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The Integrative Aspects of Cardiac Physiology and their Implications for Cell-based Therapy

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Abstract

Cardiac development is characterized by a complex interplay of chemical, mechanical, and electrical forces, which together contribute to the proper formation of the heart muscle. In adult myocardium, cardiomyocytes are elongated, well-coupled by gap junctions, and organized in spatially well-defined muscle fibers. This specific tissue architecture affects electromechanical activation and global cardiac function. Since the adult heart has only limited capacity for repair after injury, a significant loss of myocardial tissue often leads to impaired cardiac function. Recent efforts to transplant autologous cells to counteract this cardiomyocyte loss have resulted in marginal functional improvement and no evidence of myocyte regeneration. In order to achieve durable therapeutic efficiency, the transplanted cells will need to not only be cardiomyogenic, but also functionally integrate with host myocardial tissue and thereby contribute to both structural and functional restoration.

Keywords

Cell therapy; ischemic heart disease; functional integration; cardiac architecture

INTRODUCTION

The heart is a muscular organ with a unique three-dimensional (3D) architecture that is optimized for efficient electrical and mechanical activation. This heart muscle tissue remains active throughout the entire lifespan with only limited, or no degree of self-renewal after injury.^{1,2} The disruption of the myocardial architecture, as a result of myocardial infarction, bears serious consequences for the electrical activation and proper pump function of the heart leading to malignant conditions, such as ventricular arrhythmia and heart failure.

Pioneering work in the past century to document the physiology and pathology of the cardiovascular system has provided us with novel insights on the myocardial structure and function. This knowledge has led to the development of the most effective strategies to diagnose and treat cardiovascular diseases. While traditional therapeutic options targeting symptomatic relief of patients with heart failure have been reasonably successful, there has been no effective therapy developed targeting the underlying cause – cardiomyocyte loss or dysfunction. More recently, cell therapy was introduced as a potential therapeutic treatment modality for patients

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with heart failure due to ischemic heart disease. Although still in its infancy, cell-based therapy has the potential to reconstitute damaged heart by repopulating infarcted areas with new cells. These cells, if capable of integrating functionally with residual cardiomyocytes, may improve or restore cardiac function through a variety of mechanisms, including cardiomyogenic differentiation and paracrine effects. For cell therapy to become successful it is not only necessary to select the most appropriate cell type for transplantation and route of delivery, but also to understand the way these new cells will have to be incorporated into the myocardium in order to prevent rhythm and conduction disturbances. In this article we will focus on these integrative aspects of cardiac cell therapy. First, the architecture of the normal myocardium will be described. This is followed by a brief overview of the current status of cardiac cell therapy for ischemic heart diseases and aspects of functional integration of transplanted cells with host myocardium tissue. And finally, we will conclude with the future perspective on cell-based cardiac therapy.

MYOCARDIAL ARCHITECTURE AND FUNCTION

To understand how transplanted cells might integrate with host cardiac tissue and contribute to positive force generation, it is essential to first consider normal myocardial tissue architecture and electrical and mechanical activation of the myocardium. During cardiac development, different types of progenitor cells contribute to the formation of muscular, vascular, conductive, and interstitial cells. Developmental processes, such as differentiation, proliferation, and migration are integral in the generation of organized structures. Only recently it was recognized that these cardiovascular progenitor cells belong to a family of closely related cells.³⁻⁷ During early development, round-shaped cardiomyoblasts elongate through unidirectional growth and align in a specific orientation in response to directional stress.

How the process of elongation and alignment is governed, to generate a short and a long cell axis, is still not fully understood. Processes, such as electrical activation⁸ and mechanical stretch^{9,10} appear to be involved. Extracellular matrix is likely to play an important role. Existing literature suggests that extracellular matrix malformations, which give rise to increased structural heterogeneity, can be responsible for some cardiac pathologies.¹¹

As early cardiomyoblasts begin to mature into spontaneously contracting cardiomyocyte precursors, intercalated disc components, such as gap junction proteins (e.g. connexin 43), become clustered at the short cell axis of cardiomyocytes.¹² Consequently, these rod-shaped cells are predominantly coupled in the longitudinal direction while each cardiomyocyte can act as a single excitable and contractile unit. These initially one to two-dimensional myofibers subsequently connect in the long axis of the fiber to form a 3D myocardium. The formation of a 3D myocardial structure allows the establishment and maintenance of efficient electrical conduction. This 3D multicellular functional syncytium, consisting of myofibers with specific architectural characteristics, is required to generate enough force to extrude the blood from the ventricles.¹³ Importantly, the presence of normal myocardial architecture has functional implications for electrical conduction across the cardiac muscle. In particular, conduction of the electrical impulse parallel to the myocardial fiber axis (longitudinal) is about 3 times faster than perpendicular to the fiber axis (transverse), indicating anisotropic conduction¹⁴ (i.e. heterogeneity of a physiological property for a certain material when measured along different axes). In contrast, isotropy refers to *homogeneity* in each direction. The preferential anisotropic conduction is determined by 3 factors: cell geometry, cell size, and gap junction distribution patterns.¹⁵

During electrical activation of the heart, a wavefront is propagated from the endocardium to the epicardium in a spiral-like fashion, guided by the orientation of myofibers in the working myocardium. As a result of this fiber arrangement and the resulting electrical activation pattern,

the left ventricle wall shortens, thickens, and twists along the long axis during normal cardiac cycles, extruding a maximal volume of blood from the ventricles. In addition, this typical cardiac architecture also influences diastolic function.¹⁶

A fine balance between the different cell types, their distribution patterns, and degree of communication allows the heart to be optimally efficient as an electromechanical pump. This diversity of physiologically interdependent cell types is markedly disturbed after myocardial infarction, which results in replacement of well-coupled electrically active cardiomyocytes by scar tissue, containing primarily unexcitable fibroblasts and an electrically insulating extracellular matrix.¹⁷ Consequently, contractile performance and impulse propagation across the scarred myocardium are hampered.

These conditions may seriously affect global cardiac function and eventually lead to symptoms of heart failure.

CELL THERAPY FOR ISCHEMIC HEART DISEASES

Given the inability of the adult heart to fully repair itself, the concept of using cells as the basis for therapy holds tremendous promise to improve cardiac function after myocardial infarction presumably through a variety of mechanisms including improved vascularization, reduced adverse remodeling, prevention of cardiomyocyte apoptosis, and enhanced electromechanical activation of damaged areas.¹⁸ In recent years, a significant number of experimental studies, and a smaller number of clinical trials, have examined the therapeutic potential of cell transplantation in the damaged heart. Transplantation of almost any cell type has been shown to improve cardiac function. These beneficial effects were associated with reduced infarct size, ischemic burden, and improved cardiac contractile function. So far, cell-based therapies have been performed in patients from a few clinical scenarios including acute myocardial infarction, chronic ischemia, and heart failure. Most of these studies involve transplanting bone marrow mononuclear cells because of their autologous source, low tumorigenic risk, and the ease of cell harvesting. Other cell types, such as endogenous cardiac stem cells, mesenchymal stem cells, hematopoietic stem cells, endothelial progenitor cells, and skeletal myoblasts have also been extensively studied in animal models. These different cell types are likely to all induce functional improvement but via distinct mechanisms. Therefore, it will be of great interest to determine the most optimal cell type for transplantation in each specific cardiac disease.

Interestingly, although most experimental cell therapy studies reported improvement in cardiac function after cell transplantation, the cell engraftment rate was shown to be rather low, reaching only 2–5% of the total number of transplanted cells.¹⁹ The low engraftment rate suggests that paracrine effects are responsible for the observed improvement in cardiac function. Yet, for cell therapy to provide a longer-term durable solution to patients with MI or heart failure, the transplanted cells should be capable of undergoing cardiomyogenic differentiation into adult cardiomyocytes and functionally integrate with surrounding host cardiac tissue.

SPATIAL INTEGRATION OF TRANSPLANTED CELLS WITH HOST CARDIAC TISSUE

In most experimental and clinical studies of cell-based therapy thus far, transplanted cells have been introduced by either intracoronary infusion or direct injection into damaged myocardial areas. In both of these cases the resulting graft is not properly guided in its spatial orientation relative to the host myocardial tissue. It had been hoped that the extracellular matrix would somehow guide the proper alignment of the transplanted cells or else the cells would have the intrinsic capacity to organize themselves into a fully structured and integrated myocardium

following transplantation. Nevertheless, due to the presence of a deteriorated extracellular matrix and disturbed electromechanical activation patterns in these damaged regions, no cell transplantation studies have yet shown the creation of a fully organized, functional, and electrically integrated myocardium. It had been hypothesized that if cells are implanted in the border-zone of an infarction, where viable myocardial tissue are present, these transplanted cells may receive enough stimuli from normal myocardial tissue nearby to align properly and regenerate normal tissue architecture within the scar region. To date, data for successful cell engraftment and tissue creation from the border-zone into the scar region remains lacking. Much work will be required to fully understand how the implanted cells might be able to integrate within the surrounding myocardium.

The consequences of misalignment of transplanted cells within native tissue may be quite severe. The increased electrical heterogeneity around the graft area could potentially lead to lethal ventricular arrhythmias, dyssynchronous contraction, and decreased cardiac output. Approaches that enforce transplanted cells to adapt to the native tissue structure, either with or without the use of tissue engineering approaches or pharmacological adjuvants, may contribute to improved therapeutic efficiency and safety.

Of note, in the developing heart, cells are guided in their alignment and arrangement by a well-structured extracellular matrix, and by electro-mechanical stimuli. However, all of these are missing or deteriorated in the infarcted myocardium. Nevertheless, clinical cell therapy with bone marrow mononuclear cells appears to be relatively safe. This may be due to the low percentage of transplanted cells that actually engrafts in the recipient myocardium and, therefore, does not create enough electrical heterogeneity within the already distorted scar region. This further confirms that the therapeutic effects associated with bone marrow mononuclear cell therapy are mainly attributed to paracrine effects, instead of formation of *de novo* cardiac cells in the areas of damaged myocardium. In the future, with improved delivery methods, the engraftment rate of cells after transplantation might increase resulting in increased risk of arrhythmia and adverse effects. It would be of great interest to develop innovative ways to repair the damaged myocardium that allows recapitulation of the normal myocardial architecture.

FUNCTIONAL INTEGRATION OF TRANSPLANTED CELLS WITHIN HOST CARDIAC TISSUE

While transplantation of new cells in the infarcted myocardium may lead to improved cardiac function through a variety of different mechanisms, these transplanted cells must functionally integrate with surrounding cardiac tissue, and thereby contribute to restoring electrophysiological homogeneity of the damaged myocardium. If differentiated into functional CMCs, these cells should approximate the same mechanical properties as host cells (i.e. rigidity and force generation) in order for proper contribution to cardiac function.²⁰ To do so, these transplanted cells must couple to neighbouring cardiomyocytes and conduct electrical impulses with the same velocity as the adjacent endogenous cardiomyocytes.²¹ Furthermore, these transplant-derived cardiomyocytes must also align with native cardiomyocytes to restore tissue structure thereby contributing to anisotropic conduction.

Currently, the alignment, or spatial integration, of transplanted cells with host cardiac tissue has mostly been studied *in vitro*.²² Soonpaa *et al.* showed more than 15 years ago the presence of gap junctions between transplanted fetal CMCs and host myocardium,²³ which most likely allowed low-resistance trafficking of ions and signalling molecules up to 1 kD to take place between these cells. In addition, a two-photon molecular excitation laser scanning microscopy was later used to prove functional integration of transplanted cells at the level of single cell, by simultaneous imaging of calcium transients in donor and host cells within the myocardium.

²⁴ Interestingly, one of the potential beneficial effects of cell therapy appears to involve improved gap junction coupling between cells within the scar area by transplanted cells.²⁵ In support of this, transplantation of cells lacking Cx43 significantly worsened cardiac function, as these became electrically isolated from surrounding host cardiac tissue, thereby increasing electrical heterogeneity and giving rise to harmful arrhythmia.²⁶

To further emphasize the importance of gap junctions in functional integration and therapeutic efficacy, Cx43 was forced expressed in skeletal myoblasts that are typically devoided of Cx43. This resulted in the elimination of the malignant arrhythmia associated with skeletal myoblast transplantation.²⁷ These experiments highlighted the importance of gap junction coupling of transplanted cells with native cells in order to gain therapeutic benefit from cell transplantation in the heart. However, while gap junction coupling seems to be mandatory for a beneficial outcome, the extent of gap junction coupling between excitable and unexcitable cells, in terms of ratios, appeared to affect this outcome. As an example, investigators have shown that increasing the number of unexcitable mesenchymal stem cells (MSCs) (>10%) relative to the number of cultured, excitable CMCs, significantly increased the risk for arrhythmias, whereas a lower number of MSCs (<10%) did not result in these harmful conduction abnormalities.²⁸

Beyond electrical coupling between transplanted cells and the host myocardium, these cells must also function in synchrony with the existing myocardium. This issue is highlighted by the fact that in human heart failure, despite the presence of electrical coupling in all of the myocardial cells, they do not always produce synchronized contraction. Although the origin of this dyssynchrony is due most commonly to conduction block, the effect of dyssynchronous contraction clearly contributes to impaired systolic function. This suggests that for the transplanted cells to achieve functional, and not just electrical, integration, they must be electromechanically similar if not identical to the endogenous adult myocardium. As most stem/progenitor cells used in cell therapy do not easily differentiate into mature adult cardiomyocytes, alternative approaches may be necessary to enhance this process. One possibility is to employ new technologies found in the emerging field of cardiac tissue engineering.^{29,30}

FUTURE PERSPECTIVES

Much work remains as we now enter the second decade of cell-based therapy for cardiovascular diseases. For autologous cell transplantation, in particular, bone marrow-derived cell studies, we will need to better define the mechanisms of benefit as well as demonstration of efficacy in hard clinical endpoints, such as decreased mortality and heart failure-related hospitalizations. Future success of cell-based therapy will depend on our understanding of the most optimal cell type, the best time point of delivery, the selection of the most appropriate patient, and the development of pharmacological or tissue engineering-based adjuvant to cell therapy that could promote survival, engraftment, expansion, differentiation, and functional integration of transplanted cells.

With regards to the cell type used for therapy, the recent derivation of pluripotent stem cells from somatic cells raises exciting prospect that one day these cells may be used as autologous cells for clinical application. Takahashi and Yamanaka made a remarkable breakthrough when they showed that adult mouse and subsequently human somatic cells could be reprogrammed into pluripotent cells by enforced expression of Oct4, Sox2, Klf4, and c-Myc.^{31,32} These induced pluripotent stem (iPS) cells behave very similarly to embryonic stem (ES) cells in various aspects including morphology, expression of pluripotency markers, ability to generate teratoma, and contribution to germline in chimeric mice. They have also been described to differentiate into beating CMCs *in vitro*.³² The advantages of iPS cells over ES cells include less ethical controversy, easier to derive, and autologous in origin. Furthermore, the recent

demonstration that viral integration is not essential for the derivation of iPS cells has further enhanced its potential for clinical applications.³³

As iPS cells can be derived from adult somatic cells, it will be possible, in the future, to derive patient's own iPS cells for cell-based cardiac therapy. Furthermore, derivation of iPS cells from patients suffering from selected genetic cardiac diseases may offer a unique opportunity to study the pathogenesis of these diseases *in vitro*. These cells, if able to recapitulate disease phenotype, may also be amenable to high-throughput drug screening to identify compounds that may prevent the onset or reverse the progression of disease. As this exciting field of genetic reprogramming moves forward, one may anticipate the development of novel therapies and screening tools, provided that the differentiation and maturation of iPS cell-derived cells are identical to those derived from human ES cells.

CONCLUSIONS

Cell-based therapy for ischemic heart diseases holds great promise to enhance cardiac function, by improving or restoring both electrical and mechanical activation of damaged myocardial regions. An important goal for this new decade of cardiac cell therapy will be to improve cell engraftment, survival, and functional integration of cells at the site of implantation. The development of new technologies to deliver these cells and support their survival and cardiomyogenic differentiation within the damaged myocardium will be essential to recreate normal myocardial anatomy and anisotropy.

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Figure 1.

Embryonic myocardial development. (A) At embryonic day 8.5 in mouse, the cardiomyoblasts (myosin heavy chain positive cells stained in black in top panel) that give rise to the eventual compact and trabecular layers of the heart lie circumferentially along the linear heart tube structure. The same two layered cells (yellow) are depicted in cartoon (bottom panel). (B) At embryonic day 9.5, upon stimulation from signals provided by the endocardium (labeled Endo), the inner layer of cardiomyoblast (labeled Myo and stained in black) delaminate to form the first trabecular myocardial cells. The same cells (yellow) are depicted in cartoon (bottom panel). (C). Subsequent proliferation of cardiomyoblasts along the circumferential wall of the heart tube creates multiple layers of cells that become eventually the compact myocardium.

Figure 2.

Effects of myocardial infarction on electromechanical activation. (A) Proper cardiac function is ensured by coordinated electrical activation (indicated by equally spaced isochronal (dashed) lines, which connect points of equal local activation times) and subsequent mechanical activation of the working myocardium, resulting in efficient pump function, associated with optimal cardiac output (thick dark blue arrow). (B) Myocardial infarction results in replacement of well-coupled, excitable, and contractile myocardial tissue in poorly-coupled, nonexcitable, noncontractile scar tissue, containing mainly fibroblasts and accumulating extracellular matrix. Hence, electrical (indicated by distorted shape and distribution of isochronal lines) and mechanical activation of the myocardium are severely disturbed, affecting global cardiac

function and resulting in decreased cardiac output (thin dark blue arrow). Cardiac cell therapy may counteract these detrimental effects by repopulating the damaged areas with new cells. Figure by S. Blankevoort (department of Anatomy and Embryology, LUMC, Leiden, the Netherlands).

Figure 3.

Myocardial tissue architecture and spatial aspects of cardiac cell therapy for myocardial infarction. The following scenarios are postulated for situations in which high engraftment rates could be achieved after cell transplantation. (A) Normal ventricular tissue structure and the oblique fiber orientation across the myocardial wall. In cardiac cell therapy, cells are directly injected into regions of infarcted myocardium (oval area), to improve cardiac function. (B) Potential effects of misalignment of implanted cells on therapeutic outcome and hazardous potential. In addition, these implanted cells may differentiate into functional, contractile CMCs*. (C) Potential benefits from forced alignment of implanted cells. As a result, the therapeutic potential of cardiac cell therapy may increase, while the hazardous potential decreases. Figure by S. Blankevoort (department of Anatomy and Embryology, LUMC, Leiden, the Netherlands).