade coronary perfusion of SDS and were recellularized with neonatal cardiomyocytes, fibrocytes, endothelial cells, and smooth muscle cells.  Decellularized sheets of human myocardium were used as a scaffold for human mesenchymal progenitor cells suspended in fibrin and implanted into infarcted rat hearts. Poly(glycerol sebacate) scaffolds were prepared at different stiffnesses by altering the curing time and were subsequently seeded with neonatal rat cardiomyocytes.	Decellularization and recellularization were confirmed by histological analysis and staining. The perfused recellularized heart showed synchronous contraction as measured by electrocardiogram (ECG) and left ventricular pressure (LVP) after electrically stimulated depolarization. Ejection fraction totaled 25% of an equivalently aged healthy fetal heart. Histological staining of extracellular matrix (ECM) proteins and tensile testing showed that decellularized tissues were similar to native tissue. Echocardiographs performed on constructs transplanted on rat hearts preserved left ventricular systolic area and fractional area change. Low stiffness groups were found to have the greatest functional change (contraction amplitude) and also the greatest compressive stiffness.	Ott et al. Nat Med 2008 [25]  Godier-Furnemont et al. Proc Natl Acad Sci 2011 [26]  Marsano et al. Biotech Prog 2010 [55]

Continued

**TABLE 38.1** Design parameters for cardiac tissue engineering—*Continued*

Native cardiac attribute	Engineering method	Results	References
Cardiomyocyte Hypertrophy	Molded rings of neonatal rat cardiomyocytes, Matrigel, and Collagen I, and cultured in different hypertrophic stimuli (angiotensin II and phenylephrine versus hypertrophic inducing serum) on a cyclic stretch device for 12 days.	Immunofluorescent analysis of single cells in the angiotensin II and phenylephrine group showed no change in length but displayed increased width and total volume suggesting concentric hypertrophy. Similar analysis on the 'hypertrophic inducing serum' group displayed significant elongation without a widened morphology suggesting eccentric hypertrophy. Further evidence for hypertrophy in both groups is supported by high levels of gene expression of ANP and low levels of gene expression of alpha/beta myosin heavy chain ( $\alpha/\beta$ -MHC).	Tiburcy et al. Circ Res 2011 [29]
Electrical stimulation	Carbon electrodes field stimulation.	Ultrastructurally improved contractile apparatus and gap junctions. Histology and immunostaining showed increased linear organization. Contracting force increased and improved electrical maturity.	Radisic et al. Proc Natl Acad Sci 2004 [48]
	Gold nanowire impregnated alginate scaffolds.	Electrical conductance through gold impregnated scaffolds was increased. Connexin 43 expression was doubled compared to non-impregnated controls.	Dvir et al Nat Nanotech 2011 [56]
	hES cardiomyocytes transplanted in ablated large animal heart model.	ECG mapping co-localized injected cardiomyocytes to ectopic ventricular pacing, demonstrating pacing potential of injected hES pacemakers.	Kehat et al. Nat Biotech 2004 [57]
Vascular Perfusion	Perfusion bioreactor through channeled scaffolds.	Perfused channeled scaffolds showed nearly 50% increase in viable cells compared to non-perfused controls. Finite element modeling provides a rational approach for vascular perfusion design through engineered cardiac tissue.	Radisic et al. Nat Protoc 2008 [39]
	Omental pre-vascularization of cardiac patch.	Prevascularization improved engraftment on the infarcted heart, and mitigated decline in cardiac function based on echocardiography.	Dvir et al. Proc Natl Acad Sci 2009 [41]
Force generation	Engineered heart tissues, ring-shaped cardiomyocyte aggregates in a mixture of Matrigel and collagen, were placed onto load-adjusted coils to apply a passive, auxotonic, load on the tissues.	Engineered heart tissues on auxotonic load displayed a greater twitch tension by isometric force contraction analysis. Four weeks after implantation onto an infarcted rat heart, echocardiographs, MRI, and catheterization together demonstrated decreased left ventricular volumes, lower left ventricular end diastolic pressures, and shorter relaxation times when compared to sham-operated rats.	Zimmerman et al. Nat Med 2006 [30]
	Cardiomyocytes in porous chitosan-collagen scaffolds were stretched by moving four pins on the scaffold. Nominal strain of approximately 20% were applied at a frequency of 1 Hz for 6 days.	Regions of high local stress were determined using a mathematical model. Immunohistochemical analysis showed high levels of connexin-43 staining at the regions of high stress. Histological analysis and scanning electron microscopy (SEM) also demonstrated elongated morphologies in areas of high stress in comparison to areas of lower stress	Zhang et al. J Tissue Eng Regen M 2011 [33]





**FIGURE 38.1**  
Cardiac tissue engineering approach.

and colleagues recently demonstrated conclusively that hESC-CMs can electrically couple and suppress arrhythmias in hearts upon MI induced by cryoinjury [63]. As cell death is a major problem for cell injection studies – with up to 90% of injected cells dying or being washed away from the injection site – the authors used a pro-survival cocktail consisting of Matrigel and various growth factors to enhance cell persistence upon injection [38].

Another issue is the cell maturity [18–20]. Being recently differentiated, iPSC-derived cardiomyocytes are relatively young, and further phenotypic maturation may be needed to grow them into adult-like myocytes with their inherent contractile properties. Furthermore, even after differentiation and maturation, it is unclear whether a pure population of cardiomyocytes would be most apt for building cardiac tissue, since co-culture effects improve function, mimicking the heterogeneity of cells found natively [21–24]. The cell-related parameters of interest for building a cardiac patch include cell identity that accounts for specific cardiomyocyte type, relative ratios of different cells, cell density, and cell maturity.

## Scaffold

Scaffolds provide a three-dimensional environment in which the cells are cultured. The constituents and organization of the scaffold dictate the organization, maturation, and function of the forming tissue constructs. The scaffold material itself ranges from the native heart matrix itself [25–27] to natural hydrogels such as collagen and Matrigel [7,28–30] to synthetic polymers, such as poly(glycerol) sebacate (PGS) or polyacrylamide [31–33]. The scaffolds differ in the way they are processed (e.g., decellularization for native heart matrix versus temperature related gelation for Matrigel), their mechanical properties, their ultrastructure, and their biodegradability. With the wide library of materials to choose from, scaffolds can be made into virtually any size or shape, depending on the application. Micro-patterning has been employed as a way to control cardiomyocyte alignment and cell-cell interactions on a single cell level [34–36], while macro-sized constructs offer amenability to force generation and animal implantation studies [24,37,38].

Due to the large metabolic demand of cardiomyocytes, scaffold design must take oxygen and nutrient delivery into account. The cardiac tissue engineering scaffolds are in general porous and perfusable to enable oxygen supply *in vitro* [19,39,40] and designed to promote angiogenesis to enable oxygen supply *in vivo* [37,41]. Some methods of cardiac tissue engineering do not utilize scaffolding material; for example, the cell sheet method [8,42–44] relies on stacking confluent sheets of cardiomyocytes and the extracellular matrix these cells alone produce.

### Biophysical stimulation

Further phenotypic maturation of cardiac constructs can be achieved using biophysical stimulation. Perfusion of engineered constructs helps alleviate the diffusional limitations [37,39,40], which is of particular importance for the highly metabolically active cardiac tissue. The flow of medium across or through the engineered construct mimics the vasculature found in native tissue in providing fresh media and nutrients while removing toxic metabolic products from the cells. These approaches are critically important for generating large, thick, clinically sized cardiac constructs with homogenous distributions of cells.

Other stimulation modalities rely on the excitation-contraction coupling property that is inherent to heart tissue [45]. Stimulation systems can excite cells using either electrical depolarization or mechanical strain. Mechanical stimulation systems use active or passive tension enhancing cellular organization, morphology and contractile force generation. Auxotonic systems maintain passive tension on engineered cardiac constructs, providing a tonic resisting force for the cells to pull against [30,46]. Phasic systems provide active, cyclic strain to improve cardiac function, though some studies have shown improved twitch forces created using the auxotonic method [23,30,33]. Electrical field stimulation uses electrodes to provide a depolarizing stimulus [20,40,47–50]. The electrical field which excites the cells of the construct creates a voltage difference across the two electrodes. This cyclic depolarization improves electrical synchronization of cardiac constructs, while also improving contractile function and cellular organization. Both of these modalities of biophysical stimulation lead to functional enhancements, such as improved cardiomyocyte ultrastructure, improved sarcomeric linearization and organization, and increased functional gap junctions.

## DIRECTED CARDIAC DIFFERENTIATION OF HUMAN STEM CELLS

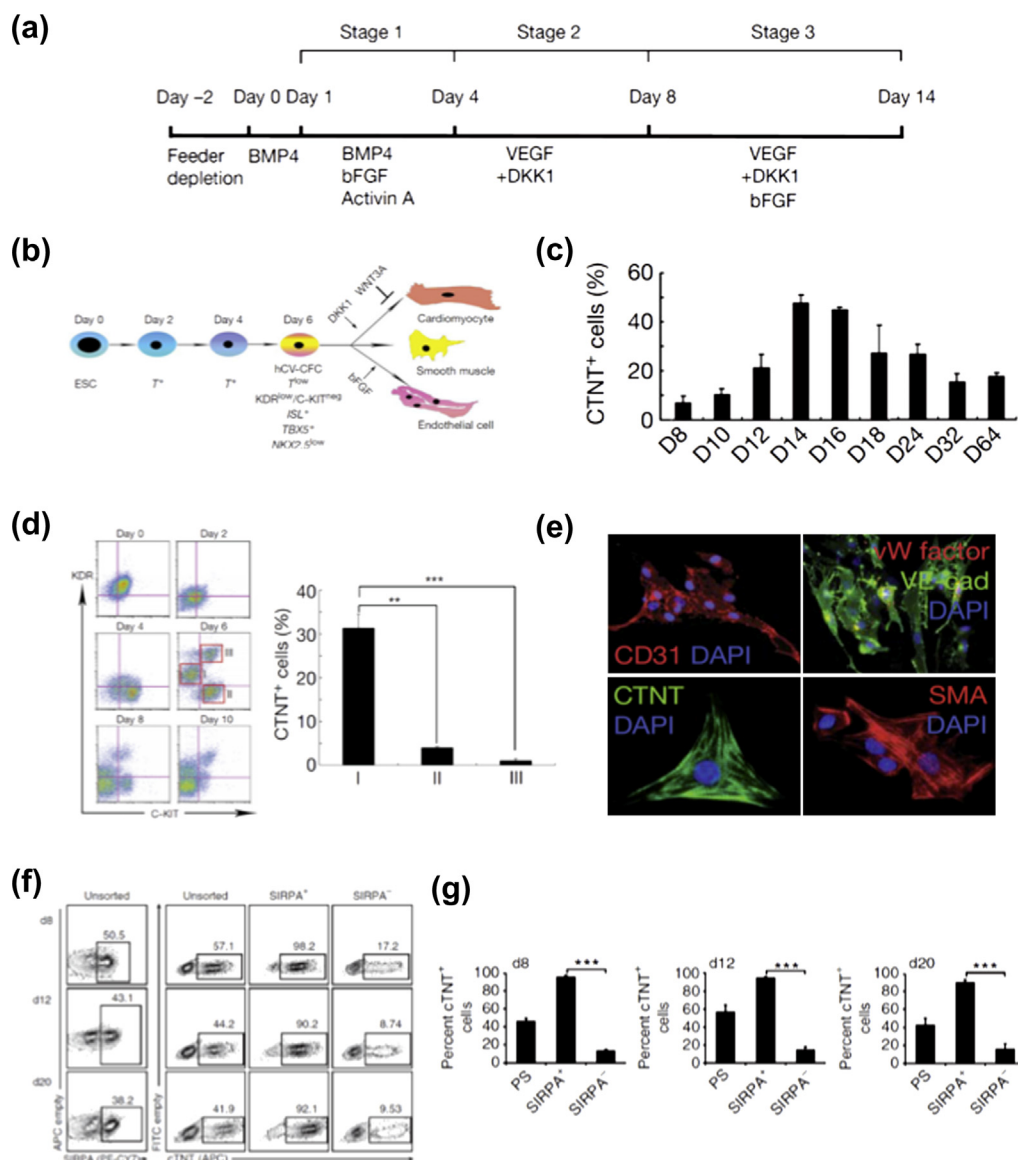
Cardiac tissue engineering requires a reliable source of cardiomyocytes. Human adult cardiomyocytes are unsuitable as they do not have the ability to proliferate. Recent advances in hESC and iPSC technologies have allowed for the generation of human cardiomyocytes from healthy progenitors as well as from diseased individuals. We describe here the differentiation of cardiomyocytes from an embryonic stem-cell-derived progenitor. We further discuss the purification and characterization of cardiomyocytes, as well as current efforts to generate patient-specific cardiomyocytes.

### Derivation of cardiomyocytes from embryonic stem cells

One of the first accounts of generating human cardiomyocytes from an embryonic stem cell source comes from Yang et al. in 2008 [53]. This study focuses on the discovery of a common cardiovascular progenitor capable of generating the different cell types dominant in the adult heart, that is, cardiomyocytes, endothelial cells, and smooth muscle cells. This method uses a combination of several factors to recapitulate cardiac development (Fig. 38.2a,b), resulting in a large percentage of troponin positive cells (Fig. 38.2c). The embryonic stem cell cultures were first induced with activin A and BMP4, generating a  $T+$  population, or one which overexpressed brachyury, a transcription factor important for defining the primitive streak and mesoderm. The cultures were next induced with Dickkopf-related protein 1 (DKK1), a canonical WNT inhibitor, and vascular endothelial growth factor (VEGF). DKK1 was necessary for generating cardiomyocytes, as demonstrated by a subsequent increase in ISL1, a marker for the secondary heart field, NKX2.5, a marker for cardiac differentiation, and increases in cardiac-specific proteins MLC2A and cardiac troponin T.

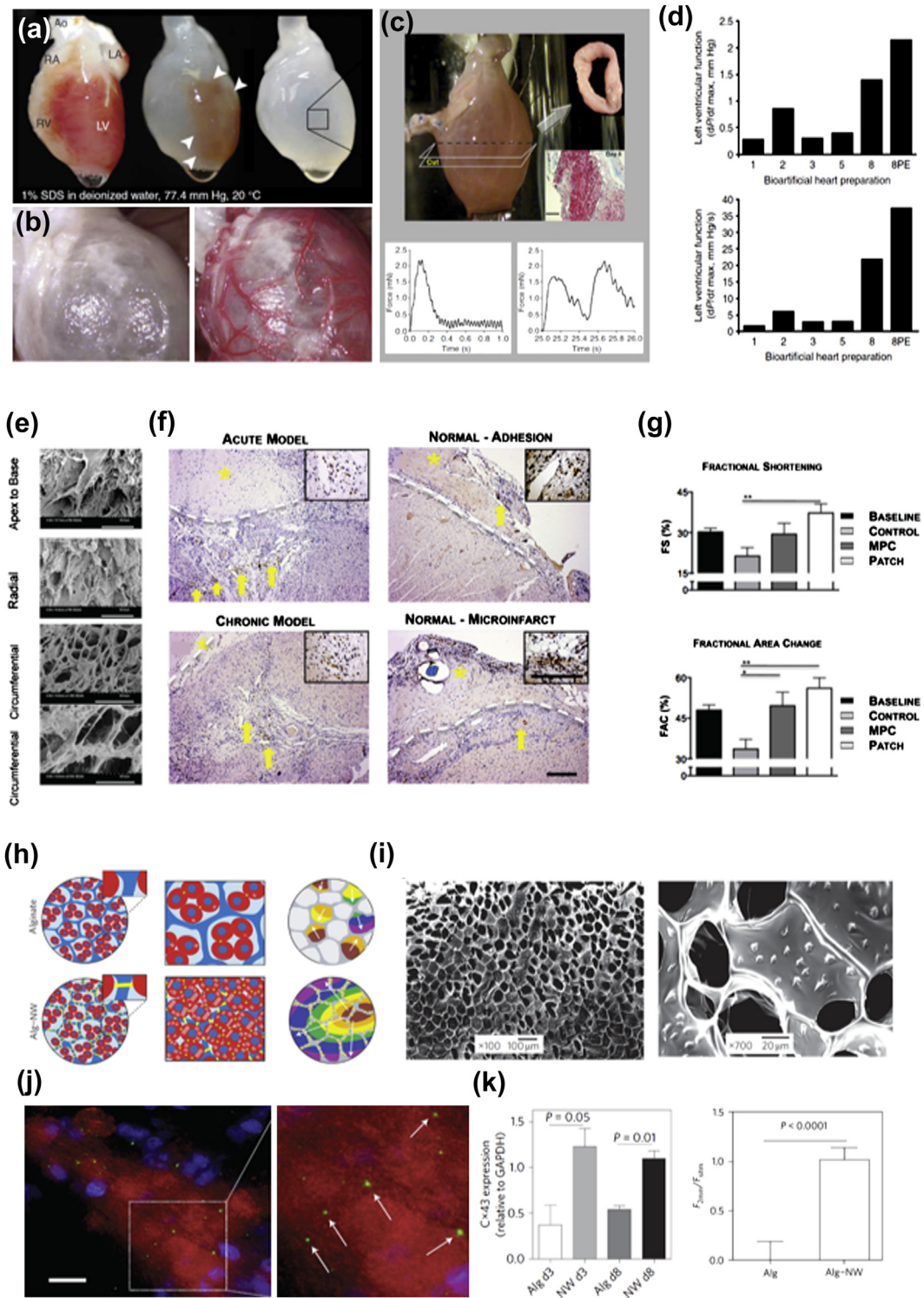
Interestingly, flow cytometric data of embryoid bodies at various stages of cultivation demonstrated three distinct populations at day 6 of induction (Fig. 38.2d). Stage I corresponds to a  $KDR^{low}/C-KIT^{neg}$  population, stage II corresponds to a  $KDR^{neg}/C-KIT^{pos}$  population, and stage III corresponds to a  $KDR^{high}/C-KIT^{pos}$  population. Kinase insert domain receptor (KDR)



**FIGURE 38.2**

**Generation of cardiomyocytes from embryonic stem cells.** (a) Cardiomyocyte derivation protocol and (b) corresponding schematic of development of cardiovascular lineages. (c) Percentage of troponin positive cells over time using the protocol in (a). (d) Flow cytometry reveals three populations on day 6 of differentiation KDR<sup>low</sup>/C-KIT<sup>neg</sup> (I), KDR<sup>neg</sup>/C-KIT<sup>pos</sup> (II), and KDR<sup>high</sup>/C-KIT<sup>pos</sup>, where differentiation of the KDR<sup>low</sup>/C-KIT<sup>neg</sup> population yielded the highest percentage of troponin positive cells. (e) Immunostaining for endothelial cell markers CD31 and vWF, cardiomyocyte marker cTNT, and smooth muscle marker smooth muscle actin (SMA) on cells differentiated from the KDR<sup>low</sup>/C-KIT<sup>neg</sup> lineage. (f) Flow cytometric analysis of human embryonic stem cell (hESC) derived cardiomyocytes sorted for signal regulatory protein alpha (SIRPA) on various days (8, 12, 20) of differentiation. (g) SIRPA positivity selects for a cardiomyocyte progenitor, resulting in up to 98% troponin positive cells.

and C-KIT were chosen for further investigation based on their significance in mouse stem cell studies. In particular, in mice KDR was shown to give rise to cardiac progenitors, and C-KIT was shown to derive hematopoietic and vascular lineages [58]. Low KDR expression and C-KIT positivity defined a cardiac progenitor, developing into cells with a greater number of cardiac troponin positive cells (>50%) compared with the other subpopulations (Fig. 38.2d). As lineages of cardiovascular cells are induced, significant populations of cardiomyocytes, endothelial cells, and smooth muscle cells form in culture (Fig. 38.2e). Transplantation of these progenitors into murine hearts has led to differentiation into the same three cell types — cardiomyocytes, endothelial cells, and smooth muscle cells, and resulted in an increase in

**FIGURE 38.3**

**Scaffolds for cardiac tissue engineering.** (a) Decellularization of whole neonatal heart with 1% SDS over the course of 12 hours. (b) Perfusion of the decellularized heart with the host vasculature through the aorta demonstrates maintenance of the decellularized blood vessels. (c) Hearts recellularized with cardiomyocytes beat spontaneously after four days in culture and can generate force when paced at 1 Hz or 2 Hz. (d) Maximum pressure and dP/dt after 8 days in culture and after stimulation with phenylephrine (PE). (e) Scanning electron microscope images of various slices of heart yield different pore sizes. (f) Migration of mesenchymal progenitor cells (MPCs) from MPC/Scaffold/transforming growth factor beta (TGFβ) constructs to acute infarcts, chronic infarcts, or normal myocardium four weeks after implantation. (g) Fractional shortening and fractional area change for 3d post-infarction rats (baseline) which were subsequently stratified into three groups: a control which received no additional intervention, an MPC group which received an injection of MPCs, and a patch group, which

ejection fraction (56% versus 39%). Whole cell patch clamp and microelectrode arrays of the cardiomyocytes demonstrated results consistent with cardiac phenotype. Together, these results suggest the identification of a cardiovascular progenitor which gives rise to cardiomyocytes, endothelial cells, and smooth muscle cells.

### Purification and use of stem cell derived cardiomyocytes

While this original study and subsequent studies have identified ways of generating cardiovascular lineages [59], one major challenge was to separate the cardiomyocytes from the non-myocyte cell types. In a screen for a marker of human embryonic stem-cell-derived cardiomyocytes, signal regulatory protein alpha (SIRPA) appeared to specifically select for cardiomyocytes above the other cell types (including endothelial cells and smooth muscle cells) [60]. Indeed, cell sorting with this marker and depletion of endothelial cells and smooth muscle cells resulted in a 98% troponin positive population of cells (Fig. 38.2f,g). The generation of a pure cell source is of particular importance since implantation of undifferentiated cells may lead to teratoma formation. Further, a purified cell source allows for greater control over the cell densities and types present in tissue-engineered constructs.

Given the precision required to generate specific cell types from stem cells, protocols must be optimized. For example, the procedure originally proposed by Yang and coworkers has been further modified and applied to multiple cell lines, including iPSCs [51]. Use of micro-bioreactor arrays allows control over the three-dimensional cellular microenvironment in a multiplexed fashion, for experimental optimization of cell derivation procedures on a small scale [61]. Additionally, these devices allow to control more than simply the concentration or type of cytokines. Various electromechanical cues can be added in order to further recapitulate the cardiogenic niche, allowing true optimization of stem cell derivation procedures [62].

## SCAFFOLDS

Cells alone do not compose functional tissues; other critical components include the substrate and extracellular matrix that surrounds and instructs the cardiac cells. Natively, the extracellular matrix provides microenvironmental cues, mechanical support and architectural guidance, acting as the scaffold upon which cardiac cells grow and function. Previous tissue-engineering scaffolds have used polymeric materials, lyophilized collagen sponges, and micro-patterned anisotropic materials. These artificial materials have design advantages in that they can be microfabricated, functionalized and are highly reproducible, but other approaches have used decellularization of native matrix as a different starting point, providing a scaffold with improved biological activity at the cost of some of the versatility of the synthetic approaches.

### Decellularization approach

Decellularization of native heart material is a powerful approach to easily recapitulate the *in vivo* architecture and extracellular composition of the heart. In 2008, Ott et al. decellularized whole rat hearts using a perfusion system of 1% sodium dodecyl sulfate (SDS) in deionized water, as shown in Fig. 38.3a [25]. The subsequent washes with detergent removed all of the cellular components from the native heart, leaving behind a 'ghost' heart, translucent in appearance, composed of thin walls of native heart matrix retaining the macroscopic and microscopic architecture.

received composite scaffolds containing MPCs in the decellularized scaffold. (h) Schematic of cardiomyocytes (red) in an alginate matrix or in the nanowired composite. (i) Transmission electron microscope images of alginate-nanowire composite scaffolds, where nanowires of approximately 5  $\mu\text{m}$  in length were incorporated into the scaffold material. (j) Connexin-43 (green), troponin (red), and 4',6-diamidino-2-phenylindole (DAPI) (blue) immunostained cardiomyocytes organized in the nanowired scaffold. (k) Connexin-43 and actinin expression by western blot relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in nanowired group versus the alginate only group 3 or 8 days after seeding with cardiomyocytes.

When reperfused with a native blood supply, the decellularized hearts clearly showed maintenance of vascular channels, demonstrating the preservation of overall native morphology (Fig. 38.3b). When reseeded with neonatal rat cells, the heart regained a cellularized appearance, and sections cut from the reseeded heart were capable of beating and matched the pacing rate applied through an external electrical field, either at 1 or 2 Hz (Fig. 38.3c). Over the eight days of culture, there was an increasing trend of contractile function and pressure generation. The whole heart preparations were also exposed to phenylephrine, which increased contractile pressures, suggesting pharmacological responsiveness (Fig. 38.3d). Overall this study provided a basis for whole heart decellularization with subsequent repopulation, providing a cardiac pump function at 2% of the adult rat heart output.

Since cardiac MIs are localized within the whole heart, sometimes only a specific section, importantly the left ventricle may need to be the target of therapy [26]. Decellularized scaffold patches can be made from native human heart sections using sequential detergent washes. However, the direction of sectioning is important, as different planes of section result in different scaffold architectures and different pore sizes (Fig. 38.3e).

When vascular progenitor human MSCs are seeded onto these native scaffolds, they act as depots of vasculogenic factors, improving the recovery of left ventricular function damaged by infarction. As shown in Fig. 38.3f, the presence of infarct improved the migration of the vasculogenic MSCs into the damaged tissue, in both acute and chronic models, and suggested that the stimulus provided by injury improved the responsiveness of cardiac tissue to the therapeutic cells. Notably, cells did not stay at the surface epicardium of the heart; instead they penetrated into the myocardium even when only microinfarcts were created using sutures alone to damage the myocardium. These native cardiac patches acted as a vehicle for a vasculogenic cell-based therapy and showed improved heart function, using echocardiographic metrics such as fractional shortening and fractional area change of the regions infarcted (Fig. 38.3g). The use of the native matrix scaffold improved recovery above cell injection alone, proving additional benefit as a delivery vehicle for the vasculogenic cells.

### Artificial scaffolds

Despite advances in using native matrices as scaffolding agents for the delivery of cells to the injured heart, the rational design of artificial scaffolds is still an active area of research. One primary limitation of fabricated scaffolds was their lack of electrical conductivity, a property that might enhance cell-cell communication and synchronization of the heart. Dvir et al. used gold nano-wires impregnated in a conventional scaffold material (alginate) to bestow conductive properties to the material [56]. Fig. 38.3g outlines how the conductive scaffold allows electrical signal propagation. Since alginate was chosen as the base scaffolding material, it maintained many of its familiar features. Even with the addition of gold nano-wires (Fig. 38.3h), the viscosity and material properties of alginate were not altered enough to change the porosity of the final scaffolds. The presence of the nano-wires improved the expression and organization of connexin 43, the primary gap protein associated with electrical-mechanical coupling and communication between cells, as assessed using immunofluorescent staining and western blot quantification (Fig. 39.3i). When calcium transients were investigated, the nano-wire-impregnated scaffolds showed fluorescent signals consistent with electrical propagation through the scaffold material, providing electrical stimulation to the cells. Engineered materials can thus add unique properties to cardiac scaffolds and tailor their functionality to that found in native heart muscle.

Freed and colleagues created an accordion-like scaffold using laser boring of 250  $\mu\text{m}$  thick poly(glycerol sebacate) layers [31]. The accordion-like honeycomb was made by overlapping two 200 by 200  $\mu\text{m}$  squares at an angle of  $45^\circ$ . The pore walls and struts were  $\sim 50 \mu\text{m}$  thick. The scaffolds were pretreated with cardiac fibroblasts followed by seeding of enriched cardiomyocytes. At the end of cultivation, the authors obtained contractile cardiac grafts with



mechanical properties closely resembling those of the native rat right ventricle. In addition, the cells in the pores were aligned along the preferred direction.

In another study, Feinberg et al. seeded a layer of neonatal rat ventricular cardiomyocytes on a polydimethylsiloxane membrane that could be detached from a thermo-sensitive layer of poly(isopropylacrylamide) at room temperature. Called Muscular Thin Films, these cell-covered sheets could be designed to perform tasks such as gripping, pumping, walking and swimming by careful tailoring of the tissue architecture, thin-film shape and electrical-pacing protocol [23a].

## BIOPHYSICAL CUES

Biophysical stimulation has been used to improve the phenotypic maturity of cardiac tissue-engineered constructs. These stimuli are designed to be biomimetic, and simulate the native heart environment to facilitate proper cardiac growth and maturation.

### Electrical stimulation

One of the primary features of the heart is its electrical connectivity and synchronization. Previous studies have shown improved conductive and functional characteristics of heart tissue grown in the presence of a stimulating electrical field. These results validate the use of electrical stimulation as a functional cue to improve phenotypic maturation of cardiac tissue, however, the precise techniques to apply electrical stimulation to engineered tissues is a topic of further exploration.

To establish baseline parameters, Tandon et al. compared multiple electrode materials and stimulation regimes to optimize electrical field conditioning of engineered heart tissue [50]. Petri dishes were outfitted with 1/8 inch rod shaped electrodes, spaced 1 cm apart, and 4 cm in length made from the following materials: carbon, steel, titanium nitride, and titanium. When comparing injected charge and recovery, the electrodes made of carbon recovered the most charge (95%), meaning had the least amount of lost charge due reaction products in the solution (Fig. 38.4a,b). The carbon electrodes were also best able to maintain a current through the bioreactor over the 2 ms time range for the depolarizing stimulus. With respect to effect on cell function, the results comparing various electrode materials was more modest, but carbon electrodes still had a favorable trend in their ability to properly capture the pacing of cardiac constructs as well as a lowered excitation threshold (Fig. 38.4c,d). This suggests that the carbon best communicates with the tissue-engineered constructs with minimal side reactions.

As seen with these and other studies, the effect of field stimulation improves cardiac tissue function, shown by increased Troponin and Connexin 43 markers, key proteins in both cell-cell connectivity and contractile apparatus (Fig. 38.4e).

### Mechanical stimulation

Mechanical stimulation approaches the electrical-mechanical coupling of heart tissue from the stretching and mechanical contraction perspective. It has been shown that passive or active tension can increase the cardiac functionality and orientation of cardiac cells. In one study, chitosan, a polysaccharide-based scaffolding material, was processed to have defined channel pores for perfusion and high porosity for cell seeding (Fig. 38.4f–h). Due to its attractive mechanical properties (Fig. 38.4i), it was used with a radial stretch device to characterize contraction using active tension. Strain maps of the scaffolds could be generated modeling the surface mechanics of the chitosan, showing stereotyped stress fields around the large pore areas (Fig. 38.4j). Interestingly, increased cell density and organization followed the predicted stress map suggesting that the mechanical stress provides a biophysical cue for cellular organization and communication (Fig. 38.4k).

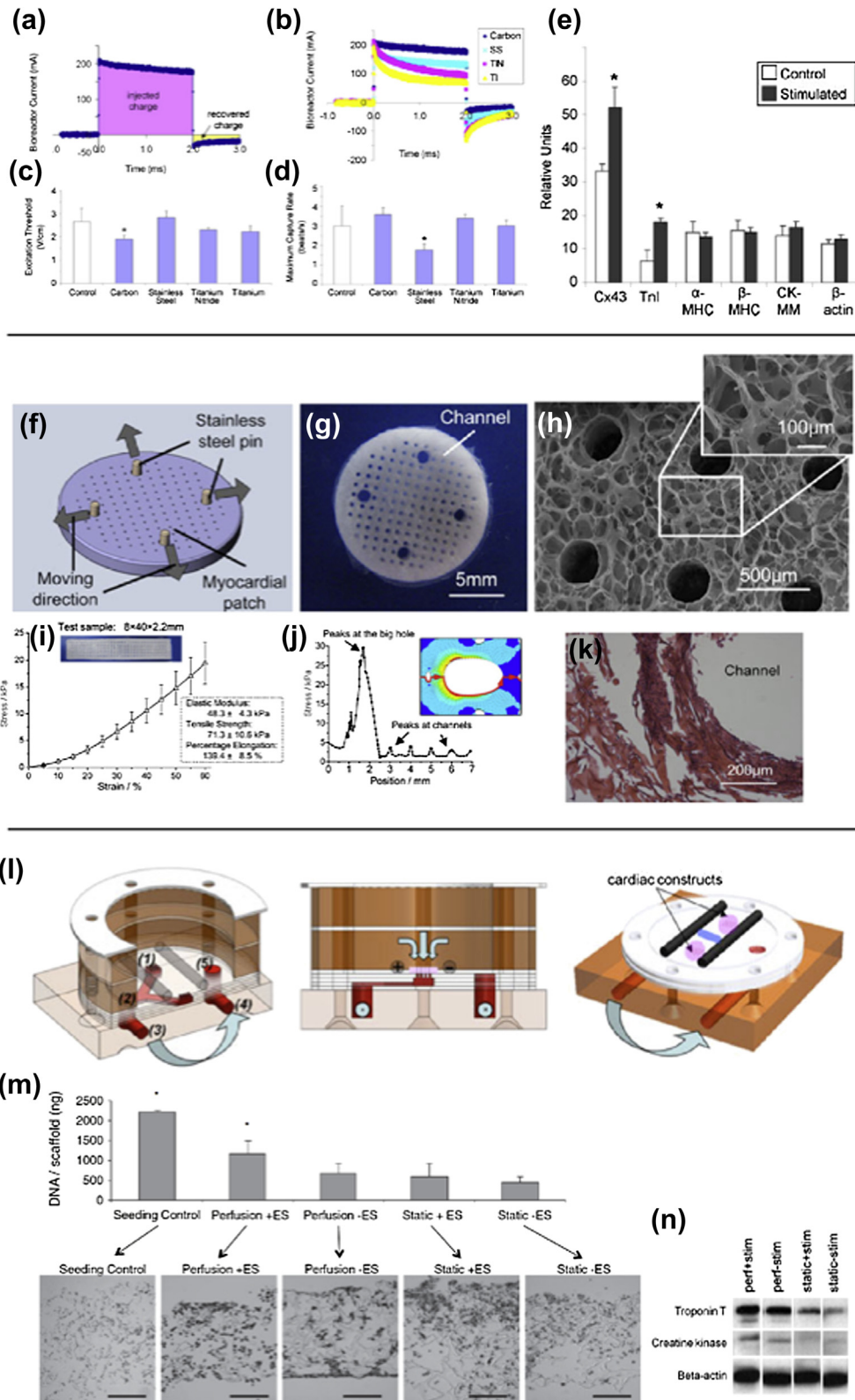


FIGURE 38.4

**Biophysical cues for cardiac tissue engineering.** (a) Bioreactor current over time depicting injected and recovered charge. (b) Current-time curves for different materials (carbon, stainless steel, titanium nitride, and titanium). (c) Excitation threshold and (d) maximum capture rate for cardiomyocytes stimulated with different materials or not stimulated at all (control). (e) Relative protein amounts of cardiac-related proteins in stimulated groups versus control. (f) Schematic scaffold design with pins for mechanical stimulation and pores for perfusion. (g) Macroscopic image and (h) scanning electron microscope image of



## Perfusion

Another critical biophysical stimulus — perfusion — is required for proper metabolic function. The transport of fresh oxygen and nutrients along with removal of metabolic products is particularly essential for cardiac tissue. Native tissue combines active perfusion and electrical-mechanical coupling, and in Maidhof et al, these two critical stimuli were combined in a novel bioreactor to enhance cell density and contractile protein expression of troponin [40]. A perfusion system, forcing fluid through a channeled scaffold was built around the standard paradigm of carbon electrodes. (Fig. 38.4l). This resulted in a more uniform cell distribution due to removal of diffusion limitations encountered when growing thicker pieces of cardiac tissue. When electrical stimulation was also applied, the cells were both more numerous while maintain an even density distribution throughout the tissue. (Fig. 38.4m) The two stimuli, perfusion and electrical stimulation, had an additive effect on troponin expression (Fig. 38.4n). The three biophysical stimuli — electrical stimulation, mechanical stretch and perfusion — all can contribute to the functional maturity of engineered cardiac tissues.

## IN VIVO APPLICATIONS OF CARDIAC TISSUE ENGINEERING

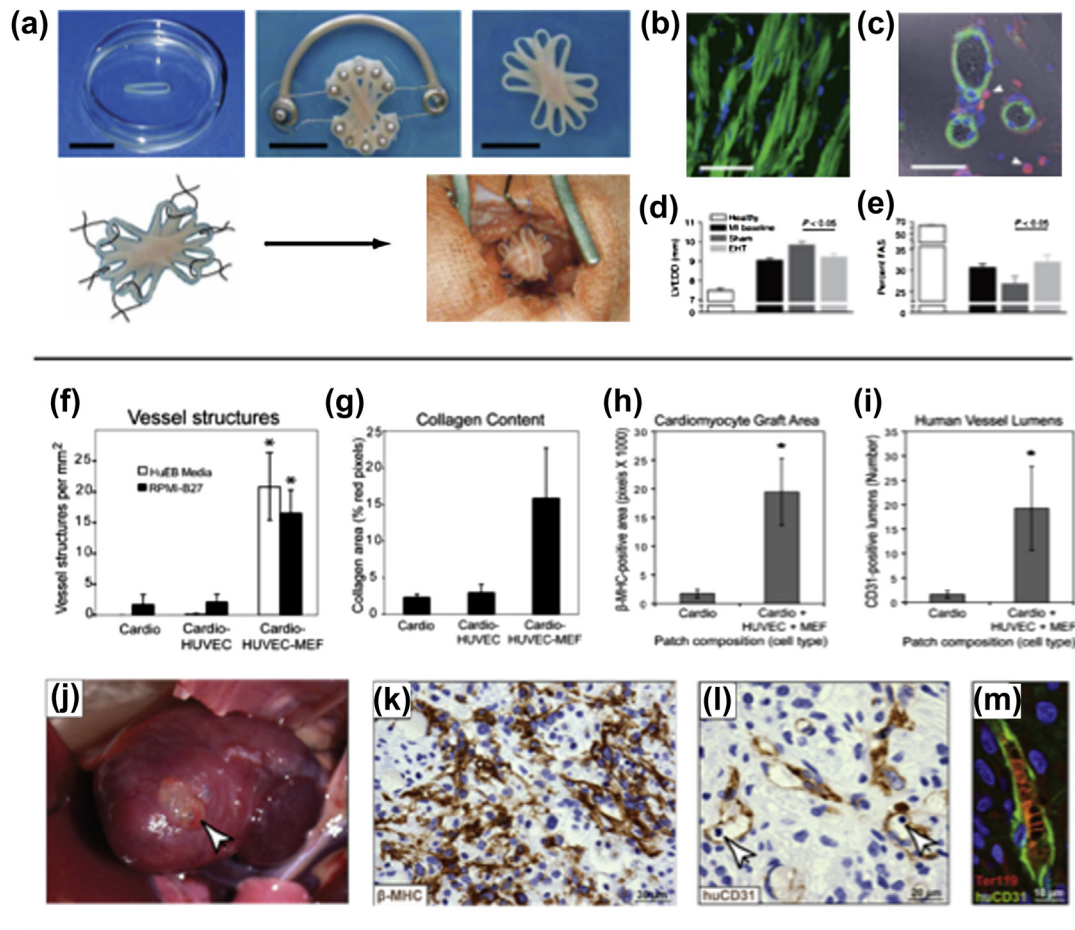
The ultimate goal of cardiac tissue engineering is the implementation of cells or tissue constructs in an injured heart and subsequently improving cardiac function. An 'ideal' cardiac patch would not only beat spontaneously, but would also connect with the host vasculature, couple electrically with the surrounding myocardium, and generate force to improve the function of a failing heart. We describe in this section two methods of fabricating a cardiac patch, the first of which focuses on using mechanical load to create force-generating constructs, and the second of which focuses on introducing multiple cell types to enhance vascularization of the cardiac patch. Open questions for *in vivo* systems include the functional differences between cell injection and scaffold implantation, and the type of arrhythmogenic or immunogenic response the foreign cells will have when near the host tissue.

### Engineered heart tissue

Zimmermann and colleagues (2006) outlined an approach to generate contracting rings of cardiac tissue which can be implanted into infarcted rat hearts, resulting in an improvement in cardiac function [30]. Engineered heart tissues were created by casting neonatal rat cardiomyocytes, collagen I, and Matrigel in a circular mold, followed by seven days of culture in 40% oxygen, under static, 'auxotonic' load, and in media containing insulin. Five of these loops were then stacked to create a large (15 mm diameter x 1–4 mm thick), fused, synchronously beating tissue assembly amenable to implantation (Fig. 38.5a).

Structural and electrical integration with the host myocardium as well as whole heart function were examined in rats with infarcted hearts implanted with engineered heart tissues. Immunostaining of the engineered heart tissue four weeks after implantation showed an elongated, sarcomeric pattern suggestive of highly differentiated cardiomyocytes (Fig. 38.5b). Additionally, there was neovascularization within the tissues from the donor cells that connected to the host vasculature (Fig. 38.5c). Electrical coupling was improved in the engineered heart tissues, as demonstrated by lower total activation times and higher QRS amplitudes. *In vivo* studies allow for the functional examination of whole hearts. Rats with engineered heart tissues demonstrated shorter left ventricular end diastolic diameter and a lower maximum left ventricular volume when compared to sham-operated rats, suggesting no additional dilation

fabricated chitosan-collagen scaffold. (i) Stress-strain curve of scaffold material with corresponding elastic modulus, tensile strength, and percent elongation. (j) Stress distribution along the length of the scaffold. (k) Hematoxylin and eosin (H&E) staining of cardiomyocyte laden scaffold in a region near a channel. (l) Schematic of perfusion-electrical stimulation bioreactor. (m) DNA content per scaffold and H&E stained sections of cardiac constructs that underwent different stimulation procedures. (n) Western blot of troponin T, creatine kinase, and beta-actin for constructs that were perfused and/or electrically stimulated.

**FIGURE 38.5**

**In vivo applications of cardiac tissue engineering.** (a) Single ring constructs are stacked in an auxotonic loading system and sewn on a rat heart using six sutures. (b) Troponin (green) and DAPI (blue) staining of engineered heart tissue four weeks after implantation showing cardiomyocyte elongation and sarcomeric alignment. (c) Vessel structures where asterisks represent the vessel lumens and arrows represent associated macrophages. (d) Left ventricular end diastolic diameter and (e) Percent fractional area shortening of healthy rat hearts compared to myocardial infarction (MI) baseline, sham surgery, and engineered heart tissue (EHT) implanted groups. (f) Number of vessel structures present in the cardiomyocyte only group, cardiomyocyte-HUVEC group, and tri-cultured group for two different media compositions after eight days of culture. (g) Collagen content of constructs after 8 days of culture. (h)  $\beta$ -MHC positive area and (i) CD-31 positive lumens based on histological immunostains in constructs implanted in skeletal muscle for one week. (j) Tri-cultured patches on the rodent heart one week after implantation. (k)  $\beta$ -MHC and (l) CD-31 immunostain of the constructs. (m) Vessel lumen in engineered tissue construct filled with Ter119-positive red blood cells from the host vasculature.

of the infarcted rat hearts as might be expected (Fig. 38.5d). Further, there was no decrease in fractional area shortening of the heart after the operation (Fig. 38.5e).

### Vascularized cardiac patches

Vascular integration of the cardiac patch with the host myocardium is important for the prolonged survival of thick tissue patches, especially given the high oxygen demand of cardiac tissue. Stevens et al. demonstrated the utility of prevascularizing cardiac tissue prior to transplantation into the infarcted area [24]. Cardiac patches were created from human embryonic stem-cell-derived cardiomyocytes and human umbilical vein endothelial cells, on mouse embryonic feeders. Several experimental groups were investigated: a cardiomyocyte only group, a group consisting of cardiomyocytes and endothelial cells, and a group consisting of all three cell types. Histology showed that the group consisting of all three cell types resulted in the largest number of vessel-like structures (Fig. 38.5f), and the stiffest construct with higher collagen contents, that were most similar to native myocardium (Fig. 38.5g). Upon

implantation into a skeletal muscle environment, the tri-culture group demonstrated great area staining for  $\beta$ -myosin heavy chain, the greatest number of vessel lumens (Fig. 38.5h,i), and neovascularization and anastomosis with the host vasculature (Figs. 38.5j–m).

### Electrical coupling of cardiomyocytes in the heart

One question remains whether or not cardiomyocytes, when applied to the heart, will form adequate connections with the host tissue, or if the engineered tissue constructs are proarrhythmic, thus potentially negating any therapeutic effect. In one recent study, Shiba et al. showed that these grafts may electrically couple with the host and suppress arrhythmias [63]. Human embryonic stem-cell-derived cardiomyocytes were transplanted onto a guinea pig cryoinjured heart and were subsequently analyzed for electrical coupling with the host tissue. They showed not only 1:1 coupling with the host myocardium, but also that the heart had reduced susceptibility to premature ventricular contractions, and sustained ventricular tachycardia. As a result, this study represents one of the first accounts that cardiomyocytes are non-arrhythmogenic when implanted. This supports the continued study of the interactions between the native host myocardium and the tissue-engineered construct.

### MODELING OF DISEASE

*In vitro* models of disease represent an important avenue for studying disease and for identifying potential therapeutic options. Here we describe the use of patient specific cells to generate diseased cardiomyocytes. We further describe the utility of tissue engineering in providing a faithful representation of diseased myocardium, and how this may be used in high throughput screens and drug studies.

#### Generation of patient-specific cardiomyocytes

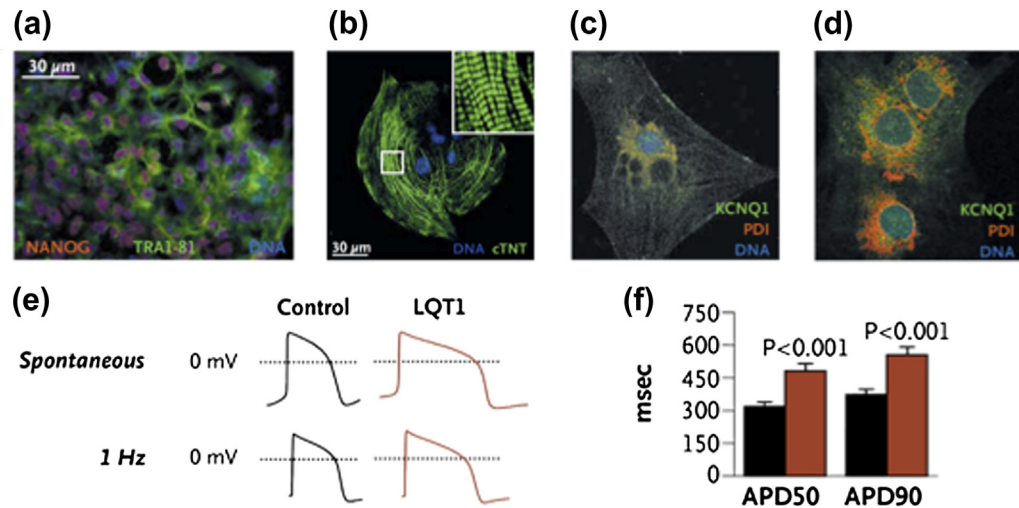
Induced pluripotent stem cells have allowed for the generation of patient-specific cardiomyocytes. One of the first accounts came from Moretti et al., who generated iPS cells from patients with a particular genetic mutation leading to Long QT syndrome and subsequently differentiated them into cardiomyocytes (Fig. 38.6a,b) [11]. Localization of the mutated channel (KCNQ1) in the endoplasmic reticulum confirmed this channel as the likely cause of disease (Fig. 38.6c,d). These cardiomyocytes displayed the electrophysiological phenotype of Long QT syndrome, including the classic lengthened action potential duration (Fig. 39.6e,f). The use of induced pluripotent cells to recapitulate the disease phenotype is particularly amenable to patients with genetic mutations. Since the study on Long QT syndrome, cardiomyocytes from patients with other diseases, including arrhythmogenic right ventricular cardiomyopathy and familial dilated cardiomyopathy, have been generated [13,14]. The iPS cells from patients harboring genetic cardiac mutations have been differentiated into CMs. These include cells from Timothy [12], Long QT [11], and LEOPARD [24a] syndromes and dilated cardiomyopathy patients [14].

#### Engineered heart tissue model for diabetes

While induced pluripotent cells are useful in generating cells from patients with specific genetic mutations, many cardiac diseases are multifactorial and require a biomimetic environment to faithfully reproduce the disease phenotype. Engineered heart tissue has been used by Song and colleagues to study the cardiac effect of diabetes and the effect of drug therapy on diabetic engineered heart tissues [64]. Briefly, the diabetic rat heart and high glucose cultivation conditions exhibited diminishing electrophysiological properties and increased ratio of myosin heavy chain isoform  $\beta$  to  $\alpha$ , indicative of diseased states.

#### Tissue engineering as a platform for pharmacologic studies

Given the active nature of cardiac tissue, new methods have been developed to study the function of engineered cardiac tissues in multiplexed *in vitro* systems. [65] Particularly, Schaaf

**FIGURE 38.6**

**In vitro models of disease.** (a) Immunostain of induced pluripotent cells from LQT1 patients for Nanog and Tra1–81. (b) Troponin stain showing sarcomeric organization in induced pluripotent stem cell (iPS) derived cardiomyocytes. Potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) (green) and protein disulfide isomerase (PDI) (red) staining for control cardiomyocytes (c) and patient cardiomyocytes (d). (e) Single cell action potentials of control and patient derived stem cells spontaneously beating or stimulated at 1 Hz. (f) APD50 and APD90 for control (black) or patient derived (red) cardiomyocytes.

et al. cast human embryonic stem cell derived cardiomyocytes in fibrin across a 24-well format which allowed the real-time measurement of force generation. [66] This system was then used to examine the effect of various proarrhythmic drugs (e.g., E-4031, quinidine, procainamide) on the beating dynamics of the heart tissue. As expected from the known electrophysiological effects of the drugs, the engineered tissues displayed irregular beating at low relaxation velocities.

In a separate study, sheets of engineered cardiac tissues were fabricated in a similarly multiplexed format which allowed the measurement of stress exerted by the cells [67]. Myocytes were seeded on a micropatterned surface to facilitate alignment of cardiomyocytes. The system was imaging compatible, allowing for the quantification of images or videos to determine contractility, action potential propagation, and cytoskeletal architecture.

## SUMMARY AND CHALLENGES

The increasing population of patients with heart disease, and the limited availability of transplant organs, motivates the field of cardiac tissue-engineering, which aims to generate robust tissue for implantation and subsequent improvement in cardiac function. The stringent requirements of cardiac tissue, including: the ability to generate contractile force, substantial metabolic requirements, and the need to electrical integrate with host tissue have resulted in a multitude of techniques to develop *in vitro* cardiac tissue. Different scaffolds, including decellularized native heart and various artificial materials have been used to recapitulate the native architecture of the heart while providing conduits for vascularization. The addition of biophysical stimuli, including mechanical stretch and electrical stimulation, are aimed to phenotypically mature the cells. When considering these various strategies implemented to design and fabricate improved cardiac tissue, one overall obstacle as the field advances is standardization of experimental and measurement techniques.

This is even more critical with the recent advent of high yield differentiation strategies to generate iPS and ES derived cardiomyocytes, enabling for the first time, a human cell source with the potential to generate patient-specific cardiac tissues. The field of pluripotent stem cell mediated therapies is still in early development, and many fundamental obstacles still remain

before their full promise is realized. However, we are primed to leverage autologous, nonimmunogenic, patient-specific cardiomyocytes with advanced techniques for the cultivation of engineered heart tissue. The creative combination of these cells with three-dimensional vascularized matrices and biophysical stimulation will allow the generation of functional tissues and provide an opportunity to intervene on the global epidemic of heart disease [25a–29a].

## Acknowledgments

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