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Tissue Engineering Approaches to Heart Repair

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Abstract

The heart is a large organ containing many cell types, each of which is necessary for normal function. Because of this, cardiac regenerative medicine presents many unique challenges. Because each of the many types of cells within the heart has unique physiological and electrophysiological characteristics, donor cells must be well matched to the area of the heart into which they are grafted to avoid mechanical dysfunction or arrhythmia. In addition, grafted cells must be functionally integrated into host tissue to effectively repair cardiac function. Because of its size and physiological function, the metabolic needs of the heart are considerable. Therefore grafts must contain not only cardiomyocytes but also a functional vascular network to meet their needs for oxygen and nutrition. In this article we review progress in the use of pluripotent stem cells as a source of donor cardiomyocytes and highlight current unmet needs in the field. We also examine recent tissue engineering approaches integrating cells with various engineered materials that should address some of these unmet needs.

Keywords

cardiomyocytes; cardiac progenitors; pluripotent cells; hydrogels; engineered heart tissues

I. Introduction

Despite the promise of regenerative medicine, cardiovascular disease remains the leading cause of death in the United States.¹ Cardiovascular regenerative medicine presents many unique challenges. First, unlike other muscles in the body, the human myocardium possesses only limited cell division² and a limited ability to repair itself after injury. A recent study using apical resection of neonatal mouse hearts suggested that murine hearts have significant regenerative capacity for some days after birth.³ This capacity was lost within the first week of birth, however, and a recent attempt to repeat these studies using a different inbred mouse strain showed only incomplete regeneration of heart tissue.⁴ These results suggest that many as yet unknown factors may impact the regenerative capacity of the young mouse heart. Spontaneous regeneration of human hearts on a scale that would be required to repair a typical myocardial infarction (MI) has not been observed to date. Indeed, it has been suggested that a typical infarct episode in the human heart might damage as many as one

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billion cells,⁵ well beyond even the most optimistic estimates of the heart's ability to replace damaged cells. Carbon-dating experiments indicate that under normal conditions cells within the adult human are renewed at a rate of about 1% per year until age 25 and at only 0.45% per year by age 70,² suggesting that there is minimal turnover of cells in healthy hearts. By contrast, the heart may be able to activate a program of renewal after injury. One study showed significant cell renewal in mouse hearts following pressure overload or infarct⁶; however, this type of spontaneous, functional recovery after a major cardiac event has not been observed in adult human hearts. For these reasons, it has long been suggested that the most feasible approach to cardiac regeneration after MI would be the engraftment of cardiomyocytes or cardiac progenitors that have been expanded *ex vivo* from stem cell populations. Here we review recent progress in the use of both transcription factor-mediated reprogramming within the heart and the isolation of cardiac cells or cardiac progenitors from pluripotent cell types such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). We also examine how bioengineers are using tissue-engineering approaches that involve both cell grafts and hydrogels to improve the integration, differentiation, and survival of cells to be grafted.

II. Characteristics of an Ideal Cell Population for Cardiac Grafts

Cells that are useful as potential donors for cardiac repair should be readily available, be expandable in culture, show an excellent natural ability for self-renewal, and have contractile and electrophysiological characteristics consistent with their roles within the heart. Cells isolated from unrelated donors raise immunological concerns. In addition, the use of human ESCs raises ethical concerns. Because of this, noncardiac contractile cells such as skeletal muscle cells and/or nonpluripotent stem cells derived from adult tissues were long considered to be the most desirable sources of potential donor cells for cardiac repair. The more recent development of protocols to differentiate large numbers of bona fide cardiac cells from iPSCs has overcome these ethical and immunological concerns, while providing hope that these cells may overcome the problems of functional integration and arrhythmias.

Several protocols for the efficient production of cardiac cells from ESCs have been developed in recent years, and these (or slight modifications of them) have proven to be equally effective for the differentiation of both mouse and human iPSCs. Most notably, coculture of human ESCs (hESCs) with the visceral endoderm-like END2 cell line⁷ has induced 20–25% cardiac differentiation, whereas protocols using either carefully timed addition of growth factors⁸ or a combination of growth factor addition and flow cytometry-based selection of cardiac progenitors⁹ have activated 30% and 40–50% of cardiac cells, respectively. These protocols are, in turn, based on a large body of work using frog, chick, and mouse embryos, as well as ESCs, to elucidate the embryology and molecular genetics of heart induction.

III. Studies Elucidating the Molecular Mechanisms of Cardiac Differentiation

The mammalian heart is made up of cells from at least 3 sources. First, multipotent cardiac progenitors that form during gastrulation give rise to the original linear heart tube and are referred to as the first heart field (FHF). In addition, 2 groups of cells that lie outside this

initial heart tube also contribute to the adult heart: the so-called second (or secondary/ anterior) heart field (SHF)^{10–15} and the neural crest.¹⁶ We previously reviewed the embryology and molecular genetics of primary (FHF) induction in detail^{17,18}; however, a few features that are particularly relevant to stem cell differentiation of cardiac cells should be mentioned here. Heart formation is a multistep process that begins with the formation of mesoderm during gastrulation. In all vertebrate embryos and in ESCs the activities of transforming growth factor (TGF)- β family members and Wnts are required to form the mesoderm as cells exit the primitive streak (the dorsal lip in amphibian embryos).^{19–28} Once formed, the mesoderm immediately begins to migrate away from the streak and toward its final position in the embryo, where it will begin to differentiate according to its location within the embryonic axis.^{29,30} When Wnt signals are depleted from the endoderm of early mouse embryos, multiple beating hearts form all along the embryonic axis,³¹ suggesting that there is a broad potential for cardiac formation within the mesoderm of the early embryo. These studies also suggest that Wnt signaling from the endoderm actively represses myocardial formation outside of the normal heart field. Thus the migration of mesoderm away from the primitive streak may serve not only to bring cells into their final positions within the embryo but also to protect the future heart field from Wnt signals that are present in the primitive streak.

This finding is consistent with earlier studies of chicks, frogs, mice, and zebrafish demonstrating that the transition from uncommitted mesodermal cell to cardiac progenitor involves both cell migration away from the primitive streak (or its embryological equivalent) and the presence of signals that inhibit Wnt.^{32–34} In the embryo (and almost certainly in differentiating ESCs) these signals come from the adjacent endoderm. Later, other growth factors including Fibroblast growth factor act on the myocardium to activate cell proliferation.^{17,18} Thus a hallmark of FHF induction across all vertebrates is the transient activation of Wnt and TGF- β signaling to initiate mesoderm formation from the pluripotent epiblast, followed by a period of Wnt inhibition that is necessary for mesodermal cells to adopt a cardiac fate^{35,36} (Figure 1). As with mammalian embryos, a combination of Wnt and TGF- β signaling can be used to activate a primitive streak–like activity from ESCs³⁷, and timely modulation of TGF- β and Wnt signaling have proven to be necessary and sufficient to activate the formation of cardiac progenitors and beating cardiomyocytes from both mouse and human ESCs and iPSCs.^{8,9,14,38–44}

As they proliferate and move toward the heart tube, differentiation of cardiomyocytes from the SHF is delayed through a mechanism that is not fully understood. At about embryonic day (E) 8.5, SHF cells begin to contribute to the growing heart by migration through the arterial and venous poles of the heart. The right and left atria contain derivatives from both the FHF and the SHF. By comparison, the left ventricle develops primarily from the FHF and the right ventricle and outflow tract (OFT) primarily from SHF progenitors.^{13,45,46} Whether there are equivalents to the FHF and SHF when cardiomyocytes are differentiated from ESCs is unclear. Since the major force generating cardiomyocytes of the heart (left ventricular cardiomyocytes) are derived from the FHF, however, understanding the factors that mediate the switch between FHF and SHF development may lead to improved protocols for the *in vitro* differentiation of cardiac cells enriched for the left ventricular fate.

IV. Cardiac Progenitors

When ESCs are differentiated as embryoid bodies (EBs), they readily form cardiac cells but also produce many other cell types. A major area of research over the past decade has therefore been the search for markers that could identify a cardiac-specific progenitor population (Figure 1). This would allow researchers to isolate, using flow cytometry or another selection method, just those cells that had the potential to differentiate as myocardium.

Genetic fate mapping experiments in the mouse indicate that both the FHF and SHF are derived from a progenitor population expressing *Mesp1*. *Mesp1*-expressing mesoderm emerges from the primitive streak of the mouse embryo during early gastrulation (~E6.5)^{47,48} and migrates around the epiblast cylinder, coalescing across the anterior midline to form the cardiac crescent at E7.5. By E8.5 the cardiac crescent has undergone a series of morphogenetic changes to form the beating linear heart tube. Mice lacking both *Mesp1* and *Mesp2* form axial mesoderm at the streak, as assessed by the expression of the pan-mesodermal marker *Brachyury/T*, but these mice do not form the migratory “mesodermal wings”⁴⁹ and as such are devoid of most mesodermal lineages. Only 2–3% of cells within a mouse EB typically differentiate as cardiac. When *Mesp1* is transiently overexpressed in EBs, this percentage increases to as much as 10%, suggesting that *Mesp1* expression encompasses a cardiac progenitor population. Since most mesodermal lineages are marked early on by the expression of *Mesp1*, however, this proved to be too nonspecific to be an effective marker of cardiac progenitors.⁵⁰

Some of the earliest markers of the cardiac primordium are the transcription factors *Nkx2.5* and *Tbx5*. Although there is considerable overlap between the expression patterns of these 2 genes, fate mapping reveals that *Nkx2.5* is expressed in derivatives of both the FHF and SHF,⁵¹ whereas the expression of *Tbx5* is biased (but not exclusive) to the SHF.⁵² Because of this, *Nkx2.5* also was examined as a potentially specific marker for cardiac progenitors. *Nkx2.5*-expressing cells that are isolated either *in vivo* from mouse embryos or *in vitro* from differentiating ESCs are bipotential and give rise to both cardiac and smooth muscle cells. In addition, this progenitor population can give rise to multiple cardiac lineages (based on cell shape and action potential morphology), including atrial, ventricular, and conduction system cells.⁵³

During the cardiac crescent and linear heart tube stages, SHF cells, identified by the expression of *Islet-1 (Isl1)*^{10,12,14,54} and *Fgf1*,¹³ reside outside of the heart tube.¹² Initial genetic fate mapping experiments suggested that cells that express *Isl1* give rise to both the coronary vasculature and multiple cardiac lineages, including cells in the atrium, ventricles, conduction system, and OFT,^{12,55,56} with the majority of cells located in the right atrium and OFT and only a small contribution to the left ventricle.⁵⁵ A recent reassessment of the *Isl1* fate map suggests that *Isl1* is expressed in all cardiac progenitors.^{51,57} Not surprisingly, *Isl1*-expressing cells that are isolated from differentiating hESCs give rise to smooth muscle, cardiac, and endothelial cells.⁵⁴

One marker that has proven to be extremely useful in identifying and isolating cardiac progenitors is fetal liver kinase-1 (Flk-1). Flk-1 is the major receptor for vascular endothelial growth factor A. Because of this, it was thought that it would primarily mark endothelial and hematopoietic lineages. However, Cre-mediated fate mapping in mouse embryos revealed, surprisingly, that Flk-1-expressing cells also give rise to several mesodermal lineages,^{58,59} and Flk-1-expressing cells isolated from differentiating murine ESCs gave rise not only to endothelial cells but also to cardiac and smooth muscle cells.^{60–62} Differentiating ESCs that are sorted using fluorescence-activated cell sorting based on the simultaneous expression of both Flk-1 and the chemokine receptor CXCR4 comprise a cardiopoietic lineage that is largely depleted of endodermal cell types.⁶³ The combination of selection, based on expression of Flk-1 (KDR in humans), and the addition of procardiogenic growth factors ultimately served as the basis for the efficient differentiation of cardiac cells from hESCs.⁹

Unfortunately, of the potential cardiac progenitor markers that have been identified to date, no single marker identifies cardiac and only cardiac lineages. Therefore the isolation of highly purified cardiac cells may require further isolation steps based on several lineage markers. For example, sorting based on the expression of several markers might produce a population that is more enriched by cardiac cells or could be used to isolate specific subpopulations within the myocardium. A potential problem with the protocols currently used to isolate cardiac cells from stem cell sources is that these protocols result in the formation of multiple cardiac sublineages, including atrial, ventricular, and conduction system cells,^{64–66} raising the possibility of arrhythmias if cells with inappropriate electrophysiologies become established within grafts.

V. Progress and New Challenges for Cell-Based Therapies

Recent work by Chong and colleagues⁶⁷ highlighted concerns about the potential for engrafted cells to cause arrhythmias. Their study demonstrated both the progress that has been made in generating donor cardiac cells from pluripotent cell sources and some important new challenges. They showed the feasibility of large-scale production of cardiomyocytes from hESCs that can be frozen in sufficient numbers (1×10^9 cells) and with sufficient subsequent viability for therapeutic applications in humans. When these cells were injected directly into the infarct zone of a nonhuman primate model of MI, these cells were retained within the heart for at least several weeks (and in the one case for up to 3 months) and showed calcium transients that were synchronized with the rest of the myocardium, suggesting functional engraftment.⁶⁷ During these studies, they made 2 observations that highlight the next major challenges for this field. First, recovery of ventricular function was not statistically significant. Although this finding may be because of the small group size, it raises the possibility that these cells lacked some of the mechanical properties of mature ventricular cells. Indeed, myofibril alignment, sarcomere registration, and cardiomyocyte diameter suggested that these cells were not fully mature. Advances in the field of physiological maturation of cardiomyocytes were recently reviewed by Yang *et al.*⁶⁸ Electrophysiological maturation of these cells was not assessed; however, grafts resulted in arrhythmias not observed in sham-injected animals, suggesting either incomplete electrophysiological maturation, scarring, or the presence of nonventricular cardiac cells within the graft.

One of the great unmet needs in the field of cardiac regeneration is to determine the extent to which the characteristics of a cardiomyocyte are determined by its final position within the heart and to what extent they are determined by extrinsic factors. In short, when does a cardiomyocyte “know” that it will be part of the atrium, ventricle, or conduction system? If a cell fated for the atrium is grafted into the ventricle, what happens to it? Does it change its physiological characteristics according to its new position? Does it die? Does it fail to functionally integrate? Or, does it become a potential source of arrhythmias? Early fate maps of chick embryos suggest that atrial and ventricular fates within the myocardium are sorted out before they exit the primitive streak.⁶⁹ This occurs during the midblastula phase in the zebrafish.⁷⁰ Explants of the prospective chick heart field that are grown in isolation differentiate according to their fate. That is, an atrial-specific myosin heavy chain is expressed only in posterior explants and not in anterior explants that are fated to become the ventricle.⁷¹ These data suggest that some degree of lineage determination occurs well before cells differentiate as cardiomyocytes. There is also evidence, however, that the fate of cardiac progenitors remains flexible for some time. For example, explants of the future ventricle could be induced to express atrial markers by treating them with retinoic acid⁷²; when cells fated to the atrium were grafted into the ventricle, they changed their rate of beating.⁷³ These early studies suggested that plasticity was maintained throughout the cardiac progenitor phase and ended only after differentiation as beating cardiomyocytes. The implication for regenerative medicine is that transplanting cardiac progenitors, rather than beating cardiomyocytes, may be more therapeutically beneficial. Although, as discussed above, a progenitor population that gives rise exclusively to cardiomyocytes has not yet been identified.

To date, few groups have attempted to differentiate cells of specific cardiac lineages. The underlying assumption has been that cardiac progenitors function as generic cardiac cells that will develop mature electrophysiologies that are appropriate for their ultimate position within the heart. However, this hypothesis has not been directly tested. It was shown recently that a small-molecule inhibitor of the canonical Wnt/ β -catenin signaling pathway seems to direct cells with high preference to a ventricular fate.^{74–76} Interestingly, other small-molecule inhibitors of Wnt and the protein Dkk1 do not have this effect,⁷⁵ suggesting that our understanding of the molecular genetics of this process is not yet fully understood. The ability to direct myocardial differentiation to specific cardiac cell types may represent an extremely important next step in realizing their full regenerative potential.

VI. Transcription Factor–Mediated Re-Programming

Another area currently being explored in cardiac regenerative medicine is the use of transcription factor–mediated reprogramming of cells. The development of iPSCs,⁷⁷ which demonstrated that essentially any differentiated cell could be restored to a pluripotent state by activating a small number of transcription factors, renewed interest in the concept that any cell might be converted to a different cell type by ectopic expression of the correct combination of transcription factors. Previous work on genes such as *MyoD* and *Pax6* suggested that at least some cell fates might be controlled by single master regulatory genes. Indeed, overexpression of the *Pax6* homolog in drosophila legs and wings was sufficient to induce ectopic eye formation.⁷⁸ Before that, *MyoD* was shown to be capable of converting

fibroblasts to a myogenic state.^{79,80} However, further research quickly demonstrated that few cell fates are controlled by the activity of a single master regulatory gene. Current work in the field of transcription factor–mediated reprogramming is focused on identifying minimal sets of transcription factors that might control given cell fates. With regard to the cardiac fate, overexpression of 3 transcription factors (Gata4, Mef2C, and Tbx5) both in isolated mouse cardiac fibroblasts and *in vivo* can activate many characteristics of cardiomyocytes, including beating, in noncardiomyocytes.^{81,82} Attempts to repeat this work using tail fibroblasts, however, showed only inefficient reprogramming, as well as the absence of spontaneous action potentials and a contractile phenotype.^{81,83} In addition, this combination of transcription factors did not induce full reprogramming in human fibroblasts.⁸⁴ It may be that a different, or expanded, set of transcription factors is required to accomplish full reprogramming in human fibroblasts. The efficiency of this approach also will have to be improved, given that full reprogramming using current protocols is rare and the average infarct in human hearts may involve injury to as many as a billion cells.⁵ In addition, the current route of delivery of these reprogramming factors *in vivo* is genetic modification of cells with viruses, and this approach may present some regulatory barriers.

Until recently, it has been assumed that reprogramming approaches would involve the conversion of a generic cell type, such as a fibroblast, to desired cell types; however, using transcription factors to convert cells from one fate to another closely related fate may be possible (and more straightforward). For example, Kapoor *et al.*⁸⁵ reported that they were able to convert neonatal rat ventricular cardiomyocytes to a pacemaker-like fate by viral transduction of the transcription factor Tbx18.

These early studies are encouraging but await improvements in efficiency. Also needed are studies to determine the extent to which reprogrammed cells recapitulate normal cardiac mechanics and electrophysiologies.

VII. Engineering Cardiovascular Tissues

Although tremendous progress has been made, there are still many challenges for cardiac regenerative medicine that remain to be solved. Bona fide cardiac cells grafted into a nonhuman primate seemed to be functionally linked to the host myocardium, as demonstrated by synchronized calcium transients. However, they caused arrhythmias and did not statistically improve overall ventricular contractility.⁶⁷ These findings are possibly due to a failure of these cells to mature in place. In addition, thinning of the ventricular wall after MI often results in remodeling that affects overall heart function (Figure 2). Although this was not assessed by Chong *et al.*,⁶⁷ remodeling could also contribute to the poor recovery of contractile function.

Solutions to the unmet challenges in cardiovascular regenerative medicine may involve the use of engineered materials to enhance cardiac differentiation and electrophysiological maturation and/ or to preserve or replicate the 3-dimensional (3D) structure of the heart following MI. Here we focus specifically on the potential of hydrogels, or the combination of hydrogels and cells, to meet some of these challenges. These could be injected directly into an area of cardiac damage or be used in the context of 3D printing to generate patches.

Using bioinks or other types of scaffolds, bioengineers are attempting to create microenvironments conducive to cardiac differentiation or maturation and/or that maintain the 3D structure of cardiac tissue after MI. Hydrogel scaffolds have facilitated the growth and expansion of vascular tissues within myocardial grafts. In addition, by varying the mechanical and chemical properties of hydrogel scaffolds, they can be designed in ways that allow researchers to test the roles of mechanical stress⁸⁶ or electrical pacing (reviewed by Vunjak-Novakovic *et al.*⁸⁷).

A. Hydrogels

A number of research groups are now exploring the feasibility of using hydrogel/cell combinations as patches, injectable fillers, or printable bioinks. Natural hydrogels include Matrigel (a commercially available combination of laminin, type IV collagen, and heparin sulfate),⁸⁸ collagen,⁸⁹ fibrin,⁹⁰ and alginate.⁹¹ Each of these separately has enhanced the retention and integration of injected cells and preserved the normal morphology of the ventricular wall after MI.⁸⁸ In addition, a number of synthetic hydrogels are being explored.⁹² However, the cytotoxic and immune potential of these various synthetic compounds is largely unknown but should be explored. For example, injection of a nondegradable synthetic polyethylene glycol (PEG) hydrogel into the infarct zone of a rat MI model resulted in significant infiltration of macrophages, suggesting an immune response.⁹³

The precise physical properties of hydrogels will likely vary somewhat depending on the experimental and therapeutic context; however, there are some general characteristics that would be highly desirable for use in cardiac regenerative therapies.

First, the ability to vary the viscosity of a hydrogel would be highly desirable. Less viscous hydrogels could be injected into the wall of a damaged heart with lower injection pressure so as not to create further damage to the wall of the heart during injection or cause damage to the cells being injected. This would include hydrogels that could be injected in a liquid or semiliquid state but that would become more rigid at body temperature. Alternatively, slightly more viscous hydrogels would be useful for 3D printing of organs or patches. For example, Duan and colleagues⁹⁴ recently reported the development of bioinks with high viscosity and low stiffness that were practical for 3D printing of structures that encapsulated human aortic valvular interstitial cells (HAVICs). This was accomplished by manipulating the relative amounts of methacrylated hyaluronic acid and methacrylated gelatin. Increased relative methacrylated gelatin resulted in improved cell spreading and maintained fibroblastic phenotype. Using 3D bioprinting Duan *et al.* produced 3D trileaflet valve conduits with HAV-ICs encapsulated by a hybrid hydrogel. After 7 days of culture, the encapsulated HAVICs showed high cell viability and cell-type appropriate morphologies and expressed all target genes that were tested, including α SMA, Vimentin, Periostin, and collagen.⁹⁴

Second, for hydrogels that would be injected into heart tissue, having gels with sufficient flexibility so as not to interfere with the contractility of cardiac cells within or near the site of the injection would be desirable.

In addition, temporary hydrogels that could degrade over time would also be highly desirable. In the short run the gel itself could be used to maintain the geometry of the ventricular wall of the heart during repair. This would give cells encapsulated within the gel time to expand and integrate into the host myocardium or provide a scaffold for endogenous stem cells to migrate into the infarct zone.

Finally, hydrogels that could be linked to, or encapsulate, small molecules, peptides, or proteins that enhance growth, differentiation, or physiological function would be tremendously useful. Similarly, attachment of nanoparticles could be used to delivery drugs in a spatially and temporally controlled fashion. For example, Paul *et al.*⁹⁵ recently demonstrated the feasibility of using a hydrogel for localized delivery of the angiogenic factor Vegf that was complexed with a functionalized graphene oxide nanosheet.

B. Hydrogels and Cardiac Repair

Pathological remodeling of the ventricular wall is a common and deleterious effect of MI. Not only is there thinning of the wall at the site of the infarct, the intact wall nearby is also susceptible to remodeling that can ultimately lead to heart failure (Figure 2). Several groups have tested the ability of hydrogels to maintain the geometry of the left ventricular wall after MI. Dobner and colleagues⁹³ injected a PEG hydrogel or saline into infarct areas within 2 minutes of coronary artery ligation. At 2 and 4 weeks after ligation, hearts injected with PEG had less ventricular wall thinning and significant reduction in end diastolic diameter increase. By 13 weeks, however, there was no difference in end diastolic diameter increase between the groups treated with PEG and those treated with saline. This suggests that the impact of hydrogel alone was relatively short lived. Alginates^{96,97} and fibrin⁹⁸ also have been tested for their ability to moderate ventricular remodeling after infarct, with similar positive effects. Hydrogels derived from decellularized ventricular wall extracellular matrix (ECM) have been studied more recently to determine whether ECM can be used, either as sheets to form patches⁹⁹ or as fully injectable fillers to repair damage to infarct zones.^{100,101} Increasing the percentage of native ventricular wall ECM in the injection facilitated the differentiation of encapsulated hESC-derived cardiac progenitors, increasing the expression of cardiac-specific genes. The addition of growth factors to these ECM-derived hydrogels did not further increase cardiac differentiation, suggesting that the matrix alone was sufficient to support cardiac differentiation.¹⁰¹ In a similar study, however, a hydrogel consisting of a hybrid of ventricular ECM, fibrin, and a low dose of TGF- β increased the vascular differentiation of mesenchymal progenitor cells.⁹⁹ A likely next step would be the use of hydrogels including both cardiac and vascular progenitor cells with or without the addition of growth factors (Figure 3).

Other approaches used specifically to increase vascular formation within cardiac grafts include work by Cui and Boland,¹⁰² who used inkjet printer technology for 2-dimensional printing of a fibrin scaffold that facilitated the growth of vascular structures with only minor deformation. After 21 days of culture, the proliferated cells formed a tubular microvasculature within the fibrin channels, suggesting that inkjet printing technology could be used for the biofabrication of human microvasculature with high resolution.

Similarly, Vollert and colleagues¹⁰³ fabricated 3D structures with microchannels that served as artificial vessels for the perfusion of engineered heart tissues (EHTs). To accomplish this, thin alginate fibers were embedded in a matrix of cells, fibrin, and thrombin. After polymerization, the fibers were removed using alginate lyase or sodium citrate. These artificial vessels improved the oxygen concentration in the center of the EHT and were ultimately populated by endothelial cells.

By combining approaches similar to those described above, Vukadinovic-Nikolic *et al.*¹⁰⁴ recently generated a large sample of EHT. This cardiac construct consisted of 3 separate layers. The bottom layer was decellularized porcine small intestinal submucosa, the middle layer was a monolayer of rat neonatal cardiomyocytes, and the top layer comprised rat heart endothelial cells. Using this approach, Vukadinovic-Nikolic *et al.* were able to engineer an artificial tissue of substantial size, with an average beat rate of 208 ± 78 beats/minute on day 3 and 154 ± 48 beats/minute on day 10 compared with 43 ± 27 beats/minute in cardiac cells grown without the other tissues, suggesting that this approach may improve the physiological or electrophysiological maturation of cardiac cells. Rat endothelial cells seeded in the top layer migrated through the cardiac compartment within 7 days and colocalized with the vessel bed of the submucosal layer. These studies demonstrate that effects caused by cell engraftment alone can be greatly enhanced using engineering approaches.

The most ambitious projects in cardiac bioengineering are efforts to completely rebuild hearts either by 3D printing or using decellularized hearts as a scaffold for repopulation by cardiac progenitor cells. In 2008 Ott *et al.*¹⁰⁵ developed a technique to efficiently decellularize rat hearts and subsequently use them as a natural platform to fabricate a beating bioartificial heart. At first, they carried out coronary perfusion with a mild detergent over 12 hours to generate a decellularized construct with a perfusable vascular tree, patent valves, and an intact ECM. They then reseeded the construct with rat neonatal cardiac cells. By day 8, these repopulated structures beat and were able to generate a constant, albeit weak, contractile force (about 2% of the force generated by a healthy adult heart). While far from clinical usefulness, this study showed proof of principle that ECM scaffolds could be used to create bioartificial heart tissue. More recently, Lu and colleagues¹⁰⁶ reseeded decellularized mouse hearts with human iPSC-derived cardiovascular progenitors. After 26 days of culture, including the addition of growth factors to promote the differentiation of cardiac tissue and blood vessels, the repopulated hearts showed a spontaneous beat rate of 40–50 beats/minute. This rate was accelerated to 90 beats/minute by perfusing isoproterenol. Subsequent studies showed that the perfused multipotent cardiac progenitors had differentiated into cardiomyocytes, smooth muscle cells, and endothelial cells.

VIII. Conclusions

Work by Chong *et al.*⁶⁷ highlights the advances that have been made in cardiac regenerative medicine based on cell-based therapies alone. However, the studies described here also highlight the unique challenges presented by this field. We highlighted several recent studies that demonstrate the potential power of combining cells with engineered materials (Figure 3). These studies suggest that encapsulating cardiac or cardiac progenitor cells within

hydrogels may greatly enhance their regenerative capacities. This is accomplished by providing scaffolds that facilitate migration and differentiation, mechanically protect cells, and preserve the 3D structure of the damaged heart tissue during repair and recovery.

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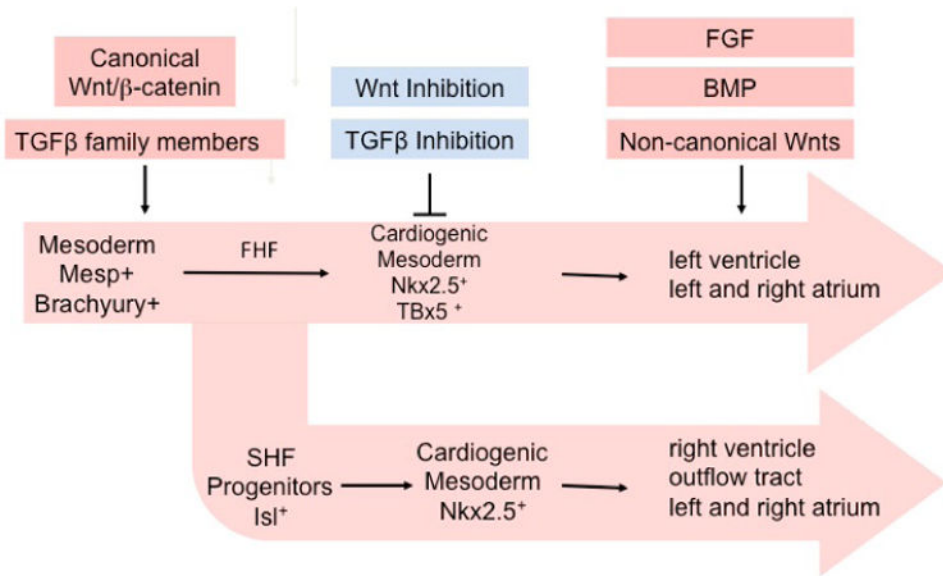


Fig. 1. A graphical representation of cardiac differentiation showing the stages of cardiac differentiation, including characteristic markers of each stage. Also noted are key indicators that differentiate between the first heart field (FHF) and second heart field (SHF). Key regulators of each developmental step are indicated. BMP, bone morphogenic protein; FGF, fibroblast growth factor; TGF, transforming growth factor.

Wall thinning and remodeling after injury

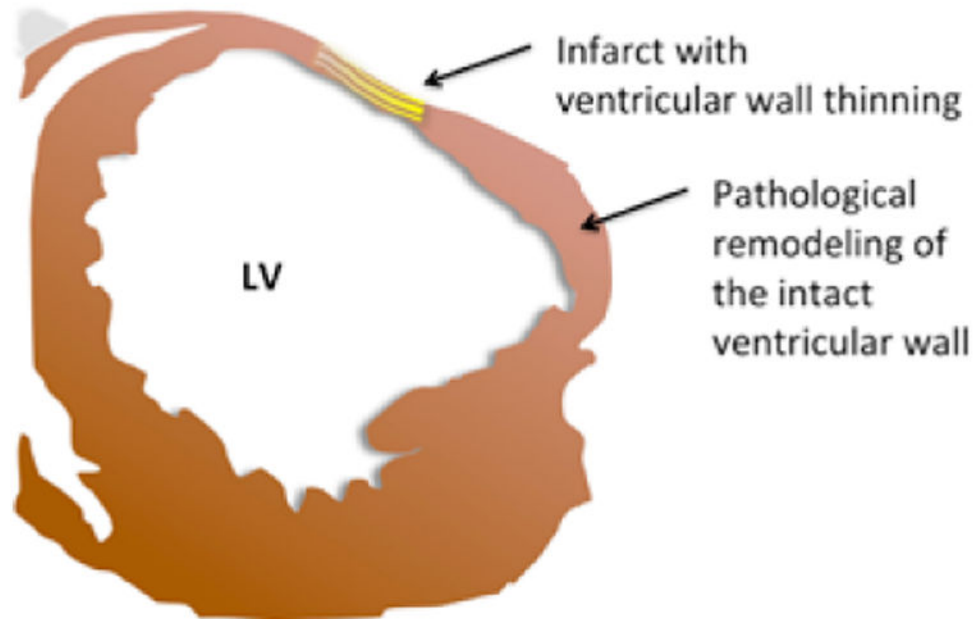


Fig. 2. A graphical representation of a typical infarcted left ventricle (LV), showing wall thinning in the damaged area (yellow). Note that while not thinned, the wall adjacent can undergo pathological remodeling that can interfere with normal functioning.

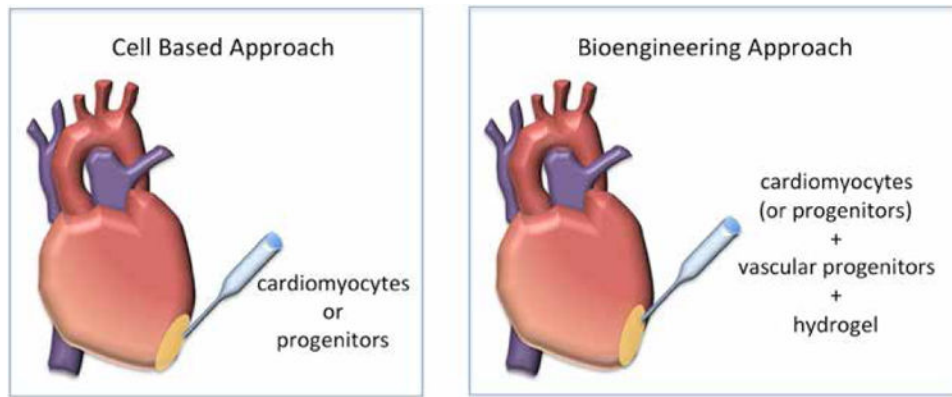


Fig. 3. A comparison of cell-based therapies for heart repair with a hypothetical bioengineering approach. In cell-based therapies cells are injected directly into the infarct (yellow). Bioengineering approaches involve injecting cells (or progenitors) of both the cardiac and vascular lineages that have been encapsulated or coated on hydrogels. Hydrogels have been shown to effectively preserve the 3-dimensional structure of the ventricular wall after myocardial infarction. They have also been shown to facilitate engraftment of donor cells and promote the physiological maturation of engrafted cells. Finally, hydrogels can be used for localized delivery of growth factors and drugs.