High-Throughput Contractility Assay for Human Stem Cell-Derived Cardiomyocytes

One Beat Closer to Tracking Heart Muscle Dynamics

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With the high rate of cardiovascular disease and cardiovascular comorbidities, the burden on the healthcare system in the United States has reached $329.7 billion dollars per year.[1] As developing countries become more industrialized, they also become susceptible to entering a phase, in which cardiovascular disease (a noncommunicable disease) begins to outrank communicable diseases such as malaria, tuberculosis, and HIV/AIDS. However, the rate of novel drug discovery dropped 33% between the years 2000 and 2009 as compared to the prior decade, whereas the price of bringing a new drug to market cost over $50 billion in the United States in 2008.[2] Consequently, great effort has been made to identify new therapeutics, yet few therapeutics have been found because of the limitations of current techniques.

Article, see p 1172

Fortunately, cardiomyocytes are one of the most readily produced and distinguishable cell types that can be differentiated from pluripotent stem cells (PSC).[3] Human PSC-derived cardiomyocytes (hPSC-CMs), while immature, have the machinery at hand to express all the electrophysiological ion channels found in an adult human cardiomyocyte, along with their critical sarcomere structures. These repeating sarcomeres, along with the sarcolemma-binding costamere structures, provide cardiomyocytes with their mechanotransductive properties. Because of the indispensable nature of these protein complexes, mutations affecting sarcomere and costamere protein-coding genes lead to severe and often lethal diseases. Additionally, gene editing technologies, such as CRISPR/Cas9, have progressed alongside improved hPSC culture and patient-derived somatic cell reprogramming methods to enable controlled in vitro studies of these diseases. With these combined tools, researchers can now identify specific drugs that may aid these patients in a precision medicine approach. This new approach to human cardiac disease modeling has now reached a point where drugs can be screened for off-target cardiac toxicity, proarrhythmic events, and patient-specific cardiac diseases.

As advances in automation, high-content imaging, gene editing, stem cell technologies, and sequencing enable researchers to model diseases at increasingly large scales, there are still several challenges that prevent researchers from fully recapitulating disease phenotypes in vitro. Specifically, hPSC-CMs continue to exhibit fetal-like phenotypes in the transcriptional, protein, and functional space.[4] The sarcomere structures that form in hPSC-CMs exhibit a misaligned, often rounded morphology, unlike the uniformly oriented, consistently spaced structures that are observed in primary tissues.[5] Several approaches have been implemented to improve this sarcomere structure, to push the hPSC-CMs toward a more mature-like state. Thus, there is a high demand for methods to quantify sarcomere structure in a dynamic culture, as they would allow researchers to screen for both hPSC-CM disease phenotypes, treatment corrections, as well as maturation factors for protocol optimization.

Currently, in the field, it has been a challenge to obtain dynamic assessment of contraction parameters in beating hPSC-CMs. The current gold standard in determining adult cardiomyocyte contractile properties is the IonOptix system.[6] In this system, a region of interest is drawn over a horizontally aligned cardiomyocyte, from which a Fast Fourier Transform is derived to achieve sarcomere spacing. However, this system is best utilized with single-cell adult cardiomyocytes because a clear contractile axis is required for the imaging software. Because adult cardiomyocytes are not available from humans and current techniques to mature hPSC-CMs are not yet able to achieve full adult-like structure, other single-cell assays have been developed to quantify sarcomere structure. Higher throughput means of determining relative displacement of monolayers of cardiomyocytes includes correlation-based correction quantification[7] and Fast Fourier Transform to directly monitor changes in sarcomere length.[8] There are additional methods that more directly quantify the forces applied by cardiomyocytes on their surroundings, such as micropost platforms[9] and traction force microscopy.[10] Both techniques examine contractile function by measuring the deformations of materials that hPSC-CMs are seeded on, rather than observing the sarcomeres themselves.

In this issue of *Circulation Research*, Toepfer et al[11] generated a new computational pipeline, coined SarcTrack, which uses wavelet transforms to noninvasively determine the contractile properties of hPSC-CMs in a robust manner. What is exciting about the SarcTrack software is it can robustly

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identify multiple sarcomere units at varying directionalities and distances to asses many sarcomeres in a given field of view from multiple cells. As a result, the resolution is much greater than other techniques as it is at the individual sarcomere level.

Like Fast Fourier Transforms, wavelet transforms are often used in image processing for denoising, compression, and object detection. These transforms are similar in that they both convert a signal or image into the frequency domain, allowing a researcher to quickly quantify any repeating pattern that is present in a signal (such as a specific audio pitch) or a repeating pattern that is present in an image (such as a striated myofibril). The advantage of wavelets is that they provide both the frequency of a repeating pattern within an image, along with the location of that repeating structure. In contrast, Fast Fourier Transform only contains frequency information and thus is more effective if the entire analyzed region contains a uniform, unidirectional repeating structure.

To use SarcTrack, a user provides a discrete set of distances and angles that could define the relative distances of any 2 neighboring Z-disks/M-bands. The algorithm then generates pairs of standard Mortlet mother wavelets and transforms these pairs based on the possible list of distances and angles provided. This creates a bank of small images that resemble ideal sarcomeres under a variety of distances and rotations. The algorithm then convolutes these wavelet pairs frame-by-frame over a video of contracting cells. The convolution algorithm finds regions within the image that closely resemble any of the defined wavelet pairs, labeling that region as a sarcomere of known rotation and distance. These wavelet pairs allow quantification of distances and angles between Z-lines and M-lines at the single sarcomere scale, with no requirement that myofibrils be unidirectional and have uniform sarcomere lengths.

One of the interesting features of this analysis technique is the ability to track various alignments of sarcomeres in hPSC-CMs. Because global tracking of various sarcomeres in a cell can be performed, a diseased cell or a drug that results in malformation/disassembly of sarcomeres will manifest as a large distribution in analyzed values. Immature hPSC-CMs have poor arrangement of myofibrils. Whereas many of the dominant myofibrils in an hPSC-CM will probably be parallel to the long axis of the cardiomyocyte, there will be many other myofibrils randomly distributed. Consequently, these other myofibrils with random orientations to the long axis or the axis of contraction will most likely have different contractile properties compared with the main myofibrils in the cell. As a result, there will be a great deal of variation found within the assessed sarcomere analysis. It is impressive that we now have tools that have the resolution to measure so many myofibrils in the cell. It would be exciting to combine this imaging technology with current cell patterning strategies to generate well-aligned cardiomyocytes or other maturation techniques to obtain mature and aligned sarcomeres for analysis.[7] This will help to reduce the amount of noise generated in the data and be well suited to disease modeling and drug discovery studies.

The current software tool is restricted to contractile measurements. However, this imaging modality could be coupled with other engineered cell lines that express calcium or voltage sensitive fluorophores. This multiplexing feature would broaden the field of view for the screening potential performed as both contractile and electrophysiological properties could be assessed at once. The requirement of user-defined wavelet kernels spanning a discrete set of distances and angles does allow for misaligned sarcomere detection, however, at a significant cost of computational power. Additionally, this forces the analyst to balance between detection resolution and speed of analysis. A first-pass script to automatically determine sarcomere directionality and distance, even coarsely, may help to address this challenge, as well as reduce the likelihood of user error when handling the script.

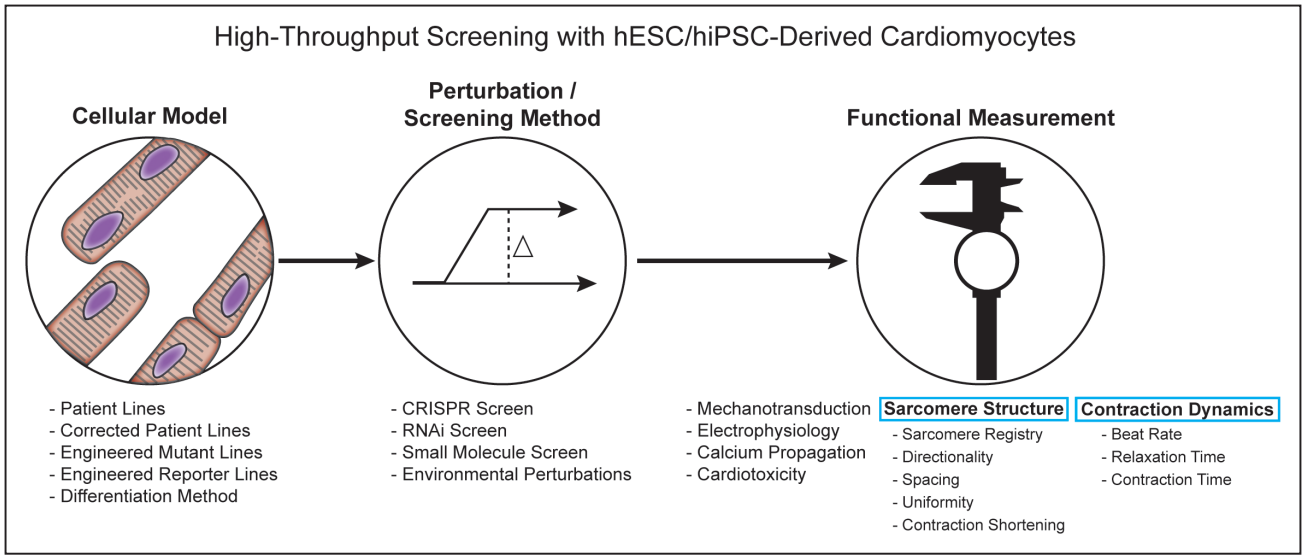

Figure. Components to consider when performing a high-throughput screen on human pluripotent stem cell–derived cardiomyocytes. Modern techniques allow for a plethora of different parameters to consider when setting out to perform a high-throughput screen including cellular model, type of screen, and end point functional measurements. Choosing the optimal conditions will depend on the disease/biological perturbation being studied, which will dictate the desired functional measurement to record and whether the cell line will require modifications to accommodate the functional measurement. hESC indicates human embryonic stem cell; hiPSC, human induced pluripotent stem cell; and RNAi, RNA interference.

Finally, there has been a great deal of effort to find novel ways to mature hPSC-CMs to a more adult-like state. Tissue engineering is a prominent technique used to recapitulate the 3-dimensional structure of the native myocardium and to introduce multiple cell types that would help promote a more mature cardiomyocyte.[12] To assess force of contraction in bulk tissues, the constructs are tethered to deflectable posts that can be used to determine force of contraction of the whole tissue.[13,14] Whereas single-cell force of contraction and sarcomere dynamics cannot be assessed in this paradigm, bulk tissue assessment to drugs or maturation cues can be achieved.

To capitalize on hPSC-CMs, complementary technology needs to be generated to assess the functional state of the hPSC-CMs during screens: genetic, small molecule, and pharmacological (Figure). These technologies need to be noninvasive, nondestructive, provide a robust assessment of cardiac function and be easily scalable to efficiently assess many cells and merge into the pharmaceutical pipeline. Developing tools such as SarcTrack helps to push the utility of hPSC-CMs as a model to study and find novel interventions of cardiac disease. Further development of such tools and multiplexing tools to assess multiple concurrent cardiomyocyte functional outputs will help generate robust in vitro platforms for rapid, reliable and cost-effective drug discovery.

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References

1. Benjamin EJ, Virani SS, Callaway CW, et al; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. *Circulation.* 2018;137:e67–e492. doi: 10.1161/CIR.0000000000000558
2. Fordyce CB, Roe MT, Ahmad T, et al. Cardiovascular drug development: is it dead or just hibernating? *J Am Coll Cardiol*. 2015;65:1567–82. doi: 10.1016/j.jacc.2015.03.016
3. Burridge PW, Keller G, Gold JD, Wu JC. Production of de novo cardiomyocytes: human pluripotent stem cell differentiation and direct reprogramming. *Cell Stem Cell.* 2012;10:16–28. doi: 10.1016/j.stem.2011.12.013
4. Mummery CL. Perspectives on the use of human induced pluripotent stem cell-derived cardiomyocytes in biomedical research. *Stem Cell Reports.* 2018;11:1306–1311. doi: 10.1016/j.stemcr.2018.11.011
5. Pasqualini FS, Sheehy SP, Agarwal A, Aratyn-Schaus Y, Parker KK. Structural phenotyping of stem cell-derived cardiomyocytes. *Stem Cell Reports.* 2015;4:340–347. doi: 10.1016/j.stemcr.2015.01.020
6. Gorski PA, Kho C, Oh JG. Measuring cardiomyocyte contractility and calcium handling in vitro. *Methods Mol Biol.* 2018;1816:93–104. doi: 10.1007/978-1-4939-8597-5_7
7. Macadangdang J, Miklas JW, Smith AST, Choi E, Leung W, Wang Y, Guan X, Lee S, Salick MR, Regnier M, Mack D, Childers MK, Ruohola-Baker H, Kim D-H. Engineered developmental niche enables predictive phenotypic screening in human dystrophic cardiomyopathy. *bioRxiv*. 2018:456301.
8. Pasqualin C, Gannier F, Yu A, Malécot CO, Bredeloux P, Maupoil V. SarcOptiM for ImageJ: high-frequency online sarcomere length computing on stimulated cardiomyocytes. *Am J Physiol Cell Physiol.* 2016;311:C277–C283. doi: 10.1152/ajpcell.00094.2016
9. Beussman KM, Rodriguez ML, Leonard A, Taparia N, Thompson CR, Sniadecki NJ. Micropost arrays for measuring stem cell-derived cardiomyocyte contractility. *Methods.* 2016;94:43–50. doi: 10.1016/j.ymeth.2015.09.005
10. Kijlstra JD, Hu D, Mittal N, Kausel E, van der Meer P, Garakani A, Domian IJ. Integrated analysis of contractile kinetics, force generation, and electrical activity in single human stem cell-derived cardiomyocytes. *Stem Cell Reports.* 2015;5:1226–1238. doi: 10.1016/j.stemcr.2015.10.017
11. Toepfer CN, Sharma A, Cicconet M, et al. SarcTrack: an adaptable software tool for efficient large-scale analysis of sarcomere function in hiPSC-cardiomyocytes. *Circ Res*. 2019;124:1172–1183. doi: 10.1161/CIRCRESAHA.118.314505
12. Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song L, Sirabella D, Morikawa K, Teles D, Yazawa M, Vunjak-Novakovic G. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. *Nature.* 2018;556:239–243. doi: 10.1038/s41586-018-0016-3
13. Boudou T, Legant WR, Mu A, Borochin MA, Thavandiran N, Radisic M, Zandstra PW, Epstein JA, Margulies KB, Chen CS. A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. *Tissue Eng Part A.* 2012;18:910–919. doi: 10.1089/ten.tea.2011.0341
14. Zhao Y, Rafatian N, Feric NT, et al. A platform for generation of chamber-specific cardiac tissues and disease modeling. *Cell.* 2019;176:913–927.e18. doi: 10.1016/j.cell.2018.11.042

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