Can We Engineer a Human Cardiac Patch for Therapy?

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Abstract: **Some of the most significant leaps in the history of modern civilization—the development of article in China, the steam engine, which led to the European industrial revolution, and the era of computers—have occurred when science converged with engineering. Recently, the convergence of human pluripotent stem cell technology with biomaterials and bioengineering have launched a new medical innovation: functional human engineered tissue, which promises to revolutionize the treatment of failing organs including most critically, the heart. This compendium covers recent, state-of-the-art developments in the fields of cardiovascular tissue engineering, as well as the needs and challenges associated with the clinical use of these technologies. We have not attempted to provide an exhaustive review in stem cell biology and cardiac cell therapy; many other important and influential reports are certainly merit but already been discussed in several recent reviews. Our scope is limited to the engineered tissues that have been fabricated to repair or replace components of the heart (eg, valves, vessels, contractile tissue) that have been functionally compromised by diseases or developmental abnormalities. In particular, we have focused on using an engineered myocardial tissue to mitigate deficiencies in contractile function. (*Circ Res*. 2018;123:244-265. DOI: 10.1161/CIRCRESAHA.118.311213.)**

Key Words: bioengineering ■ heart ■ pluripotent stem cells ■ stem cells ■ tissue engineering

Clinical Needs and Opportunities for Tissue Engineering

Clinical Need

Despite major advances in cardiovascular medicine, heart disease remains a leading cause of death worldwide. The adult mammalian heart has only a limited capacity for regeneration and, consequently, the cardiomyocytes (CMs) that are lost to ischemic injury are typically replaced by fibrotic scar tissue. To date, the only viable option for patients with the end-stage heart disease is whole heart transplantation. However, the shortage of donor hearts makes this approach unavailable for most of patients. The development of new and effective techniques for regenerating injured myocardium, or for correcting the fundamental molecular defects that lead to disease onset and progression, would thus have important therapeutic implications.

The high incidence of acute myocardial infarction, almost half a million annually[1] and subsequent heart failure are major and global health issues. Preclinical and clinical studies have demonstrated that cell therapy attenuates myocardial damage and the progression to heart failure, although the detailed mechanisms have not been deciphered.[2–4] In addition to ischemic heart disease, cell-based therapies have been effective in treating nonischemic heart diseases such as pressure-overload–induced concentric left ventricular (LV) hypertrophy and nonischemic dilated cardiomyopathy.[5,6] The clinical impact of cell-based therapy is limited by the low rate of cell engraftment.[4] Engineered heart tissues (EHTs), designed to morphologically and functionally resemble native myocardium, could provide unique advantages for enhancing cell engraftment compared with the direct myocardial injection of cells.[4,7,8] Clinical studies have demonstrated that application of hydrogels alone, which form a part of EHT, can prevent the progression of postinfarction LV remodeling and restore, to some extent, the normal cardiac function.[9,10]

Cell-Based Therapy

Pilot studies of cell-based cardiovascular therapies as summarized in the Table, began in the early 1990s using contractile cells (skeletal myoblasts and CMs) and continued through the early 2000s using noncontractile cells (fibroblasts, smooth muscle cells [SMCs], and bone marrow-derived mesenchymal stem cells [BM-MSCs]).[11–13,21,48] The results from phase I and phase II clinical trials suggest that these approaches may eventually become an effective strategy for treating ischemic and congenital heart disease, cardiomyopathy, and a variety of other cardiovascular disorders.[2,4] Currently, the most common methods for cell delivery used in clinical trials are direct intramyocardial injection and intravascular infusion. In both cases, the proportion of cells that are retained and survive at the site of administration (ie, the engraftment rate) is low and is believed to limit the treatment effectiveness.[2,4,48] Animal studies indicate that the engraftment rate can be substantially higher when the cells are administered as an EHT compared with the cell injection or infusion.[4,7,48]

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Nonstandard Abbreviations and Acronyms

Ang-1	angiopoietin-1
BM	bone marrow
CM	cardiomyocyte
CPC	cardiac progenitor cell
cTn	cardiac troponin
EC	endothelial cell
EHT	engineered heart tissue
hCMPs	human cardiac muscle patch
hESC	human embryonic stem cell
hiPSC	human induced pluripotent stem cell
IGF-1	insulin-like growth factor-1
LV	left ventricular
MSC	mesenchymal stem cell
PDGF	platelet-derived growth factor
SDF-1	stromal cell-derived factor 1
SMC	smooth muscle cell
ssTn	slow-skeletal troponin
TGF-β	transforming growth factor-β
VEGF	vascular endothelial growth factor

Cardiac Tissue Engineering

Concept and Goals

Tissue engineering is a branch of engineering science that focuses on developing living tissue matrices under laboratory conditions, with the objective of using these living tissues as model systems for drug testing or to repair injured tissues or organs. Although this is a relatively new field, successful engineering of almost all tissues found in the human body has been reported. Cardiac tissue engineering aims to manipulate the microenvironment that cells interact with, to facilitate cell assembly and build functional tissue. Its main goal is to provide a functional human cardiac muscle for drug discovery, studies of cardiac pathophysiology, and ultimately for therapy by repairing the diseased or damaged myocardium. In vitro approaches are aimed at organizing human stem cell-derived CMs into a functional tissue that is large enough for the intended use and capable of generating a contraction force (≥2–4 mN/mm^2) and propagating electric signals (at conduction velocities of ≥25 cm/s).

According to the classical tissue engineering paradigm, cells are cultivated on a scaffold (structural and logistic template for tissue formation) in a bioreactor (a culture system providing conditions designed to achieve a desired degree of functionality). Cultivation of scaffold-free tissues that are based on cells alone has also been explored.[49,50] Most studies to date demonstrated that a mixed cell population representing the diversity of the native myocardium (CMs, fibroblasts, and endothelial cells [ECs]) rather than the CMs alone, enhances the outcomes of the tissue engineering process and the survival of cells on transplantation of cardiac patches.[51–53] In addition, most studies now recognize that some kind of physical stimulation is required (mechanical, electrical, or both), to enable contraction of CMs in synchrony and drive their differentiation, maturation, and functional assembly into functional tissue units.[54–57]

Historical Perspective

Cardiac tissue engineering is maturing as a field, since the early studies that appeared in the late 1990s (Figure 1). The first studies utilized cells derived from either neonatal rat hearts or chick embryo hearts[58–61] as human CMs were not available and could not be appreciably expanded from cardiac biopsies.[62,63] The pioneering study describing the derivation of human embryonic stem cells (hESC) came out in 1998, but the directed differentiation protocols were yet to be perfected to increase the yield and quality of CMs.[64–66] Because spontaneous ESC differentiation yields only 2% to 4% CMs, these early protocols were not suitable for obtaining millions of CMs needed for cardiac tissue engineering, even if only for research purposes. Instead, neonatal CMs were obtained by digestion of the embryonic chick or neonatal rat heart ventricles. These studies have shown, for the first time, the ability to form a 3-dimensional (3D) cardiac tissue in the laboratory using cells, biomaterials, and bioreactors. Importantly, many of these initial approaches that worked for embryonic and neonatal animal cells were found useful later on for the cultivation of human tissues starting from human induced pluripotent stem cell (hiPSC)-derived CMs.[47,54] Therefore, the early studies that used animal cells informed the main guiding principles for the cultivation of human tissues, once the human cells became available.

In 1999, using a variation of approach described by Moscona,[67] Rob Akins et al[61] demonstrated that it is possible to create cardiac spheroids using ventricular cells dissociated from neonatal rat hearts. The method relied on the cells' own capacity to reassemble small tissue-like aggregates, in the absence of external organizational cues derived from a scaffold or physical signals. In parallel, our laboratory compared the 2 cell sources (neonatal rat versus chick embryo hearts) and types of bioreactors (rotating vessels versus mixed flasks) in their ability to induce the formation of cardiac tissue on fibrous poly(lactic-co-glycolic acid) PLGA scaffolds, relying on the classical tissue engineering paradigm.[60] These studies demonstrated the importance of appropriate supply of oxygen and nutrients for maintaining the metabolic function and elongated cell shape in engineered cardiac tissues. In the same year, we demonstrated that cardiac tissues based on fibrous scaffolds and neonatal rat CMs can be used as models for electrophysiological studies.[68] This area has grown appreciably over the past decade with a variety of approaches to generate models of healthy and diseased myocardium for studies of tissue development, physiology, and pathophysiology.[54,69–71]

The concept of a CM populated matrix was introduced by Eschenhagen et al[58] in 1997 as an in vitro tool for target validation using embryonic chick CMs and collagen hydrogel. In 2000, Zimmermann et al[59] introduced the term EHT to describe a system more relevant for mammalian heart repair. EHT were constructed from neonatal rat heart-derived CMs in a collagen-Matrigel hydrogel matrix that was either anchored at each end using Velcro or assembled into a ring using cylindrical molds. This approach is widely used by these investigators and has been adopted by other laboratories with appropriate modifications for cultivation of both rat and human cell-based tissues.[69–71] This important study measured the contractile force generated by EHTs, at the baseline level

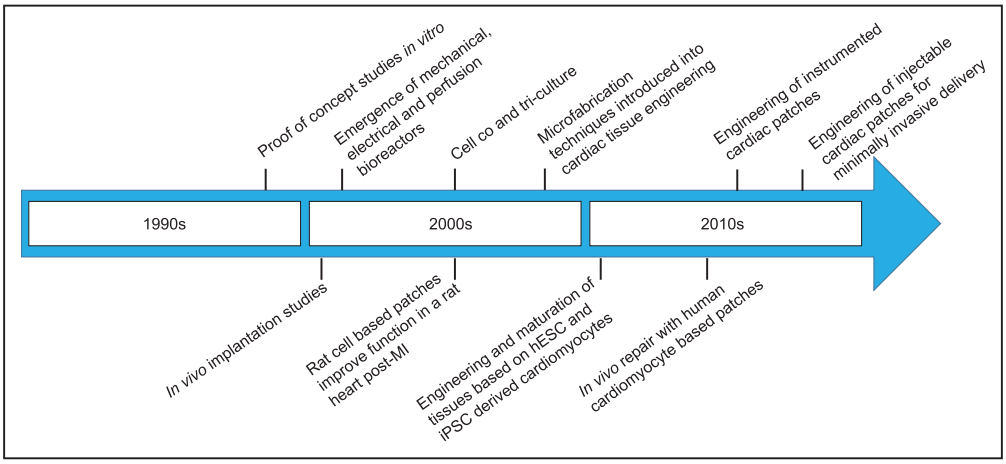

Figure 1. Time line for key milestones in the field of cardiac tissue engineering. hESC indicates human embryonic stem cell; iPSC, induced pluripotent stem cell; and MI, myocardial infarction.

and in response to isoprenaline, and established the first force-length relationship (positive) and force-frequency relationship (negative) for the engineered cardiac tissues.

After the field has conclusively demonstrated that it is possible to cultivate contractile cardiac tissues using the tissue engineering paradigm, it remained to be shown that these tissues can make a difference when implanted in vivo, by improving contractile function. Toward this goal, Li et al[22] used commercially available porous collagen sponges seeded with fetal Lewis rat CMs. These cardiac patches were implanted atop of the cryoinjured Lewis rat heart ventricles and maintained for 5 weeks in vivo. Although this study did not report any significant structural or functional improvements, it was the first to implant an engineered cardiac patch. A year later, Leor et al[23] demonstrated that it was indeed possible to improve cardiac function by patch implantation. Here, CMs derived from fetal Sprague-Dawley rat hearts were cultivated in alginate scaffolds and implanted onto the adult rat Sprague-Dawley myocardium damaged by coronary artery occlusion. The patches were cultivated for only 4 days in vitro, under static conditions, and implanted for 9 weeks. Both the maintenance of LV function (by echocardiography) and the attenuation of LV dilatation (by histology) were demonstrated relatively to untreated controls.

Overview of Approaches

These early studies faced challenges related to the cell survival in thick constructs and achieving the differentiation and maturation of CMs. As the field advanced after the initial proof-of-concept studies, several approaches emerged that enabled the investigators to create cardiac patches better resembling the native myocardium. The density and spatial distribution of the cells in tissue constructs were increased by cultivation in bioreactors with perfusion (interstitial flow) of culture medium and by coculture with ECs to promote vascularization. The differentiation and maturation of the cardiac patches were further improved by mechanical and electrical stimulation.

Culture With Medium Perfusion

Native myocardium contains >90% of CMs by volume and an extremely high physiological cell density of $>10^8$ cells/cm^3 that is required to display appropriate functional properties. However, when one considers the metabolic demand of CMs for the limiting factor, oxygen, and the low solubility of oxygen in aqueous media, it becomes clear that it is possible to satisfy that demand at a physiological cell density only in constructs that are thinner than a few hundred micrometers. Using oxygen microelectrodes, we confirmed that oxygen concentration drops to very low levels in the interior of 2 mm thick cardiac constructs grown under static conditions.[72]

To maintain full viability of cardiac constructs at a physiological cell density, we developed a suite of perfusion bioreactors that enable cell seeding and cultivation in porous collagen scaffolds under perfusion.[73–75] We further investigated, with the aid of mathematical modeling, how exactly the geometry and spacing of channels in the scaffolds perfused with culture medium may result in more uniform distributions of oxygen and nutrients. This study was guided by biomimetic principles for providing a more physiological transport between the cells and their environment. As in native vascularized tissues, rapid distribution of the fluid (blood or culture medium) was achieved by convective flow through the channels and diffusional transport into the surrounding tissue space.[76] We confirmed experimentally that the medium perfusion through the channels resulted in increased depths of viable tissue and more uniform oxygen distribution.

To increase the capacity of culture medium for oxygen, we utilized another biomimetic component—the addition of perfluorocarbon oxygen carriers mimicking hemoglobin in blood.[77] Although the capacity of oxygen carriers is much lower than that of blood, this study established some principles and methodologies for establishing convective-diffusive oxygen transport in engineered cardiac tissues using perfused bioreactors. In recent studies, these same principles were used to create AngioChip, a vascularized polymer-based microfluidic scaffold.[78]

Mechanical Stimulation

The first description of the importance of cyclic stretch in EHT lattices was published in 2000, using embryonic chick[79] and neonatal rat[59] CMs in collagen gels subjected to cyclic stretch. In 2002, Zimmermann et al[56] further perfected this approach and demonstrated the importance of mechanical stimulation in

maintaining the differentiated phenotype of a cardiac tissue in vitro. Under the conditions of linear cyclic stretch, CMs and nonmyocytes formed cardiac organoids consisting of a well-organized and highly differentiated cardiac muscle syncytium that exhibited contractile and electrophysiological properties of working myocardium. Ultrastructural features were well developed, with sarcomeres arranged into myofibrils, Z, I, A, H, and M bands, specialized cell-cell junctions and basement membranes. Contractile properties were similar to those measured for native rat heart tissue, with a high ratio of twitch to resting tension and strong β-adrenegenic responses. First, implantations in healthy rats showed survival, vascularization, and signs of terminal cardiac differentiation.[80] Mechanically stimulated EHTs were able to improve local and global cardiac function when implanted on the infarcted rat hearts and to attenuate pathological function in comparison to the untreated infarcted hearts.[57]

Cultivation of muscle tissue around and between 2 or more flexible posts was introduced to enhance contractility of EHT implants.[57] This technique proved useful for several muscle engineering approaches.[81–84] The methodology based on auxotonic mechanical stimulation was recently extended to the cultivation of EHTs from human CMs derived from embryonic and iPSCs and fibroblasts, in serum-free medium, with evidence of maturation at the cellular and tissue levels.[85]

In parallel studies, the authors collaborated with our laboratory to combine mechanical stimulation with electrical stimulation at frequencies matching physiological heart rate, to improve functional maturation.[84] To engineer cardiac tissues, neonatal rat heart cells were encapsulated in collagen type I hydrogel, and the cell-hydrogel constructs were formed >8 days of static culture between 2 flexible poles. These constructs were subjected to 5 days of electrical field stimulation at frequencies ranging from 0 to 6 Hz that was synchronized with auxotonic contractions. EHTs stimulated at 4 Hz displayed a positive FFR (frequency-following response), reduced calcium sensitivity, frequency-dependent acceleration of relaxation, and enhanced postrest potentiation. At the cellular level, these functional advances were associated with improved calcium storage and release capacity of the sarcoplasmic reticulum and increased amounts of SERCA2a (sarco/endoplasmic reticulum Ca^{2+}-ATPase) and RyR2 (ryanodine receptor 2). This study is important as it showed the structural and functional benefits of electromechanical stimulation at the physiological heart rates.

Electrical Stimulation

Contraction of the cardiac muscle is driven by the waves of electrical excitation (generated by pacing cells) that spread rapidly along the membranes of adjoining cardiac myocytes and trigger calcium release, which in turn stimulates contraction of the myofibrils. Electromechanical coupling of myocytes is crucial for their synchronous response to electrical pacing signals, resulting in contractile function and pumping of blood.[86]

We pioneered electrical field stimulation of cardiac constructs using neonatal rat ventricular cells cultured on collagen sponges and subjected to suprathreshold, square-shaped monophasic pulses (2 ms duration, 1 Hz, 5 V) for up to 8 days.[55] Electrical field stimulation induced cell alignment and coupling, increased the amplitude of synchronous contractions by a factor of 7 and resulted in a remarkable level of ultrastructural organization. Developments of conductive and contractile properties of the cardiac constructs were concurrent, with a strong dependence on the initiation and duration of the electrical stimulation. Aligned myofibers expressing cardiac markers present in stimulated samples were similar to those found in neonatal rat heart ventricles. Stimulated samples had sarcomeres with clearly visible M, Z lines and H, I, and A bands. In contrast, nonstimulated constructs had poorly developed cardiac-specific organelles and ultrastructural features. To further mimic conditions in the heart, we applied biphasic electrical stimulation and demonstrated that electrical stimulation enhanced the assembly of cardiac organoids from cocultures of CMs, fibroblasts, and ECs.[87]

In the native myocardium, connexin 43 is concentrated at the end of the cells, however, in cell culture connexin 43 is typically sporadically expressed. Lasher et al[88] developed a specialized bioreactor in which neonatal rat CMs were seeded around 2 posts to form an engineering cardiac tissue fiber. After 3 days of culture, these fibers were electrically stimulated by carbon electrodes. The stimulated samples were more elongated and had stronger expression of sarcomeric structures, which was similar in morphology to the native myocardium. Finally, connexin 43 expression was significantly increased in stimulated samples compared with the unstimulated samples and was expressed at levels similar to native adult myocardium. However, its distribution was still irregular and clustered over the cell membrane, rather than being concentrated at the cell ends as in the adult rat CMs.[88]

In an attempt to more accurately mimic adult native myocardium, bioreactors providing more complex stimulation protocols are being developed. A combination bioreactor designed to electrically stimulate and perfuse the tissues was also developed. Neonatal rat CM tissue constructs were subjected to a flow velocity of 0.1 mm/s and were stimulated with a 3 Hz monophasic square wave. The cells in the stimulated tissues were found to be the most abundant and more elongated compared with unstimulated tissues.[89]

These same principles of electrical field stimulation were further adapted for the cultivation and maturation of CMs derived from human embryonic and adult pluripotent stem cells (hESCs, hiPSCs). We first seeded cells using a collagen gel around a template suture in a microfabricated well and subjected them to electrical field stimulation of progressive frequency increase (up to 6 Hz in 1 week).[54] The engineered platform allowed for the generation of 3D, aligned cardiac tissues with frequent striations termed biological wire, or Biowire. The Biowires subjected to electrical field stimulation markedly increased myofibril ultrastructural organization, displayed elevated conduction velocity and altered the electrophysiological and Ca^{2+} handling properties versus nonstimulated controls. Future iterations of the model supported the use of vascular cells to aid in oxygen and nutrient diffusion.[54] The platform was later modified to support seeding around a polytetrafluoroethylene tube. The tubing could be perfused with various drugs and the surrounding tissue could be monitored to test for cardiotoxicity.[90]

Overall, the studies of cardiac tissue engineering that implemented in vitro a single, but critically important in vivo factor, progressively enhanced the functional tissue assembly and improved the properties of engineered myocardium at the cellular, ultrastructural, and tissue levels.

Maturation

The molecular, structural, and functional properties of iPSC-derived CMs more closely resemble fetal than adult CMs.[91] Morphologically, mature CMs are well-aligned, rod-shaped, and larger with greater cross-sectional surface areas and longer sarcomeres.[44,85,92,93] CM maturity can also be quantified via the expression of marker proteins such as connexin 43, N-cadherin, the T-tubule protein caveolin-3, and SERCA (sarco/endoplasmic reticulum calcium-ATPase), which all increased as the CM matures.[55,93,94] The ratios of adult to fetal troponin I gene products (cTn [cardiac troponin I] and ssTn [slow-skeletal troponin I], respectively) also increased.[95] The maturity of the EHT has been evaluated from action potentials recorded by patch-clamp,[96] and from the action potentials and calcium transients measured using voltage-sensitive dyes,[97] genetically encoded voltage indicator proteins,[98] and calcium-sensitive molecules.[94] Mature CMs also contract more forcefully when stimulated with β-adrenergic agonists.[85]

Both hESC-CMs[40] and hiPSC-CMs[92,99] gradually become more mature after transplantation but do not mature under standard culture conditions.[92,94,100] Mechanical and electrical stimulations have been demonstrated to promote the maturation of engineered cardiac tissues in vitro as discussed above. Maturation may also be influenced by the cellular composition of the tissue, as well as the scaffold properties and culture conditions. Tiburcy et al[85] demonstrated that a tissue containing 70% hiPSC-CMs and 30% human fibroblasts displayed greater force generation than tissues containing larger (90%) or smaller (50%) fractions of CMs. Although tissues containing murine ESC-CMs alone contracted asynchronously after 21 days of culture, the inclusion of mouse ESC-derived Nkx2.5+ cardiac progenitor cell (CPCs)[101] or neonatal rat ventricular fibroblasts improved synchronicity, contractile force, action potential propagation, and calcium transients.[102] Cultivation in EHT format promotes maturation as illustrated by microelectrode recordings of hiPSC-EHTs that revealed normal diastolic potential and an upstroke velocity of >250 V/sec, which are similar to the human ventricular tissues.[103] CM maturation has also been promoted by manipulating the scaffold structure to promote alignment[104,105] and by varying its composition. Gao et al[106] used multiphoton-excited 3D printing to generate a native-like extracellular matrix scaffold with submicron resolution. Chun et al[107] reported that a scaffold composed of 4% polyethylene glycol and 96% ε-caprolactone promoted maturation more effectively than other compositions tested. iPSC-CMs also matured more efficiently when cultured in a fibrin gel than in monolayers,[96] and when the iPSC-CM/fibrin constructs were cultured on a dynamic, rocking platform,[97] possibly because of improved nutrient availability.

It remains unclear whether matured heart tissues have higher chance to survive than immature tissues after transplantation. Recently, it was reported that heart tissue composed of hESC-CMs and collagen were mechanically stimulated for 12 to 14 days in culture before transplantation into chronically infarcted rat hearts.[41] Notably, this longer period of stimulation was associated with the evidence of graft survival for as long as 220 days after transplantation and the well-organized sarcomeres aligned with the strain axis in transplanted CMs. More studies are warranted to study the association between maturation and survival.

Recently, Gao et al[7] generated human cardiac muscle patches (hCMPs) of clinically relevant dimensions (4 cm×2 cm×1.25 mm) by encapsulating CMs, SMCs, and ECs derived from hiPSCs in a fibrin scaffold and then culturing the construct on a dynamic (rocking) platform. The hCMPs began to beat synchronously within 1 day of fabrication, and after 7 days of culture, in vitro assessments indicated the mechanisms related to the improvements in electronic, mechanical coupling, calcium-handling, and force generation (1.18 nN/input myocyte) suggesting a maturation during the culture. The hCMP transplantation into a porcine model of MI was associated with significant improvements in LV function, infarct size, myocardial wall stress, myocardial hypertrophy, and reduced apoptosis in the periscar border zone myocardium. hCMP transplantation also reversed some MI-associated changes in sarcomeric regulatory protein phosphorylation. The exosomes released from the hCMP appeared to have cytoprotective properties that improved CM survival.

Vascularization

The energy demand of myocardial tissue is exceptionally high because of the contraction. In principle, the survival of implanted cardiac tissue requires reestablishment of the oxygen and carbon substrate delivery to the EHT within the first few hours after the transplantation, which remains to be the major challenge for the field. Heart has very robust neovascularization capacity. Therefore, if the transplanted EHT could survive for the first few days after transplantation, we usually observe very robust angiogenesis of vessels growing into the grafted EHT with the vascular density almost similar to the naive cardiac tissue.[7,108,109]

Shimizu et al[110] elegantly demonstrated that it is possible to generate 1 mm thick viable and vascularized cardiac grafts in vivo, through a polysurgery approach, where thin (80 μm) nonvascularized cell sheets were sequentially implanted in vivo on top of one another in intervals of 1, 2, and 3 days. For scaffold-based cardiac tissues, vascularity and cell survival are generally greater in constructs that contain both CMs and non-CMs (eg, ECs, SMCs, pericytes, and fibroblasts) than in constructs composed of CMs alone.[44,80,108,111,112] ECs and SMCs release cytokines that stimulate neovascularization and recruit endogenous CPCs.[113] Pericytes and fibroblasts produce VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), Ang-1 (angiopoietin-1), and perhaps other mitogenic factors that stimulate the proliferation of ECs while inhibiting apoptosis.[114,115]

The formation of the new capillary beds can be promoted in CM sheets by inclusion of ECs between the sheets, during manufacturing.[7,116] However, CMs also seem to contribute to the vessel growth, as neovascularization was significantly greater in infarcted rat hearts after treatment with sheets of murine ESC-derived CMs, ECs, and mural cells, but not when

the CMs were omitted.[117] The results of experiments conducted by Iseoka et al[118] suggested that the engineered cardiac tissues composed of 50% to 70% hiPSC-CMs and 30% to 50% nonmyocytes were more stably engrafted and led to greater functional improvements in a rat MI model than the corresponding tissues containing larger (90%) or smaller (25%) proportions of CMs.

Vascularized cardiac tissues have also been created by combining neonatal rat cardiac cells with a mixture of Matrigel, IGF-1 (insulin-like growth factor-1), SDF-1 (stromal cell-derived factor 1), and VEGF, and then implanting these tissues into the rat omentum for 7 days to induce the ingrowth of native blood vessels.[119] When tested in a rat model of MI, prevascularized tissues were structurally and electrically integrated into the host myocardium 4 weeks after transplantation, and improved cardiac function and remodeling. Overall, vascularization achieved either during in vitro culture or after implantation and involving blood perfusion remains a critical requirement for the survival and function of cardiac tissue grafts.

Cell Sources

Cellular Components of Adult Cardiac Tissue

In the adult mammalian heart, CMs constitute ≈30% to 40% of the total number of cells and ≈80% of the total heart volume,[120] whereas the remaining nonmyocyte population is composed primarily of ECs and fibroblasts, with smaller proportions of hematopoietic-derived cells and vascular SMCs/pericytes.[121] Studies conducted over the last decade have identified several types of CPCs in adult mammalian hearts, including cardiosphere-derived cells, c-kit+ CPCs,[122] Sca-1+ (stem cell antigen-1) CPCs,[123,124] and side population cells.[125] The precise numbers and developmental origin of these progenitor cell populations have yet to be determined, but when tested in animal models of myocardial injury, they have been associated with significant improvements in contractile function and remodeling (Figure 2).

Skeletal Myoblasts

It is the first type of cells that were tested for myocardial repair. Pilot studies in early 1990s demonstrated that skeletal myoblasts forms grafted after being injected to normal or injured myocardium.[11–13] In 2001, Menasché et al reported the first clinical study and showed that intramyocardial injection of autologous skeletal myoblasts enhances cardiac contractile function in a patient with MI.[14] The beneficial effects of transplantation of autologous skeletal myoblasts were confirmed in clinical studies by Sawa et al.[18–20] A major concern is that myoblasts can't form gap junctions with host myocardium because of the lack of connexin-43, thus increasing the risk of ventricular arrhythmia in recipient hearts.[15–17] It seems that genetic modification of myoblasts may address this issue, as transfection of human skeletal myoblasts with Cx-43 gene has been shown to attenuate their proarrhythmic potential.[126] Notably, gap junctions do form between CMs and myoblasts or their progeny (myotubes) in culture,[127] which suggests that the results from in vitro studies with myoblasts must be considered cautiously when interpreted for an in vivo setting.

Primary Cardiac Cells

In the early 1990s, Soonpaa et al[21] reported that grafted fetal mouse cardiomyocytes survived and formed intercalated discs with host mouse myocardium, establishing the feasibility of primary CMs for myocardial repair. Souren et al[128,129] demonstrated the feasibility of generating 3D structures from neonatal rat CMs. The cells in a floating collagen matrix survived and displayed rhythmic contractions and ECG-like potentials. Later studies demonstrated transplantation of EHTs with fetal CMs attenuates LV remodeling in infarcted rat hearts.[22,23] Clinical application of primary cardiac cells-based therapy is unpractical because of the limited availability of human primary cardiac cells.

By analogy with monolayer studies in which fibroblasts significantly overgrow CMs in culture, early cardiac tissue engineering studies used the populations of neonatal rat heart cells that were enriched with CMs as much as possible, by a series of preplating steps.[68] The structure, metabolism, and electrophysiology of tissue constructs composed of neonatal rat cardiac cells (myocytes and nonmyocytes versus enriched population of myocytes) were studied in a polyglycolic acid scaffold.[68] Cell size and metabolic activity in both constructs were similar to those in freshly isolated adult and neonatal rat ventricular tissue. However, the electrophysiological properties of myocyte-enriched constructs, although subnormal, were superior to those observed in the constructs containing both myocytes and nonmyocytes. These cell subtypes exist in a balanced equilibrium and constant cross talk via paracrine signals.

In 2006, Naito et al[130] were the first to publish that a mixed cell population found in native CM isolates containing ≈47% CMs and ≈49% cardiac fibroblasts resulted in improved EHT formation, especially in defined culture media, in comparison to the population enriched by preplating to 63% of CMs and 33% of fibroblasts. In addition, our studies showed advantages of compartmentalization of CMs and fibroblasts. The polymeric scaffolds pretreated with fibroblasts, before seeding with CMs, resulted in improved structural and contractile properties of cardiac tissues, in comparison to those grown using the CMs enriched by preplating to 80% to 90%.[131]

Over time, the field has learned to harness the power of fibroblasts by introducing them strategically at defined percentages during coculture with ventricular CMs, to obtain engineered cardiac tissues with enhanced structural and functional properties and ability to survive in vivo.[51–53,115,132–134] Alternatively, MSCs have also been used as supporting cells in engineered cardiac constructs.[44] In the context of engineering cardiac tissues based on hESC-derived CMs, we demonstrated enhanced matrix remodeling and functional properties in cultures with 75% CMs and 25% hESC-derived CD90+ mesodermal cells,[53] although it is possible to obtain well-developed, stable, and relatively mature EHTs from pure hiPSC-CM preparations especially if fibrin matrix is used.[135] In those controlled cocultures, fibroblasts contributed to CM survival by paracrine signaling and matrix remodeling. There is clearly a consensus in the field right now that nonmyocytes are important for cardiac patches, both in vitro and in vivo.[51–53,115,132–134] The advances made over the last few years have enabled the generation of tissues

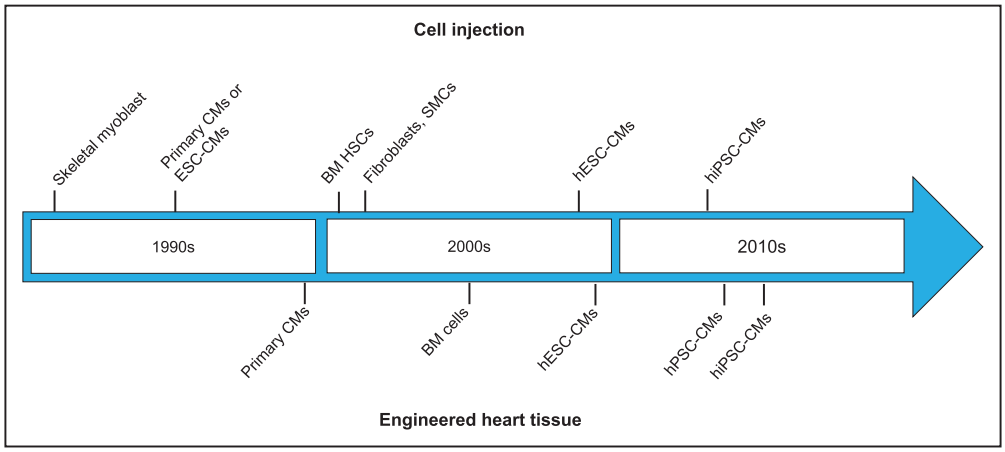

Figure 2. Time line of cell source development for cardiac cell therapy and tissue engineering. BM indicates bone marrow; CMs, cardiomyocytes; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; HSCs, hematopoietic stem cells; and SMCs, smooth muscle cells.

with sophisticated architecture and functional properties approaching those of native hearts.

Mesenchymal Stem Cells

MSCs are the most commonly used cells in preclinical and especially clinical investigations of cell therapy of the heart.[27,136] These cells are defined by their multipotency, capacity for self-renewal, and low immunogenicity,[137] but do not persist long in target tissues,[138] which likely limits the risk of any long-term complications after administration but also affects therapeutic efficiency. MSCs can be obtained from many different organs,[139] with the BM aspirates and adipose tissue being the most common sources of MSCs for experimental use.[140] The BM also contains a variety of other cell types (eg, BM stromal cells,[141,142] hematopoietic stem cells,[24] and endothelial progenitor cells[25]) that have been investigated in preclinical and clinical studies of cardiac disease but are generally not used for myocardial tissue engineering. Intramyocardial injections of autologous BM-MSCs improves cardiac function in rat cryoinjury model.[143] Clinical studies by Hare et al[33] demonstrated that both allogeneic and autologous BM-MSCs enhance cardiac function and attenuate LV remodeling in ischemic cardiomyopathy patients. By injecting different doses of cells, it was found that low-dose MSCs (20 million cells) produced greatest reductions in LV volumes and increased ejection fraction than middle dose (100 million) or high dose (200 million). It is generally thought that paracrine effects contribute to the cardiac protection from BM-MSCs. Overexpression of Akt gene in transplanted BM-MSCs enhances their proangiogenesis effects in MI rats.[26]

In 2004, Liu et al[27] evaluated the therapy of EHTs made of autologous BM-MSCs in a fibrin patch in MI sinew model. Transplantation of EHTs was associated with improvements in contractile function and with a robust increase in neovascularization in the region covered by the EHT. Autologous MSCs have also been shown to differentiate into ECs and SMCs when administered as fragmented sheets to infarcted swine hearts,[144] the sheets were grown in a thermo-responsive methylcellulose hydrogel, and the treatment preserved cardiac function while attenuating LV remodeling. The first experiments with engineered cardiac tissues containing MSCs of human origin were performed in 2011, when Godier-Furnemont et al,[31] applied a mixture of human MSCs, fibrinogen, and thrombin to a 300-μ thick section of decellularized human heart tissue and tested this EHT construct in nude rat models of acute and chronic MI; the treatment was associated with cardiac functional improvement 4 weeks after transplantation. Their results also indicated that treating the MSCs with TGF (transforming growth factor)-β promoted the release of pro-angiogenic factors, but they found no evidence that the MSCs had differentiated into CMs. This study also showed that the native tissue matrix used as a cell delivery vehicle can promote both the local and long-ranging cell function, a concept that has been extended to other therapeutic cells and formulations of the native tissue matrix.

In addition to BM, MSCs derived from other tissues such as umbilical cord matrix[32] or lining,[34,35] adipose,[29] placenta,[30] amnion,[28,145] have been shown to attenuate LV remodeling and improves cardiac function after transplantation to MI animals.

Pluripotent Stem Cells

hPSCs, including ESCs and iPSCs, can be used to generate potentially unlimited numbers and types of cells for regenerative medicine. ESCs are collected from the cluster of cells that give rise to the body of the embryo proper (ie, the inner cell mass)[146] and, consequently, can differentiate into all cell types except those in extraembryonic tissues such as the placenta. ESCs can also be obtained via parthenogenesis[147]—asexual, uniparental reproduction from an unfertilized oocyte,[148] a process that occurs naturally in some lower organisms, but can be chemically stimulated in mice and nonhuman primates[149–152] and may alleviate ethical issues about the destruction of fertilized human embryos. iPSCs also avoid ethical concerns as they can be generated from the patient's own somatic cells via the overexpression of 4 transcriptional regulators, Oct4 (octamer-binding transcription factor 4), Sox2 (sex determining region Y-box 2), Klf4 (KLF family of transcription factor 4), and Myc.[153] However, the pluripotency and unlimited proliferative capacity of ESCs and iPSCs can also lead to tumor formation; thus, pluripotent cells are typically differentiated into CMs, SMCs, and ECs, before being assembled into engineered tissues.

In 1996, pilot study by Klug et al[36] showed that intramyocardial injection of purified ESC-CMs formed stable

intracardiac graft in mdx recipient mice. Preclinical studies showed that hESC-CMs engrafted in MI rats,[37,38] guinea pigs,[39] or monkeys,[40] and attenuated LV remodeling. Large engraftment is associated with higher frequency of arrhythmia.[40] In 2006, Guo et al[154] created the first EHT with mouse ESC-CMs. After 7 days of mechanical stimulation, the resulting tissues functionally and structurally resembled immature native cardiac tissue. In the next year, Caspi et al[115] generated heart tissue combining hESC-CMs with Matrigel containing either ECs differentiated from hESCs (hESC-ECs) or human umbilical vein ECs. Transplantation of hESC-CMs via engineered muscle rings has been shown to enhance engraftment rate, lead to long-term survival and progressive maturation of these CMs.[41]

In 2011, the first cardiac tissues containing human iPSC-derived cells were generated by combining 2 million CMs (hiPSC-CMs or hESC-CMs), 1 million human umbilical vein ECs, and 1 million MSCs or murine embryonic fibroblasts with collagen type I and were cultured with uniaxial cyclic stretch.[44] The tissue constructs generated from hiPSC-CMs and hESC-CMs were indistinguishable. One week after transplantation onto the hearts of athymic rats, both types of cells had formed grafts that contained human microvessels and were perfused by blood. A long-term large animal study[45] confirmed that when sheets of hiPSC-CMs were transplanted into infarcted swine hearts, the treatment significantly improved the LV remodeling, neovascularization, and contractile function; small numbers of transplanted hiPSC-CMs were still detectable 8 weeks later. Notably, the efficiency of differentiation and subsequent function of iPSC-derived cells may be influenced by epigenetic factors that the iPSCs retain from their tissues of origin.[155–157] Transplantation of hiPSC-derived vascular cells (ECs and SMCs) has been proven to enhance cardiac function and promote angiogenesis in MI swine model.[46] For this reason, vascular cells were typically added into hiPSC-CMs containing EHTs to aid in vascularization of ETHs in vivo and hence, survival of transplanted cells.[8,47] However, in a recent report addition of ECs did not increase the survival of human iPSC-based EHTs transplanted onto guinea pig hearts.[47]

Notably, Menasche and associated have reported the first clinical trial of patients treated with a human ESC-derived CM graft, which suggested safety and efficacy of using human pluripotent stem cell product.[3,43]

Exosomes

In the treatment of heart disease, standard therapies fail to recover the injured myocardium and do not alleviate the need for heart transplantation. Stem cell therapies of the heart demonstrated only modest improvements in ejection fraction and clinical outcomes. Although the primary use of stem cells was to form de novo CMs,[21] their clinical benefits despite poor retention have led to the discovery that implanted stem cells exert their clinical benefit largely via their secretome.[127] The paracrine activity of transplanted cells is mediated, in part, by exosomes, nano-sized (<100–150 nm diameter) regulatory vesicles that are secreted by most types of cells[7,158] and contain a variety of proteins and RNAs.[159] Exosomes are used by the cells to deliver collections of bioactive components as a means of intercellular communication. In contrast to individual secreted factors, exosomes provide a unique method for cells to deliver a packaged set of bioactive components, the collection of microRNAs in their cargo. It is intriguing to think therapeutic delivery system using the circulation system with microRNAs mimic as cargo and synthetic exosome as vehicle. Exosome secretion is a multistep process requiring both transporter molecules and ATP,[160] and secreted exosomes can be readily collected from the culture medium.[161–163]

Because of their ability to regulate a broad range of cellular behaviors, exosomes have begun to be used as treatments in a variety of diseases. Importantly, exosomes from different cell types or cells in different states can carry vastly different sets of microRNAs, leading to a variety of effects. Recent research efforts have focused on leveraging exosomes as a powerful therapeutic tool. The results from pilot studies conducted by Sahoo et al[164] indicated that exosomes secreted by human CD34+ BM cells increase the viability, proliferation, and angiogenic activity of ECs, whereas Khan et al[165] have shown that murine ESC exosomes promote angiogenesis and CM survival, reduce fibrosis, and improve cardiac function after delivery to infarcted mouse hearts.

Exosomes also appeared to enhance the survival, proliferation, and angiogenic potential of cardiac cells, and to attenuate ischemic injury in both small and large animal models.[7,165,166] For example, exosomes from human cardiospheres attenuated LV remodeling and improved cardiac function when tested in swine models of both acute and chronic MI.[166] Collectively, these observations suggest that even in the absence of transplanted cells, exosomes may be useful for the treatment of heart disease, but more extensive investigations are needed to determine the optimal cellular source of exosomes for cardiovascular therapy and which molecular contents are responsible for any observed beneficial effects.

Exosomes are thought to signal directly to the myocardium and the supporting cells including fibroblasts and ECs, altering their responses to ischemic injury. For maximum effects, it will be important to extend the current studies to exosomes secreted by matured human iPSC-CMs, which may contain distinct microRNAs important for regulation of cardiac-specific processes. Furthermore, similar to the key limitation of cell-based therapies, exosome-based therapies of the heart could be improved by using biomaterial systems to provide sustained delivery of exosomes, with retention times extending to several weeks.

Manufacturing

Cardiac Cell Spheroids

During embryonic development, cells aggregate into clusters that facilitate interactions necessary to drive tissue differentiation and maturation. Cultured hiPSC-derived cardiac cells tend to self-associate, forming spheroids that can contain mixtures of CMs, CPCs, and many other cell types. Nguyen et al[167] showed that the proportion of CMs in an initially heterogeneous population of hiPSC-derived cardiac-cell spheroids increased from 10% to 40% to 80% to 100% over a 7-day culture period and that the cells became more structurally mature. Furthermore, when spheroids of murine or human CPCs

derived from hESCs or hiPSCs were injected into mouse hearts,[168–171] the engraftment rate was high, especially when the spheroids were encapsulated in an alginate/chitosan micromatrix before injection.[168] The injected cells differentiated into CMs and ECs, and the treatment was associated with improvements in cardiac function, scar size, and angiogenesis. Spheroids are also compatible with bioprinting technology. Ong et al[171] used a 3D bioprinter to assemble a cardiac patch from spheroids of hiPSC-CMs, human umbilical vein ECs, and human adult ventricular fibroblasts; these patches displayed spontaneous beating and ventricular-like action potentials and remained stably engrafted with evidence of vascularization 1 week after transplantation onto rat hearts.

Scaffolds

Cardiac tissues can be constructed (1) from one or more sheets of cells that were grown in monolayers and released, intact, from the culture surface,[172] (2) by seeding the cells into the extracellular matrix of decellularized myocardial tissue[173,174] or, most commonly, (3) by suspending cells in a scaffold. Materials such as collagen,[175] fibrin,[27] and polyglycolic acid[176] have been widely investigated, and some novel compositions (eg, silk fibroin and hyaluronic acid,[177] alginate/chitosan polyelectrolyte complexes[178]) have recently been introduced. For nanofibrous scaffolds, the fiber diameters can be controlled via electrospinning,[179,180] a technique that enables manufacturing of scaffolds with mechanical properties that closely mimic the native extracellular matrix.[181] Electrospun scaffolds have porous architectures with a high surface area to volume ratio, to promote cell adhesion and migration.

Microfabricated Systems

Adopting techniques from the materials microfabrication industry has transformed the way cardiac patches are engineered using both synthetic and natural biomaterials. Scaffold architectures can be precisely controlled using these techniques to effectively guide the orientation of CMs. We now have methods to generate anisotropic tissue structures similar to the native myocardium even in the absence of specific physical cues such as electrical or mechanical stimulation. Engelmayr et al[105] pioneered the use of microfabrication in creation of 3D cardiac tissue engineering scaffolds. They created an accordion-like scaffold using laser boring of a 250 µm thick poly(glycerol sebacate) layer. The accordion-like honeycomb was designed by overlapping two 200 by 200 µm squares at an angle of 45°. The pore walls and struts were ≈50 µm thick. The scaffolds were pretreated with cardiac fibroblasts followed by the seeding of enriched neonatal rat CMs. During pretreatment, rotating culture was used, whereas static culture was used on CM seeding. 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.

The Bursac laboratory investigated another method to replicate in vitro the microstructure of heart tissue defined by diffusion tensor magnetic resonance imaging. From the 3D reconstructed image, a specific 2D plane was chosen and the cardiac fiber directions on this plane were converted into soft-lithography photomasks, and later into fibronectin-coated polydimethylsiloxane sheets. By adjusting the width and spacing of fibronectin lines, the cell elongation, distribution of gap junctions, and cell distribution could be altered without affecting cell direction. This approach enabled systematic studies of structure-function relationships in healthy and structurally remodeled hearts.[182,183] In other studies, a high degree of anisotropy, correlating with high conduction velocities in the longitudinal direction (≈35 cm/s), could be achieved for neonatal rat CMs cultured on micromolded poly(ethylene glycol) hydrogels with submicrometer features, by alternating 800 nm by 800 nm groves and ridges. Interestingly, the submicrometer features forced the cells to align focal adhesions along the grove/ridge direction, and the cytoskeleton followed.[184]

Using injection-molding of a polymer poly(octamethylene maleate (anhydride) citrate), known as POMaC, through microfabricated polydimethylsiloxane molds, we have developed new shape memory scaffolds that enable injection of fully functional tissues.[185] Cardiac patches made using these shape memory scaffolds (1 cm×1 cm) were delivered through an orifice as small as 1 mm, recovering their initial shape on injection without affecting CM viability and function. In a subcutaneous rat model, injection of cardiac patches was equivalent to open surgery in terms of vascularization, macrophage recruitment, and cell survival. The cell-polymer patches significantly improved cardiac function on MI in a rat, compared with untreated controls. Successful minimally invasive delivery of human cell-derived patches to the epicardium, aorta, and liver in a porcine model were also achieved. The scaffold's shape memory was because of the anisotropic microfabricated mesh design; isotropic meshes could not open up after injection. To further increase the polymer elasticity for cardiovascular applications, we synthesized a new polymer, poly(octamethylene maleate (anhydride) 1,2,4-butanetricarboxylate; 124 polymer).[186] This polymer supported rat cardiac cell attachment in vitro and decreased fibrous capsule formation in vivo, compared with poly(lactic acid), a common polymer in Food and Drug Administration approved devices.

Using a new 3D stamping techniques and master molds of precise shape, we were able to develop generic, polymer-based vasculature (termed AngioChip) suitable for organ-on-a-chip engineering, tissue engineering, and direct surgical anastomosis. AngioChip supported the assembly of parenchymal cells on a mechanically tunable matrix surrounding a perfusable, branched, 3D microchannel network coated with ECs. The design of AngioChip decoupled the material choices for the engineered vessel network and for cell seeding in the parenchyma, enabling extensive remodeling while maintaining an open vessel lumen. The incorporation of nanopores and microholes in the vessel walls enhanced permeability and permitted intercellular crosstalk and extravasation of monocytes and ECs on biomolecular stimulation.[78]

Relying on the versatile 3D stamping technique, we invented polymer scaffolds with a microfabricated hook-and-loop system that enables facile assembly and disassembly of individual cell layers, thereby enabling patterned coculture in 3D. The assembly of Hook-in-Tissue preserved the guided cell alignment realized by the topographical features in the 2D scaffold mesh and allowed for the instant establishment

of coculture conditions by spatially defined stacking of cardiac cell layers or through EC coating. The assembled 3D cardiac tissue constructs were immediately functional as measured by their ability to contract in response to electrical field stimulation. On-demand tissue disassembly was demonstrated while preserving the structure, physical integrity, and beating function of the individual layers.[187] These manufacturing approaches are summarized in Figure 3.

Cell and Tissue Implantation

Peripheral Systemic Delivery

Approaches for delivering cells and tissues fall into 2 general categories: systemic delivery versus local delivery. For all methods, low engraftment rate remains a critical issue.[2,4] Intravascular or intraventricular delivery generally results in recirculation and global distribution of cells to important organs such as lung, spleen, or BM. Despite inability to localize cells at the site of injury, some beneficial effects have been observed. Intravenous allogeneic human BM-MSCs have been proven to be safe in patients after acute MI in a randomized, double-blind, placebo-controlled, dose-ranging study.[190] Comparing to placebo, hMSC treatment was beneficial in terms of the improved LV function and the reversal of LV remodeling. It was somewhat surprised to note that intraskeletal muscle injections of nonautologous BM-MSCs were able to improve LV function in hamsters with heart failure.[191] Systemic delivery that suggests cytokine effects is less invasive, but mechanisms of action remain to be deciphered.

Local Myocardial Injection

Intramyocardial injections of cell suspensions or scaffold-forming biomaterials have been the most common approaches tested in preclinical and clinical studies.[4,192] Local delivery can be achieved by open chest surgery[17,193] or by catheter-based transendocardial injections, when the cells are typically injected into the border zone via an infarcted related reopened coronary artery.[194] EHTs, including scaffold-based tissue patches, cell sheets, and decellularized matrix were typically sutured to the epicardium in the injured area.[192] Although these EHT patches have been safe and effective in clinical trials (Table; clinical trials by Menasche and Sawa), the requirement of open-heart surgery to suture the hEHT on the surface of the

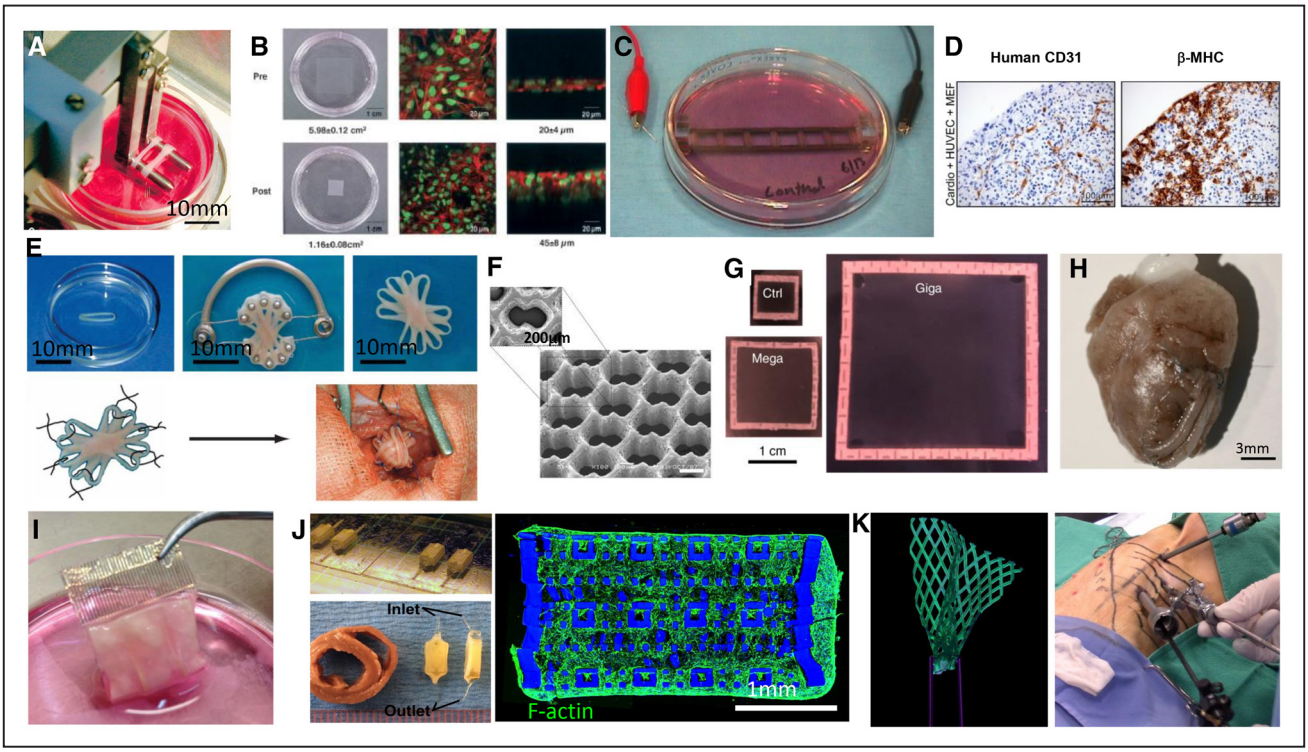

Figure 3. Overview of approaches. A, Cyclic mechanical stimulation enables engineering of differentiated neonatal rat cell-based engineered heart tissue (EHT). Reprinted from Zimmermann et al[56] with permission. Copyright ©2002, the American Heart Association. **B**, Engineered cardiac tissue can be created by stacking of cell monolayers. Reprinted from Shimizu et al[49] with permission. Copyright ©2002, the American Heart Association. **C**, Electrical field stimulation enhances cell assembly. **D**, Cell triculture enhances cardiac cell presence and vascularization in vitro and in vivo. Reprinted from Stevens et al[51] with permission. Copyright ©2009, the National Academy of Sciences of the United States of America. **E**, Auxotonic mechanical stimulation yields a rat-cell–based EHT that improves global and local cardiac function on MI in rats. Reprinted from Zimmermann et al[57] with permission. Copyright ©2006, Springer Nature. **F**, Microfabricated elastomeric scaffold introduce physiological mechanical anisotropy into engineered cardiac tissue. Reprinted from Engelmayr et al[105] with permission. Copyright ©2008, Springer Nature. **G**, Scale-up of cardiopatches to clinically relevant dimensions. Reprinted from Shadrin et al[188] with permission. Copyright ©2017, the Authors. **H**, Human induced pluripotent stem cell-based cardiac patch enhances function of a guinea pig heart. Reprinted from Weinberger et al[47] with permission. Copyright ©2016, the American Association for the Advancement of Science. **I**, An instrumented cardiac patch with built-in electrodes for recording and pacing. Reprinted from Feiner et al[189] with permission. Copyright ©2016, Springer Nature. **J**, Vascularized microfabricated scaffold enables engineering of thick cardiac patches and direct anastomosis. Reprinted from Zhang et al[78] with permission. Copyright ©2016, Springer Nature. **K**, Injectable cardiac patches based on elastomeric scaffolds allow minimally invasive delivery of human patches into large animals. Reprinted from Montgomery et al[185] with permission. Copyright ©2017, Springer Nature.

Table. **Representative Studies of Cardiac Cell Therapy**

Cell Type	Cell Source (Trial Number)*	Cell Number	Delivery Route	Disease or Myocardial Injury Model	Follow-Up	Summary/Observation		Publication Year
						Heart Function	Others	
Skeletal myoblast	Autologous skeletal muscle from dogs[11]	$0.5–1.5\times10^6$	IM	Cryoinjury in dogs	14 wk	NA	Survival of skeletal myoblasts within cardiac scar area of injured heart at 6–8 wk but not at 14 wk after cell injection	1992
	Mouse C2C12 cells[12]	$4–10\times10^4$	IM	No injury in mice	3 mo	NA	Survival of skeletal myoblasts in normal heart at 3 mo after cell injection	1993
	Autologous skeletal muscle from rabbits[13]	1×10^7	IM	Cryoinjury in rabbits	6 wk	Improved PRSW	Engraftment of skeletal myoblasts improved cardiac function	1998
	Autologous skeletal muscle from patients[14]	8×10^6	IM (after coronary bypass)	MI in patient (n=1)	5 mo	Improved LVFS	First clinical study of skeletal myoblast for myocardial repair	2001
	Skeletal muscle from newborn rats[15]	5×10^6	IM	MI in rats	26–30 d	NA	Grafted skeletal myoblasts displayed contractile activity but lack of electricomechanical coupling with host myocardium	2003
	Autologous skeletal muscle from mice[16]	$1–5\times10^5$	IM	Cryoinjury in mice	2 wk	Improved LVEF	Engraftment of skeletal myoblasts genetically engineered to express Cx43 conferred protection against induced VT	2007
	Autologous skeletal muscle from patients (NCT00102128)[17]	4 or 8×10^6	IM (after coronary bypass)	MI in patients (n=97)	3 mo	No improvement of heart function comparing to bypass alone	Engraftment of skeletal myoblasts is associated with increased risk of arrhythmia	2011
	Autologous skeletal muscle and bone marrow cells from patients (UMIN000001859)[18]	$2.7–30\times10^7$	IM	Ischemic cardiomyopathy in patients (n=4)	6–12 mo	Improved LVEF in 3 patients	No sustained VT	2011
	Autologous skeletal muscle from patients (UMIN000008013)[19]	3×10^8	Cell sheet	Ischemic cardiomyopathy in patients (n=7)	26 wk	Improved LVEF	Improved patient status (NYHA functional class, 6-min walk distance)	2015
	Autologous skeletal muscle from patients (UMIN000000660)[20]	$4.5–7.5\times10^8$	Cell sheet	Dilated cardiomyopathy in patients (n=4)	3 mo	Improved LVEF in 3 patients	Transplantation of skeletal myoblasts reduces cardiac hypertrophy	2017
Primary cardiac cells	Fetal mouse CMs[21]	$1–10\times10^4$	IM	MI in mice	Up to 19 d	NA	Grafted fetal CMs survived and formed intercalated discs with host myocardium	1994
	Neonatal rat CMs[22]	4×10^7	Gelatin mesh	MI in rats	5 wk	No significant change	Surviving grafts enhanced angiogenesis	1999
	Neonatal rat CMs[23]	3×10^5	Alginate scaffolds	MI in rats	2 mo	No significant change	Surviving grafts reduced LV dilation and enhanced angiogenesis	2000

(*Continued*)

Table. Continued

Cell Type	Cell Source (Trial Number)*	Cell Number	Delivery Route	Disease or Myocardial Injury Model	Follow-Up	Summary/Observation		Publication Year
Bone marrow hematopoietic stem cells (BM-HSC)	Mouse BM-HSCs ($CD31^{-/low}$, c-kit^+, Sca-1^+)[24]	2000	IV	MI in irradiated mice	4 wk	NA	Transplanted cells migrated into ischemic myocardium and differentiated to cardiomyocytes (at low rate, ≈0.02%) and endothelial cells (3.3%)	2001
	Human BM-derived CD34+ endothelial progenitor cells[25]	1×10^5	IM	MI in rats	4 wk	Improved LVFS	Increased angiogenesis	2003
Mesenchymal stem cells (MSCs)	Rat BM-MSCs overexpressing Akt[26]	$2.5–5\times10^6$	IM	MI in rats	3 wk	Improved LVSP and LV –dp/dt	Reduced infarction size and improved LV remodeling	2003
	Swine BM-MSCs[27]	5×10^6	Fibrin patch	MI in swines	≈18 d	Improved systolic wall thickness	Enhanced angiogenesis	2004
	Human amnion-derived MSCs[28]	1×10^6	IM	MI in rats	2 mo	NA	Engrafted cells differentiate into cardiomyocyte-like cells	2005
	Mouse adipose-derived MSCs[29]	2×10^5	IM	MI in rats	30 d	Improved LVEF	Improved LV remodeling. Grafted cells differentiated into smooth muscle cells and endothelial cells	2006
	Human placenta-derived MSCs[30]	1×10^6	IM	MI in rats	4 wk	Improved LVEF and LVFS	Improved LV remodeling and enhanced angiogenesis	2007
	Human BM-MSCs[31]	1×10^6	Fibrin hydrogel in decellularized sheet of human myocardium	MI in rats	4 wk	Improved LVFS	Enhanced angiogenesis in infarction area but not normal myocardium	2011
	Human umbilical cord matrix-derived MSCs[32]	5×10^6	IM	MI in rabbits	30 d	Improved LVEF and LVFS	Improved LV remodeling	2011
	Human BM-MSCs (NCT01087996)[33]	Allogeneic vs autologous BM-MSCs at 3 different doses (2×10^7, 1×10^8, 2×10^8)	TEM	Ischemic cardiomyopathy in patients	13 mo	No difference between 2 cell types	Low-dose MSCs (20 million cells) produced greatest reductions in LV volumes and increased EF. Allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions	2012
	Human umbilical cord lining-derived MSCs[34,35]	$2–2.5\times10^6$	Fibrin patch	MI in rats	4 wk	Improved LVEF and LVFS	Improved LV remodeling and enhanced angiogenesis	2013
Embryonic stem cell (ESC)-derived cells	Purified mouse ESC-derived CMs (mESC-CMs)[36]	1×10^4	IM	Dystrophic mdx mice	7 wk	NA	mESC-CMs (>99% pure) formed stable intracardiac graft in mdx recipient mice	1996
	Human ESC-derived CMs (hESC-CMs)[37]	1.5×10^6	IM	MI in rats	2 mo	Improved LVFS	hESC-CMs engrafted in MI rat heart and improved LV remodeling	2007

(*Continued*)

Table. Continued

Cell Type	Cell Source (Trial Number)*	Cell Number	Delivery Route	Disease or Myocardial Injury Model	Follow-Up	Summary/Observation		Publication Year
Embryonic stem cell (ESC)-derived cells (Continued)	Human ESC-CMs[38]	1×10^7	IM	MI in rats	4 wk	Improved LVFS	Prosurvival cocktail enhances the therapeutic efficacy of hESC-CMs	2007
	Human ESC-CMs[39]	1×10^8	IM	Cryoinjury in guinea pigs	4 wk	Improved LVEF	Engraftment of hESC-CMs electrically coupled with host myocardium and reduced the incidence of both spontaneous and induced VT	2012
	Human ESC-CMs[40]	1×10^9	IM	MI in monkeys	Up to 3 mo	No significant change	hESC-CMs remuscularized infarcted heart. Large engraftment is associated with higher frequency of arrhythmia	2014
	Human ESC-CMs[41,42]	2.5×10^6	Engineered heart muscle ring	MI in rats	220 d	No significant changes of LVEF. Preserved heart function revealed by tagged magnetic resonance imaging	Transplantation of EHTs increased engraftment rate and led to long-term survival and progressive maturation of hESC-CMs	2015
	Human ESC-derived Isl-1+ SSEA-1+ cells (NCT02057900)[43]	4×10^6	Fibrin patch	Ischemic HF in diabetic patients (n=1)	3 mo	Improved LVEF	No complications (eg, episodes of VT). Improved 6-min walking test, and increased wall motion and NYHA functional class	2015
Induced pluripotent stem cells (iPSC)-derived cells	Human iPSC-derived CM (hiPSC-CMs)[44]	2×10^6	A mixture of gel (collagen and basement membrane extract) and cells (2 million hiPSC-CMs or hESC-CMs, 1 million HUVECs, and 1 million MSCs or MEFs)	Normal rats	1 wk	NA	Both types of EHTs (hiPSC-CMs vs hESC-CMs) formed grafts that contained human microvessels and were perfused by the native circulation	2011
	hiPSC-CMs[45]	Unknown	Cell sheet	MI in swines	8 wk	Improved LVEF	Poor engraftment rate. Improved LV remodeling and function because of paracrine effects	2012
	Human iPSC-derived vascular cells (hiPSC-VCs)[46]	4×10^6 vascular cells (ECs and SMCs)	In a fibrin patch	MI in swines	4 wk	Improved LVEF	Increased angiogenesis via recruitment of c-kit+ cells to border zone	2013
	Human iPSC-derived trilineage of cardiac cells (CMs:ECs:SMCs)[8]	6×10^6 (CMs:ECs:SMCs=2:1:1)	IM and injection site covered by IGF-containing fibrin patch	MI in swines	4 wk	Improved LVEF	Improved myocardial metabolism and angiogenesis. Reduced infarct size, ventricular wall stress, and apoptosis. No ventricular arrhythmias	2014

(*Continued*)

Table. Continued

Cell Type	Cell Source (Trial Number)*	Cell Number	Delivery Route	Disease or Myocardial Injury Model	Follow-Up	Summary/Observation		Publication Year
Induced pluripotent stem cells (iPSC)-derived cells (Continued)	Human iPSC-derived cardiomyocytes and endothelial cells[47]	5×10^6 (CMs) and 2×10^6 (ECs)	Fibrin patch	MI in guinea pigs	4 wk	Improved FAC	EHTs displayed large engraftment and electrically coupled with host myocardium	2016

BM indicates bone marrow; CMs, cardiomyocytes; ECs, endothelial cells; EDV, end-diastolic volume; EHT, engineered heart tissue; ESV, end-systolic volume; FAC, fractional area change; HF, heart failure; IGF, insulin-like growth factor; IM, intramyocardial injection; IV, intravenous; LV −dP/dt, left ventricular rate of pressure decay; LV+ dP/dt, LV rate of pressure rise; LVDP, left ventricular developed pressure; LVEDD, left ventricular endo-diastolic dimension; LVEDP, improved left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; LVESD, left ventricular endo-systolic dimension; LVFS, left ventricular fraction shortening; LVSP, left ventricular systolic pressure; MEF, murine embryonic fibroblast; MI, myocardial infarction; NA, not applicable; NYHA, New York Heart Association; PRSW, preload recruitable stroke work; SMCs, smooth muscle cells; STEMI, ST-segment–elevation myocardial infarction; TEM, transendomyocardial injection; and VT, ventricular tachycardia.

*Unique identifiers registered in https://www.clinicaltrials.gov.

recipient heart could significantly reduce its application in patients with severe heart failure, or institutions not equipped to perform such operations.

Delivery Time

Although several clinical trials were designed to study the impact of cell delivery time on therapeutic effects, thus far, the ideal timing for cell therapy remains unknown, and it also may vary case by case.[195,196] Recently, preclinical studies from the Bolli laboratory (Table) showed that repeated intraventricular cell injections guided by echocardiography resulted in a greater cumulative effect on LV function and remodeling than a single cell injection.[197–199] Several clinical trials were initiated to evaluate the efficacy and safety of a single dose versus repeated dose regimen of cell delivery in patients with acute MI (RELIEF/URL: http://www.clinicaltrials.gov. Unique identifier: NCT01652209), heart failure (REPEAT/URL: http://www.clinicaltrials.gov. Unique identifier: NCT01693042, REMEDIUM/URL: http://www.clinicaltrials.gov. Unique identifier: NCT02248532), and dilated cardiomyopathy (REMEDIUM/URL: http://www.clinicaltrials.gov. Unique identifier: NCT02248532).

Animal Models

Immunocompromised rodents including NOD/SCID (nonobese diabetic/severe combined immunodeficiency) or NOD/SCID gamma mice and athymic nude rats were commonly used to study the engraftment and function of transplanted human cells and cardiac tissues in injured hearts. Human immune systems can also be established in immunodeficient mice via transplantation of human hematolymphoid cells, which is called humanized mice.[200] Such humanized mice were used to test the immunogenicity of transplanted human cells. Although rodents are the most practical animal model for studies of efficacy and mechanisms of cardiac repair, they are not representative of human physiology. Concerns have been raised that rodent hearts are anatomically and physiologically very different from human and that large animal models such as porcine or NHPs (nonhuman primates) are more relevant for preclinical studies.[7,40,45,201,202] The similarity of human and porcine coronary anatomy and electrophysiology renders the porcine models suitable for examining the efficacy and mechanisms of cardiac cell therapy.

Mechanisms of Action

The goal of cardiac tissue transplantation, at least for constructs containing CMs, is to replace fibrotic scar tissue with electromechanically functional, vascularized cardiac muscle. The results from investigations in mice, rats, guinea pigs,[39,47] swine,[8] and nonhuman primates,[40,203] suggest that the benefits associated with transplanted ESC- and iPSC-based tissues may be accompanied by some degree of remuscularization. Recently, we reported that overexpression of a cell cycle gene, CCND2 (cyclin D2), induces proliferation of hiPSC-CMs, significantly enhancing the potency for myocardial repair, as evidenced by remuscularization of injured myocardium in mice.[204] On the contrary, nonviable (irradiated) EHTs[41] and EHTs containing hiPSC-ECs and hiPSC-SMCs but no CMs[46] have also been associated with significant improvements in cardiac function after transplantation into infarcted mammalian hearts. Conclusive evidence that human MSCs may be able to differentiate into functional CMs[205] is still lacking. Much of the benefit associated with cardiac tissue transplantation likely evolves from paracrine activity of transplanted cells. MSCs can promote vasculogenesis by differentiating into endothelial and SMCs. Engrafted hiPSC-derived cardiac cell lineages seem to activate the cell cycle of endogenous CMs,[206] while enhancing angiogenesis, reduces scar bulging, infarct border zone LV wall stress and CM overstretch, which in turn is accompanied by a significant reduction of border zone apoptosis and infarct size, improving the LV ejection fraction and myocardial ATP turnover.[46]

Roadblocks, Prospects, and Challenges

Roadblocks

Low Engraftment Rate

Although human MI can result in a loss of as many as 1 billion CMs, current methods for cell delivery lead to grafting of only 0.1% to 10% of injected cells, a few hours after transplantation.[4] The delivery of an engineered cardiac tissue patch sutured on the surface of the infarct bed results in a 10-fold higher engraftment rate as compared with the direct myocardial injection of cells.[4,7,41,48] Our recent reports demonstrate the potential for scalable fabrication of large and thick, clinically-sized tissue patches with near adult levels of cardiac electrical

and mechanical function.[7] We demonstrated that a 3D dynamic culture of hPSC-derived CMs, ECs, and SMCs can achieve significant electrophysiological and mechanical maturation of the resulting tissue construct, which starts to resemble the native myocardium in terms force generation, action potential, and impedance. However, the action potential conduction velocity is only reaching 25% of that in the native myocardium, raising concerns about the reentry arrhythmia.

Once the low engraftment rate is overcome, the next major problem we will encounter is the integration. The myocytes in the graft can act as pacemaker cells causing arrhythmia. The different speeds of cardiac electrophysiological signals that pass through the myocardium with or without graft particularly pass through the fibrotic interface between the host myocardium and the graft can also cause the reentry arrhythmia. Therefore, the integration of graft will likely be a major problem once hEHT graft is significantly large, which can only be examined successfully in a large animal model.

Lack of Electrical and Mechanical Integration Between the Patch and the Host

Lethal arrhythmia caused by disordered electrical activity in the heart remains a critical concern associated with cardiac cell therapy. The propagation of electrical waves through the heart can be perturbed by anatomic and functional defects, leading to aberrant waveforms that are difficult to study in animal models and manage in patients.

Animal studies have shown some positive effects of implanted cardiac cells and patches but have been complicated by the emergence of arrhythmia.[40] An important study of heart repair by immature hESC-CMs in a nonhuman primate used clinically relevant cell numbers (1 billion hESC-CMs per heart), and a clinically relevant model of heart injury (MI followed by reperfusion).[40] At 2 weeks after cell delivery by direct injection into myocardium, the regions with hESC-CMs were perfused by blood, and the electromechanical junctions were established between the graft and host myocytes. In contrast to small animal models, nonfatal ventricular arrhythmias were observed in hESC-CM–engrafted primates. It was concluded that hESC-CMs can remuscularize the infarcted primate heart, however, the potential arrhythmic complications will need to be overcome before translation into patients.

Immunologic Issues

Heart therapy using autologous cells derived from the patient being treated has been considered a highly advantageous option as any immunologic issues are completely avoided. Autologous MSCs were widely studied in various types of tissue regeneration, including the heart. The advances in derivation of CMs from iPSCs offer a new and potentially powerful source of autologous cells with capacity to rebuild the heart muscle and vasculature lost to MI.[8,44–47] However, the need for detailed characterization and quality control of each batch of cells being implanted requires additional time for cell evaluation and complicates clinical protocols. Certain cell types, such as MSCs derived from BM or fat aspirates were shown to be anti-inflammatory and immunoprivileged[137] and have been used in hundreds of clinical trials. Comparisons between autologous and allogeneic MSCs have not shown differences in donor-specific immune reactions.[33] Further studies revealed that the immune-modulatory properties of MSCs can be attributed to multiple factors including TGF-β, prostaglandin E2, interleukin-10, interleukin-1 receptor antagonist, interleukin, and leukocyte inhibitory factor that act in concert.[207,208] Recent advances in derivation of CMs from iPSCs offer a source of autologous cells with capacity to rebuild the heart muscle and vasculature lost to MI.[8,44–47] However, the studies of the safety and therapeutic value of these cells are at the beginning, and many issues will need to be resolved before either autologous therapy (that will require gene editing for certain patients with genetic diseases) and allogeneic therapy (that will require immunosuppression) become a clinical reality.

Prospects

Instrumented Patches

Cardiac patches based on synthetic scaffolds are becoming highly sophisticated. They are now able to stimulate and record electrical activity of the cardiac tissues relying on built-in electrodes. Feiner et al[189] engineering an electronic cardiac patch composed of gold electrodes embedded in a thin SU-8 mesh. An electroactive polymer capable of releasing proteins or small molecules on electrical stimulation was loaded onto select electrodes. This sensor was then deposited with electrospun polycaprolactone-gelatin fibers, thus creating an electrospun biomaterial-electrode patch. The patch was then seeded with neonatal rat CMs and cardiac fibroblasts. This device supported homogenous distribution of cells that interacted with the electronic fibers. Finally, an important function of the electronic patch is the ability to remotely control the tissue contraction and propagation.

Layers in Myocardium and Engineering of Whole Ventricles

By gross anatomy, the LV is in the shape of a cone and embedded in the base are the mitral (inlet) and aortic (outlet) valves.[209] On a smaller scale, the myoarchitecture of the ventricular wall consists of 3 layers—superficial, middle, and deep—with the varying orientation (+60° to −60°) of the myocardial strands transmurally. These layers are not separated by cleavage planes or fibrous tissue, but rather form a continuum of beating CMs. The layers generate the pressure needed to expel the blood, which occurs by the dual action of (1) rotation to reduce the diameter of the ventricle and (2) contraction to pull the base toward the apex and shorten the long axis.[210] Although the twitching heart ventricles have been bioprinted, the physiological twisting motion that enables pumping of blood,[210] has not been reproduced in a tissue engineering system. By ECM patterning in 2D, highly ordered myofibril strands with defined contractile orientation and action potential propagation were generated.[211,212] Structural design and stroke kinematics (contraction/relaxation) of a jellyfish were replicated by cultivation of neonatal rat CM on fibronectin micropatterned thin films of polydimethylsiloxane.[211] Current efforts rely on electrospun scaffolds with complex fiber orientation in 3D.[213] Ultimately, these efforts may lead to a construction of a whole ventricle. Early work proved that it is possible to create a sac-like structure by molding of collagen hydrogels using agarose molds.[214] More recently a ventricle like structure formed by

freezing chitosan in a 3D printed CAD model of the LV.[215,216] These early ventricles were able to contract, but contain mostly isotropic orientation of neonatal rat CMs.

Epicardium and Endocardium

It remains to be determined how and where to apply cardiac patches for optimal therapeutic benefit. The ventricular wall is comprised of the epicardium, the outer epithelial lining of the muscle; the myocardium, composed of working CMs and support cells such as fibroblasts; and the endocardium, consisting of a specialized endothelial lining that is in contact with the blood filling the ventricle. The majority of cell therapy approaches focus on the direct injection of a cell suspension into the myocardium using either catheters or open chest surgery.[196,217] These approaches are inherently limited by the very small volume of free space available for cell engraftment. The cell density in the myocardium is extremely high (10^8 cells/cm^3[218]); on injury such as MI, the dead cells are replaced by a dense collagenous scar leaving little space for the applied cells to engraft and making it impossible to implant a cardiac patch into the myocardium. This leaves the epicardium and the endocardium as the possible options. On the endocardial side, patch deployment faces significant risks including thrombosis and embolization. This leaves the epicardium as a safe and an accessible option. However, the presence of mesothelial cells at the epicardium was reported to prevent patch integration, necessitating methods that promote migration of these cells that would enable ultimate patch integration.

Summary and Future Direction

MI often induces LV remodeling, with an initial period of hemodynamic stability that is followed by the development of severe LV dysfunction, hypertrophy, and congestive heart failure, which is one of the most significant clinical problems. With the recent developments in hiPSC technology, biomaterials, and tissue engineering, it is foreseeable that in the near future the clinically relevant size of functional human myocardial tissue patches (hMTP) will become available for patients experience MI. However, to make the engineered hMTP to be a surgical alternative for heart failure patients, requires the graft vascularization and integration. The roadblock of integration could only be overcome if large hMTP grafts can be successfully tested in large animal models allowing mechanistic deciphering and demonstrating functional integration. We will need to engineer new hPSC lines that can generate all cardiac cell types with faster rates of electrical and calcium signal transduction. As sciences continue to converge in cell and molecular biology, biomaterials, and tissue engineering, and with complementary progress in minimally invasive surgical robotics technology, the engineered human EHT patches will become an increasingly feasible option for the treatment of patients with heart failure.

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Disclosures

None.

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