Published in final edited form as:
Circ Res. 2008 July 18; 103(2): 119. doi:10.1161/CIRCRESAHA.108.180828.

Nature Versus Nurture in Cardiac Conduction: Toward Integrative Paradigm of Cardiac Tissue Engineering

Igor R. Efimov
Washington University in Saint Louis, Mo

Keywords

conduction; connexin43; stem cell plasticity; stem cells; tissue culture; tissue engineering

> "Biology today is at a crossroads. The molecular paradigm, which so successfully guided the discipline throughout most of the 20th century, is no longer a reliable guide. Its vision of biology now realized, the molecular paradigm has run its course. Biology, therefore, has a choice to make, between the comfortable path of continuing to follow molecular biology's lead or the more invigorating one of seeking a new and inspiring vision of the living world, one that addresses the major problems in biology that 20th century biology, molecular biology, could not handle and, so, avoided."[1]
>
> —Carl R. Woese

Circulation research, as many other branches of biomedical science, has motivated many young investigators by the promise of delivering to society therapies against deadly diseases. As with all branches of the natural sciences, circulation research follows periodic changes in overarching methodological approaches that dominate the field for several decades. The two extremes of this research pendulum are the reductionist and the integrative approaches to the cardiovascular system. Five years ago, Eugene Braunwald reflected on the 50th anniversary of the journal *Circulation Research*, whose inaugural Editor in Chief was Carl J. Wiggers.[2] Braunwald recalled a dominance of the reductionist approach that was personified by the leading physiologist of the beginning of the 20th century, Ernest H. Starling, followed by the integrative approach under the leadership of Carl J. Wiggers. "As cardiovascular research has unfolded in the 50 years of *Circulation Research*, the pendulum between the reductionist and holistic approaches has swung back and forth."[2] Braunwald also concluded that:

> "Contemporary cardiovascular scientists increasingly recognize that despite the enhanced appreciation of the myriad individual components that make up the circulation, a better understanding of the system in which these components operate is urgently needed. It has become quite clear that an organism's function is not simply the sum of the functions of its individual genes and their products but also depends on the interactions between these products. There is increasing interest in how these interactions are integrated to modify the behavior of the cells, tissues, and organs that comprise the circulatory system. This is leading to a resurgence in research on the circulation as a system."[2]

Correspondence to Professor Igor R. Efimov, Washington University, Biomedical Engineering, Campus Box 1097, One Brookings Drive, St. Louis, MO 63130-4899. igor@wustl.edu.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

Disclosures
None.

The reductionist approach has dominated circulation research for the last several decades. It has led to dramatic increases in the understanding of molecular mechanisms of normal and abnormal cellular electrophysiology, which in turn, led to the development of exciting new theories of arrhythmia. A number of these theories have been supported by convincing empirical evidence "from cell to bedside."[3,4] As a result, the field has been propelled by the elegant "silver bullet" solutions against lethal cardiac arrhythmias. Nearly every generation of electrophysiologists has come up with a target for their own "silver bullet": sodium channel, calcium channel, potassium channel, etc. Now the connexin hypothesis appears to suggest a new "silver bullet". Visions of several generations have crystallized in the recently developed theory of channelopathies.[5]

The current state of circulation research is reminiscent of the early history of elementary particle physics. Many decades ago it appeared to many physicists that the foundation of laws of matter can be eloquently explained by the interaction of very few elementary particles and very few fundamental laws governing these interactions. Yet, as more and more unexpected particles were discovered, or the peculiar properties of the existing particles were uncovered, increasingly more sophisticated theories have been produced, making irrelevant the elegance and eloquence of the early reductionist theories.

Cardiac electrophysiology went along a very similar path in search of an antiarrhythmia therapy. A road-map was drafted nearly 70 years ago by Carl J. Wiggers, who wrote:

> "As to the fundamental mechanisms of fibrillation we have plenty of theories, but none is universally accepted … they all center around two ideas, viz., (a) that the impulses arise from centers, or pacemakers, or (b) that the condition is caused by the re-entry of impulses and the formation of circles of excitation."[6]

The old ion channel-centered theory, which was based on a single cell paradigm, seemed to have done a satisfactory job explaining both focal and reentrant theories of arrhythmia. These early theories of arrhythmia, with their four classes of antiarrhythmic drugs, were almost Aristotelian. But they fell under the pressure of empirical evidence[7]: multiplying channel isoforms and subunits; unexpected auxiliary proteins expressed and functioning in concert with channels, numerous increasingly complex signaling pathways activated in response to a diverse array of electric and mechanical stimuli. These important players had been unknown, overlooked, or neglected in the past.

Can a cardiac arrhythmia with broad clinical impact be explained within a framework based on single channel biophysics or even single cell physiology? Most importantly, can an antiarrhythmic treatment be developed based on such a mechanism? Despite the explosion in the number of filed patents offering positive answers to such questions, it is becoming more and more apparent that these issues will not be so easy to settle. Perhaps it is time to look back and reflect on the warning of Hippocrates, who also contributed to the debate between the reductionists and the integrationists:

> "In medicine, one must pay attention not to plausible theorizing but to experience and reason together. I agree that theorizing is to be approved, provided that it is based on facts and is systematically induced from what is observed; but conclusions drawn by the unaided reason can hardly be serviceable, only those drawn from observed facts."[8]

The recent emergence of connexins as an important cause of some arrhythmias was in part driven by dissatisfaction with channel-based pharmacological therapies, which failed in many clinical trials.[7] It became clear that in many cases the most fundamental principles of arrhythmia cannot be explained solely by the abnormalities in the firing of a cell action potential that results from an ion channel malfunction. However, the discovery of the importance of an

abnormal cell-to-cell coupling reminded us of the Wigger's vision. Both focal and reentrant mechanisms are critically dependent on slow conduction and poor coupling. Cell–cell uncoupling is commonly found under numerous pathophysiologic conditions. Which of the two mechanisms will be favored by uncoupling? Probably uncoupling would enhance both the focal and the reentrant mechanisms. For example, the safety of the conduction from an abnormal focus requires source–sink matching. The cell–cell uncoupling may play a role in allowing a weak source to drive a significant electrotonic load. Similarly, an abnormal reentrant pathway has to provide safe conduction with a conduction delay sufficient to set the stage for a reentry. In numero[9] and in vitro[10] evidence suggests that slow conduction and source–sink matching may depend more on connexins than on ion channels. Moreover, these studies have presented evidence that cell–cell uncoupling improves the safety of the slow conduction.[10]

Recent introduction of an innovative concept of cardiac tissue engineering for treatment of various diseases of the heart[11] is now attracting new young scientific talent to the cardiovascular field by offering the seemingly boundless potential of tissue engineering and novel clinical therapies that may result from it. However, both basic and clinical evidence suggests that we are just starting an exciting, but long and difficult, path toward an understanding of how stem cells derived from various sources can couple with the host myocardium and form a long-term functional myocardial structure, which will one day replace the myocardium damaged by infarction or aged sinoatrial or atrioventricular nodes. An engineered tissue transplant cannot be understood solely from a single cell point of view. It must be engineered as a 3-dimensional tissue that forms an intimate functional interface with the host myocardium, including normal cardiac conduction, innervation, and perfusion.

The article by Pijnappels et al,[12] in this issue of *Circulation Research,* presents new evidence in cell culture that structure and alignment of mesenchymal stem cells is critically important in coupling of the transplanted cells with the myocardium. They demonstrate that mesenchymal cells transplanted into the damaged myocytes cell culture can be forced to align in a preferential direction by microabrasion of the coating in the dish. Such alignment facilitates the functional integration with myocardium as evident from an improved conduction velocity and from an increased expression of connexin 43. Therefore, this report shows that geometric considerations are an important independent predictor of the functional integration of synthetic cardiac tissue with the host myocardium.

The study has some limitations. It remains to be shown that the observed phenomenon can be reproduced in vivo. Before that, however, it should be reproduced in 3D tissue constructs. Most importantly, the study does not offer a solution on how such cell alignment can be performed in 3D tissue constructs that are innervated and vascularized and that would integrate with adult myocardium with extensive fibrosis. Despite these and other limitations, this article is another step toward integrative understanding of cardiovascular tissue engineering. It adds to an earlier report from Zhuang et al,[13] which shows that mechanical periodic stretch is required for maturation of electric and mechanical synapses.

Cardiovascular tissue engineering is emerging as a highly promising new field. This field requires an integrative approach. Many reports show a multitude of factors that are critically important in functional integration of an engineered construct and a host myocardium. Several groundbreaking developments present new opportunities for exploration. Recent revolutionary reports from the laboratories of Yamanaka[14] and Thomson[15] show the possibility of inducing stem cells from somatic human cells, which describes a promising new path to finding an alternative source of cells for tissue engineering. In vivo tissue engineering is already beyond proof of concept and shows how maturation and vascularization of 3D cardiac tissue constructs can be achieved.[16] An intricate design of the molecular scaffolds presents a promising platform

for micropatterning tissue engineered constructs at the cellular level while maintaining tissue-level scaling capabilities.[17]

References

1. Woese CR. A new biology for a new century. Microbiol Mol Biol Rev 2004;68:173–186. [PubMed: 15187180]
2. Braunwald E. *Circulation Research*: Reflections on the Founding Editor, Carl J. Wiggers. Circ Res 2003;92:253–254. [PubMed: 12595333]
3. Zipes, DP.; Jalife, J. Cardiac Electrophysiology: From Cell to Bedside. W.B. Saunders Company; Philadelphia, London, Toronto: 1990.
4. Zipes, DP.; Jalife, J. Cardiac Electrophysiology: From Cell to Bedside. W.B. Saunders Company; Philadelphia, London, Toronto: 2000.
5. Marban E. Cardiac channelopathies. Nature 2002;415:213–218. [PubMed: 11805845]
6. Wiggers CJ. The mechanism and nature of ventricular fibrillation. Am Heart J 1940;20:399–412.
7. The Antiarrhythmics Versus Implantable Defibrillators (AVID) Trial Executive Committee. Are implantable cardioverter-defibrillators or drugs more effective in prolonging life? Am J Cardiol 1997;79:661–663. [PubMed: 9068527]
8. Hippocrates. Precepts. Athens: V Century BC;
9. Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. Circ Res 1997;81:727–741. [PubMed: 9351447]
10. Rohr S, Kucera JP, Fast VG, Kleber AG. Paradoxical improvement of impulse conduction in cardiac tissue by partial cellular uncoupling. Science 1997;275:841–844. [PubMed: 9012353]
11. Zimmermann WH, Schneiderbanger K, Schubert P, Didié M, Münzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. Circ Res 2002;90:223–230. [PubMed: 11834716]
12. Pijnappels DA, Schalij MJ, Ramkisoensing AA, et al. Forced alignment of mesenchymal stem cells undergoing cardiomyogenic differentiation affects functional integration with cardiomyocyte cultures. Circ Res 2008;103:167–176. [PubMed: 18556577]
13. Zhuang J, Yamada KA, Saffitz JE, Kléber AG. Pulsatile stretch remodels cell-to-cell communication in cultured myocytes. Circ Res 2000;87:316–322. [PubMed: 10948066]
14. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872. [PubMed: 18035408]
15. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–1920. [PubMed: 18029452]
16. Birla RK, Borschel GH, Dennis RG, Brown DL. Myocardial engineering in vivo: formation and characterization of contractile, vascularized three-dimensional cardiac tissue. Tissue Eng 2005;11:803–813. [PubMed: 15998220]
17. Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, Freed LE, Vunjak-Novakovic G. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc Natl Acad Sci U S A 2004;101:18129–18134. [PubMed: 15604141]