Cell replacement therapy: The functional importance of myocardial architecture and intercellular gap-junction distribution

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Cellular cardiomyoplasty is emerging as a plausible therapeutic approach designed to promote cardiac muscle regeneration and neovascularization after myocardial infarction. Numerous experimental studies suggest that the mere transfer and implantation of adult stem and progenitor cells can have a measurable effect on cardiac tissue perfusion and contractile function.^{1,2} A likely explanation for the observed improvement in cardiac performance remains elusive and controversial.³ Much remains unknown in muscle biology, but the cell-based therapy “translation train” has left the platform ahead of schedule, with unforeseen stops and an indefinite destination.⁴

Indeed, we are living in interesting times (hopefully not a curse). The dogma that the heart is a postmitotic organ composed of terminally differentiated myocytes that are incapable of re-entering the cell cycle is undergoing intense scrutiny and attack.^{3,5} Anversa and Kajstura³ were instrumental in providing the rationale (and argument) for proclaiming that cardiac regeneration is part of normal cell-turnover homeostasis. A number of clinically amenable approaches for transplanting adult stem cells into areas exhibiting muscle contractile deficit are currently undergoing trials.⁶⁻⁸ Autologous skeletal myoblasts were injected directly into the wall of the injured myocardium, resulting in improved cardiac function. The mechanism of action remains largely unknown because these cells do not transdifferentiate into cardiac myocytes⁹ or functionally (electromechanically) integrate into the working muscle syncytium. This lack of communication with native cardiomyocytes is problematic and might be the basis and substrate for the observed lethal arrhythmias.⁹ In addition, the level of intercellular coupling required for skeletal cells to excite their adjoining neighbors is by necessity exceedingly high because of the inherently short (2.5 ms) action potential duration.

For these reasons, pressure has been intense to use bone marrow–derived cells that have been reported to transdifferentiate into a variety of somatic cells. The study¹ that is credited with legitimizing this approach showed that the bone marrow–derived mononuclear cells can moderate left ventricular remodeling (after induced myocardial infarction) and measurably improve cardiac function.

Much of the improvement in structure and function has been attributed to the de novo generation of cardiomyocytes. The purported frequency at which this happens has been at the center of the criticism because only a few of the millions of cells injected truly undergo transformation to a cardiac phenotype. Nonetheless, the possibility remains that donor cells release cytokines (eg, vascular endothelial growth factor, insulin-like growth factor 1, and platelet-derived growth factor) and thereby restore coronary blood vessels and promote the growth and increase of residual myocytes.⁷ The fact that regeneration of blood vessels, cardiomyocytes, and

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Received for publication Feb 7, 2006; revisions received Feb 22, 2006; accepted for publication Feb 24, 2006.

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J Thorac Cardiovasc Surg 2006;131:1310-3
0022-5223/\$32.00

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doi:10.1016/j.jtcvs.2006.02.018

Figure 1. Schematic representation of longitudinally sectioned myocardium depicting gap-junction localization domains that are grouped primarily within the intercalated disks (sites of intercellular coupling and adhesions). Exogenous stem cells are shown as having a more diffuse gap-junction spatial distribution pattern that might enable both transverse and axial spread of excitation wave front.

metabolism is most pronounced in the ischemic border zone and peri-infarct tissue margins suggests that recruitment and induced division of functionally competent endogenous cardiac stem cells might be at play.³

Functional Cellular Network Connectivity

The individual cells that make up cardiac contractile tissues are not intended to function independently of their neighboring cells. An individual left ventricular myocyte can be connected to as many as 12 neighboring cardiomyocytes.¹⁰ Each cell is recruited into a regulated collective network, a syncytium of activity that enables the rapid coordinated contraction of muscle cells along their entire length (Figure 1). The working myocardium of the human heart consists of a vast (approximately 7.5 billion $\pm$ a billion) assembly of individual myocytes (Appendix 1) orchestrated electrically (and mechanically through adherens junctions) to bridge the cell-to-cell gap-junction channels and facilitate intercellular communication. The physiologic basis for gap junctions is to provide a low-resistance pathway for flow of electrical current and small molecules from a cell to its immediate neighbors. The major purpose of bridging the extracellular space is to allow unimpeded electrical current flow necessary to achieve a rapid, coordinated excitation activation wavefront. A number of cardiac disease states (eg, ischemia, infarction, and dilated cardiomyopathy)¹¹ can give rise to heterogeneous gap-junction expression¹¹ and distribution by disturbing the normal pattern of synchronous ventricular activation. These disease states might diminish cardiac contractile performance and result in discrete areas of conduction defects.

Gap Junction–dependent Cell-to-cell communication

Connexin proteins form the intercellular channels. Three different connexins, connexin 43, connexin 40, and connexin 45, are expressed in the mammalian heart.¹² Connexin 43 is the dominant ventricular coupling protein, also localized in atrial myocardium. Connexin 40 is preferentially expressed in atrial tissue and in the specialized conduction system. Connexin 45 is mainly expressed in the sinoatrial and atrioventricular nodes.^{13,14} The connexin-formed channels enable intercellular exchange of low-molecular-weight substances, including ions, and second messengers. Within each transmural layer of the myocardial wall, the gap junctions are predominantly localized to the longitudinal ends of individual myocytes (Figure 1). This architectural topography ensures a longitudinally directed intercellular current flow (and synchronization of Ca^{2+} transients) during cardiac electrical excitation.¹⁵ One of the requisites for clinically effective cellular replacement therapy is to demonstrate that the myogenically directed donor cells (eg, bone marrow), when injected into the left ventricle, will integrate into the tissue matrix of the host cells and form functional gap junctions (ie, show a positive signal [immunofluorescence] for the presence of connexin proteins). Nonmyocyte cells are capable of connecting electrically to myocytes, effectively forming sinks (drains) for electrotonic currents, producing localized conduction slowing and a decrease in action potential peak upstroke levels. In addition, localized inhomogeneity in connexin 43 distribution was shown to correlate accurately with circumscribed conduction block and changes in the action potential peak upstroke. In areas of expected geometric cell transition, a partial electrical cell-to-cell uncoupling might actually help decrease the occurrence of conduction block.¹⁶ Electrical interaction and coupling across the extracellular space are feasible without the need of bridging the close apposition of cells by gap junctions. Mathematic modeling and computer simulation studies have been used to show that electric field-effect transmission can account for slow propagation of conduction in the absence of cell-to-cell coupling by gap-junction channels, but these theoretic findings remain highly speculative.¹⁷

Remodeling of Gap-junction Expression and Distribution

Myocardial ischemia is accompanied by progressive impairment of gap junction–mediated electrical coupling. This response, manifested as pH-mediated gating capacity, serves as a protective and compensatory mechanism that effectively uncouples the propagation of transcellular injury signals (oxidative stress and intracellular Ca^{2+} overload) from one cell to another. Logic would argue that infusing cells that encourage rapid cell-to-cell coupling might in some instances be counterproductive (ie, might further exacerbate cell injury). It might be advantageous to transiently inhibit the gap junction–mediated intercellular communica-

tion during myocardial reperfusion to help attenuate the associated injury.¹⁸ In counter distinction inhibition of cell coupling in normal myocardium is known to be a causative substrate for re-entrant ventricular arrhythmias.

Studies in animals have demonstrated that the increased incidence of arrhythmias in structural heart disease is accompanied by remodeling of the cellular distribution of gap junctions to a diffuse pattern, resembling the neonatal cardiomyocyte phenotype. It is worth re-emphasizing that in adult ventricular myocytes, gap junctions are located at the transverse-oriented intercalated disks.¹⁹ This association produces a pattern of gap junctions located primarily near the ends of the cells. In contrast, neonatal cardiomyocytes exhibit a characteristically different gap-junction topology, with relatively uniform distribution and occupancy along the perimeter of the myocytes. Importantly, this general pattern of connexin 43 distribution is also observed in human bone marrow-derived stem cells (Figure 1). As cardiac cells enlarge (eg, growth hypertrophy), the gap junctions shift and redistribute from the sides to the ends of ventricular myocytes. Normal growth produces electrical propagation patterns that are manifested differentially at the macroscopic and microscopic levels. In particular, anisotropic longitudinal and transverse conduction velocities and propagation delay are the requisite norms of working myocardium.

The relation of cell-to-cell delays of action potential transfer and associated velocity of conduction distribution can be complex. Moreover, it is now recognized that repolarization heterogeneities between subepicardial and midmyocardial cells can be the causative substrate for re-entrant ventricular arrhythmias in the failing myocardium. Previously, it was presumed that transmural action potential duration gradients were attributable to and induced by heterogeneities of ion-channel expression. One can infer from these studies that the mechanism responsible for maintaining transmural action potential duration heterogeneities in heart failure is dictated by the degree of intercellular uncoupling (downregulation of cardiac gap-junction protein connexin 43).²⁰ Specifically, the action potential duration dispersion was greatest in failing myocardium, and the largest transmural action potential dispersion gradients were consistently found in regions exhibiting the lowest relative connexin 43 expression.

These observations show that reduced connexin 43 expression produces uncoupling between transmural muscle layers, leading to slowed conduction and marked dispersion of repolarization between epicardial and deeper myocardial layers. In studies using knockout mice, cardiac-specific loss of connexin 43 was accompanied by normal heart structure and contractile function, yet these animals uniformly experienced sudden cardiac death from spontaneous ventricular arrhythmias.¹² It is now a well-established fact that tissue architecture influences on conduction are determined by the size, shape, and transmural packing arrangement of individ-

Figure 2. Schematic representation of injured myocardial tissue and associated gap-junction topography.²¹ Shown is the hypothetical partial-thickness and full-thickness epicardial infarct border zone gap-junction disorder topography. Necrotic subendocardial infarct regions are assumed to be devoid of gap junctions. Modified with permission from Peters NS, Wit AL. Myocardial architecture and ventricular arrhythmogenesis. *Circulation*. 1998;97:1746–54.

ual myocytes and by the 3-dimensional distribution and electrophysiologic behavior of the intercellular junctions responsible for cell-to-cell impulse propagation.²¹

Electromechanical Connectivity and Resultant Tissue Heterogeneity

The act of implanting cells into the myocardial tissue is subject to uncertainty in its topographic composition and distribution (ie, neighborhood cluster size and cell-cell interaction). The resultant structural heterogeneity and changes in heterocellular gap-junction coupling (non-uniform anisotropy) can serve as substrates for initiating and disrupting electrical wave propagation (Figure 2). In particular, numerous experimental and mathematic model systems^{22–24} have demonstrated that the wave propagation front can break up into spiral waves or be blocked altogether, depending on the size and distribution of local asymmetry in cell spacing and connectivity.²⁴ Wave-front directional stability is dictated in part by the nature of the anatomic obstacles encountered (eg, by the distribution and nature of electrical connectivity of infused cells). Importantly, wave-propagation breaks might occur in the absence of heterogeneity in circumstances in which the action potential duration is coupled to the recovery time of the involved tissue. Unguided infusion of exogenous cells creates a complex mélange of functioning (current sources) and nonconductive/damaged patches (current sinks) of cardiac tissue (Figure 2) that can potentially contribute to the formation of unidirectional conduction block and in the process initiate re-entrant spatiotemporal activation patterns. Theoretic considerations would suggest that propagation around obstacles is feasible without forming spiral waves provided that the length scale (object size) of the resultant (iatrogenic) heterogeneity is smaller than the length scales associated with the propagating wave front.

This brief overview is meant to serve as a reminder and fair warning that gap-junction expression patterns, spatial distribution, and topology (not merely their presence) are critical determinants of the reconstituted electromechanical conduction pathways. We still must learn a great deal from how the altered connexin 43 expression and spatial distribution produce the arrhythmia substrate (eg, mosaic coupling pattern) in the diseased heart. Electrical discontinuities (obstacles) are inherently spawned by inclusion of nonmyocyte cells within the myocardial milieu. Moreover, the complex patchwork of heterocellular functioning tissue (Figure 2), resulting from non-uniform expression of gap junctions and resultant electromechanical bridging in areas of infarction, are problematic and should be of concern for both the surgically administered, intramyocardially unguided cell delivery approach and the intracoronary diffuse route of stem-cell distribution. It must be emphasized that there are no assurances that these delivery strategies can activate the developmental cardiogenic repertoire in a temporally regulated fashion.

Evidence for recapitulated electrical cell-to-cell coupling has been difficult if not impossible to document in the setting of clinical trials. The nearly universal enthusiasm that has catapulted the bench-to-bedside application of cell replacement therapy to a fast-track necessity continues unabated.^{4,6-8} The flurry of clinical trials using cell-based cardiac repair carry the burden of proof (ie, control group, differentiated phenotypes, and safety), irrespective of the chosen stem-cell identity and mode of delivery being promulgated. It is indeed good fortune that the ongoing clinical trials that use bone marrow–derived stem cells have been relatively free of the many dangerous interludes that can beset the runaway spatiotemporal electrophysiologic heterogeneity. The avoidance of the adverse events and consequent derailing of this journey can be attributed primarily to the notable paucity of fully integrated, electromechanically coupled cells. The good fortune might prove to be the benefactor of the cytokine payload delivery to the site of injury. The cell transplantation–mediated activation of the inflammatory pathways is the likely mode of action by which antiapoptotic signals journey to induce and preserve cardiomyocyte organization, extracellular matrix collagen deposition is reduced, and neovascularization is promoted.

Caveat emptor (when boarding this fast moving “transplantation train”) . . .

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Appendix 1

HeartMath 101

Heart weight = ~300 gm; Total volume of all cells = ~300 mL (density of ~1.0 gm/mL); Cardiac myocyte volume occupancy assumed to be 50% (or 150 mL); 150 mL = $150 \times 10^{12} \mu\text{m}^3$; Volume of a cardiomyocyte (nominal size $100 \mu\text{m} \times 20 \mu\text{m} \times 10 \mu\text{m}$) = $2.0 \times 10^4 \mu\text{m}^3$; Number of cells = tissue volume/cell size = $[150 \times 10^{12}] / [2.0 \times 10^4] = 7.5 \times 10^9$ (~7.5 billion cardiomyocytes).