Comments to the Editor

Rigorous Phenotyping of Cardiac iPSC Preparations Requires Knowledge of Their Resting Potential(s)

In a recent "Biophysical Letter" (1), voltage-sensitive dye methods were used to record action potentials from populations of human-induced pluripotent stem-cell-derived cardiac myocytes (iPSC-CMs) that had been maintained in defined cell culture conditions. The main goal of this study was to determine whether chamber-specific (i.e., atrial versus ventricular) action potential waveforms (2) could be identified consistently from these cardiocytes. The data shows that the action potential waveforms depend strongly on cell culture density. In fact, this experimental parameter apparently dominates (and may obscure) detection of the well-known atrial or ventricular action potential waveforms, as well as altering intrinsic pacemaker activity. Accordingly, the main conclusions drawn by these authors are that:

1) The cell culture seeding density significantly affects the observed action potential morphology of these iPSC-CM preparations;
2) Within an iPSC-CM preparation studied at a predetermined seeding density, the action potentials exhibit a normal distribution of morphologies as opposed to any consistent chamber-specific waveforms; and
3) Alteration in the intercellular resistances of these iPSC preparations does not provide an explanation for there being no detectable chamber-specific action potential waveforms.

These interesting findings are potentially very significant. iPSC-CM methodology is importantly involved in essential aspects of both personalized regenerative medicine and safety pharmacology screening for a wide spectrum of either new or repurposed cardiovascular drugs. In these settings (3,4), and also in fundamental studies of human cardiovascular disease mechanisms, the signature provided by the action potential waveform, and the presence/absence of related pacemaker activity, are the main phenotyping criteria that provide a sign and a measure of progress toward identification of much-needed novel therapeutic agents. However, in our view, additional information is needed before the conclusions from this study (1) can be fully assessed, or accepted without significant reservations.

It is well known that the waveform and baseline stability of the mammalian cardiac action potential depends strongly on the value of the resting membrane potential (5,6) In part, this is because the resting potential regulates the excitability of both myocytes and Purkinje fiber cells. However, the same K^+ channels (I_{K1}) that regulate the resting potential in atria and ventricles also produce the outward current that is responsible for the final phase of repolarization of the action potential (7). Unfortunately, voltage-sensitive dye recordings of action potential waveforms (as used in this study) cannot yield any direct information concerning the resting potential, or I_{K1} (1). Detailed recordings of the resting potential, and voltage-clamp measurements of the highly nonlinear underlying background K^+ current I_{K1} or $K_{ir}2.1$, are needed as part of the investigation of action potential morphology.

In fact, the importance of appropriate expression levels of I_{K1} for valid phenotyping of human pluripotent stem cell-derived cardiomyocytes has been clearly established by articles from the laboratories of Bett et al. (8), and Lieu et al. (9) (see Fig. S2D). Here, I_{K1} is described as an environmental cue. This is perhaps apt, because it is very important to draw attention to the very strong (but also nonlinear) dependence on the size of I_{K1} as a function of plasma K^+ levels (7).

A very recent article on electrophysiological properties of human induced-pluripotent stem-cell-derived cardiomyocytes used the dynamic voltage-clamp technique to inject predetermined I_{K1} current waveforms into these targeted myocytes (10). The expected pattern of results was obtained (see their Fig. 3), and from the data in this article, it is clear that it is the outward limb of the I_{K1} current-voltage relationship that is of critical importance (8).

An additional reason for needing to have detailed knowledge of the resting potential and underlying K^+ current(s) when carrying out studies such as the one reported by Du et al. (1) is that the intrinsic biophysical properties of I_{K1} can also alter intercellular coupling and thus can change/modulate electrotonic cell-cell interactions (11). Variations in I_{K1}, and in particular changes in the negative slope region of its ion transfer or current-voltage relationship, can effectively homogenize the resulting action potential waveforms in a fashion that depends strongly on a number of

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cell culture conditions. This includes (but is not limited to) cell density. For example, it is well known that I_{K1} current density decreases as a function of the time during which mammalian myocytes are held in conventional two-dimensional culture conditions (12), including methods similar to those used by Du et al. (1).

Patch-on-a-chip methods, including semiautomated planar patch-clamp platforms have been advanced for the study of these hiPSc cardiocytes (13). Ideally, these experiments should include detailed assessment of the resting potential and recordings of action potentials in each targeted cell studied at physiological temperatures (13). To accomplish this, protocols must involve repeated assessments of the patch seal resistance and the input resistance of the myocyte (14). In fact, it would seem to be prudent to combine these essential measurements with a determination of the complete I-V curve for I_{K1}, and an assessment of the impedance profile of action potential during its plateau (15).

In summary, the "Biophysical Letter" by Du et al. (1) contains important new information regarding novel phenotyping procedures. Their results characterize iPSC cardiomyocytes under conventional cell culture conditions. However, the unqualified conclusion that chamber-specific action potential waveforms cannot be identified from these preparations needs to be reevaluated. To be certain of this, accurate measurements of the resting potential and the underlying background inwardly rectifying K^+ current (I_{K1}) density are both needed. As mentioned (8,9), this requirement has already been recognized for electrophysiology studies of cardiac stem cells. Thus, Bett et al. (8) have reported that adding an I_{K1} current in their action potential clamp studies of stem cells stabilizes the resting potential and action potential waveform. This result was anticipated based on a number of previous reports that, in two-dimensional cell culture conditions, I_{K1} progressively and quite quickly runs down (12). This progressive decrease in I_{K1} in ventricular myocytes can be linked to the concomitant changes in the density of the transverse tubule system (12) and the recognition that I_{K1} is strongly expressed in the T-tubule membrane (16). iPSC cardiomyocytes have only a minimal T-tubule system. The immature microanatomical phenotype of these iPSC cardiomyocytes may contribute to their propensity to show spontaneous pacemaker activity that appears to be modulated by electrogenic current flow due to Na^+/Ca^{2+} exchange activity (17). If this is the case, the value of the maximum diastolic potential or resting potential is again of critical importance due to the intrinsic biophysical properties of the Na^+/Ca^{2+} ion transfer or I-V relationship (18).

It is also known that soon after mammalian cardiac cells grow to confluence in cell culture, the density of a number of ionic currents can change significantly, and connexin expression is also augmented (19). The advantages of using patient-specific iPS cells for disease modeling and regenerative medicine are considerable (9,10,17,20). However, detailed understanding of the electrophysiological principles that regulate the resting potential, action potential waveforms, cell-cell interactions, and/or pacemaker activity and $[Ca^{2+}]_i$ homeostasis (21) is required for these applications and for safety pharmacology (22).

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