Human Biological Pacemakers
Intrinsic Variability and Stability

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In healthy individuals, the sinoatrial node (SAN) is responsible for initiating excitation > 100,000 times per day. As with all cardiac pacemaking cells in the specialized conduction system, SAN automaticity is driven by diastolic depolarization via several redundant pathways to ensure that the heart beats. Key cellular mechanisms include the hyperpolarization-activated inward current ($I_h$) and the Ca clock (inward Na/Ca exchange current activated by sarcoplasmic reticulum Ca$^{2+}$ release). However, molecular, histological, electrophysiological, and in silico studies have revealed that the SAN is extremely complex, and that the redundant systems not only provide safety of pacemaking, but also allow precise modulation of heart rate to accommodate changing physiological demands. Despite this ingenious design, physiological limitations of electronic pacemakers, biological pacemakers may also be more suitable for pediatric patients and have the opportunity to provide more precise autonomic responsiveness. Several approaches have been used in the development of biological pacemakers, including the transfer of pacemaking genes to the heart, and the implantation of exogenous pacemaking cells, and a combination of gene and cell therapies.

One of the most exciting recent developments with regard to biological pacemakers has been the exploration of human induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) as potential pacemaking cells. With this approach, human somatic cells such as hair follicles or skin cells are reprogrammed to become pluripotent stem cells and are differentiated into cardiomyocytes. This unique approach circumvents many of the ethical issues associated with the use of human embryonic stem cell–derived cardiomyocytes (hESC-CMs) and allows the creation of pacemaker cells from a patient’s own tissue, making this approach immunocompatible. Regardless of the approach or cell type used to create biological pacemakers, the template or ideal cell remains the heart’s own SAN cells, and the new pacemaker should ideally mimic the heart’s own pacemaker to the extent possible. In addition to regular automaticity, the SAN displays a unique property of beat rate variability (BRV) that includes long-term oscillations in BRV that repeat over multiple time scales. So, although heart rate stays steady in an individual over time, slight variations in heart rate occur that exhibit characteristic periodicity over time. Importantly, multiple studies have shown that relatively high BRV correlates with overall cardiovascular health and that decreases in BRV are associated with increased mortality resulting from cardiac causes.

The mechanisms by which BRV promotes cardiovascular health are incompletely understood. It is thought that a significant component of BRV is due to responses to cyclic autonomic input and circadian rhythms. However, even in denervated hearts after cardiac transplantation, BRV remains (although at a reduced level). This suggests that at least part of BRV is intrinsic to the SAN itself, whether at the level of the single cell or in multicellular behavior. Therefore, it should follow that if a biological pacemaker is to replace the SAN, it may be advantageous to evaluate the ability of the proposed pacemaking cells to mimic the heart's own SAN cells, and the new pacemaker should ideally mimic the heart’s own pacemaker to the extent possible.

In this issue of Circulation, Mandel et al did just that; they evaluated BRV and power-law behavior in both hESC-CMs and iPSC-CMs over a period of 15 days in culture. Power-law behavior refers to the repeatability of short- and long-term oscillations in BRV and can be quantified by plotting the power spectrum of the RR interval versus frequency on a log-log scale. This plot produces a linear relationship, the slope of which ($\beta$) has been estimated at $-1$ for healthy individuals and is $-2$ for denervated hearts after cardiac transplantation. The study by Mandel et al reveals that both hESC-CMs and iPSC-CMs exhibit BRV and power-law behavior remarkably similar to that seen in the normal human heart.

Furthermore, the authors also conducted phase-plane analysis of the dynamic properties of the cultures by plotting the time series of interbeat intervals against itself with a small
time delay. This approach is similar to phase-plane analysis used to reveal the organization of spiral waves during reentrant arrhythmias. Their results indicate that many cultures start out in a disorganized state at day 1 and become increasingly more organized with time, whereas other cultures show a trend to become less organized with time and eventually stop beating altogether. Taken together, power-law and phase-plane analyses represent a powerful, quantitative methodology for screening not only potential candidate cell types for biological pacemakers but also cultures within a single cell type for their self-organization and long-term pacemaking potential. Importantly, phase-plane analysis on cultured cells may prove especially useful when a patient’s own iPSC-CMs are used for biological pacemakers because each culture within and between patients may have variable self-organization and pacemaking potential. This type of analysis represents the only methodology available for evaluating the evolving self-organization of these systems over time.

However, even if a candidate cell population shows human-like BRV and power-law behavior in culture, this does not necessarily mean that these same properties will manifest once the cells are transplanted to the host myocardium. Conversely, the lack of such power-law behavior should not necessarily exclude candidate cell types. Recent work by Zhang et al demonstrated that even when a large number of SAN cells are autologously transplanted to the right ventricle of canine hearts, the range and success of pacemaking activity of the SAN cells are limited in the ventricle compared with their normal atrial environment. This underscores the importance of the host environment in influencing the resultant pacemaker activity and is likely attributable to the local anatomy, cell-cell coupling, and source-sink interactions, meaning that the resulting pacemaking activity is highly dependent on the amount of depolarizing current produced by the pacemaker cells relative to the amount of this current absorbed by the surrounding ventricular myocardium. Thus, the host environment may limit the potential of even the most ideal biological pacemaker cell populations.

The embryoid body cell clusters used by Mandel et al (and by others) are a heterogeneous population of cells, some of which behave electrically like nodal pacemaker cells, some like atrial myocytes, and some like ventricular myocytes. This is both good and bad news with respect to transplanting these clusters of cells into a real heart. The good news is that, despite this cellular heterogeneity, the pacemaker function and even BRV shine through. Thus, an appropriately selected in vitro biological pacemaker may be ready to go (plug and play) and integrate smoothly in the intact heart as a sufficient and stable source of local current injection. The potential downside is that these cells are intrinsically plastic with respect to phenotype, so they might regress from the pacemaker phenotype (spontaneously or in response to in vivo tissue cues), and the reduced current source/sink balance in vivo might limit the efficacy of a promising in vitro pacemaker.

Furthermore, the transplant location within the host myocardium can significantly influence the BRV and autonomic responsiveness of the biological pacemaker. Shlapakova et al recently showed that significant BRV and response to emotional stimuli can be achieved with pacemaking gene (HCN2) transfection to the left bundle-branch of canine hearts, whereas, when HCN2-expressing human mesenchymal stem cells were transplanted into the left ventricular anterior wall, autonomic responses were not observed. Differences in autonomic innervation at the transplant location may be responsible for these findings. Again, this might work both ways. That is, even candidate pacemakers exhibiting weak BRV and power-law behavior in culture could be encouraged if transplanted into highly innervated regions (eg, atria or conduction system). Conversely, pacemakers with good function, BRV, and autonomic responsiveness could fail in the wrong location.

Finally, it remains unknown whether the BRV and power-law behavior observed in the present study are inherent to the cells themselves or to the population of cells (in this case, embryoid bodies). For example, would each isolated cell on its own display this sort of BRV behavior, or is it the result of coupled oscillators within the complex cell population? This could potentially have important clinical implications. If the BRV characteristics are a property of the cell population, how might this behavior change once the cells are coupled to host atrial or ventricular myocardium? What happens if a number of cells die or migrate from the transplant location; will BRV remain? All of these factors remain to be determined. These issues are simply to say that despite the unprecedented opportunity and the elegant characterizations of pacemaker patient-specific iPSC-CM cultures described here, there is much left to do to achieve the ultimate clinical goal.

In summary, the work of Mandel et al represents an important step forward in screening candidate pacemaker cells by using elegant tools of nonlinear dynamics. They have presented convincing, quantitative data showing human-like BRV and power-law behavior in both hESC-CMs and iPSC-CMs. However, many questions remain regarding the origins of this behavior, the basis of its apparent physiological benefit, and the short- and long-term outcomes when transplanted into host myocardium. In addition to ideal candidate pacemaker cells, successful biological pacing will likely require fine-tuning of the behavior of the pacemaking cell population and the interactions between the pacemaker and host myocardium. Therefore, the empirical characterization tools such as those described by Mandel et al may be incredibly useful for screening candidate cells and may enhance the chances of success in the careful in vivo testing and validation that must follow.

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None.

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