One Stride Forward: Maturation and Scalable Production of Engineered Human Myocardium

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The capability of obtaining human cardiomyocytes from human pluripotent stem cells (hPSC-CM) opens up an array of medical possibilities from disease modeling, to drug screening, to heart regeneration. The hPSC-CM, however, are structurally and functionally immature relative to their adult counterparts1. To achieve the full potential of hPSC-CM, one main challenge, and a field of intense research, is to drive the hPSC-CM into a phenotype reflecting the biology of the adult cardiomyocyte. Multiple approaches have been employed including long term culture2, electromechanical stimulation3, hormonal treatment4, microRNA5, and tissue engineering6. Though researchers are able to obtain hPSC-CM with increased cell size, decreased circularity index, increased contractile force, enhanced maximum mitochondrial respiratory capacity, and improved calcium dynamics, the achieved hPSC-CM maturation level, in every single aspect, is still far from complete. For example, the immature hPSC-CM typically has minimal phospholamban (PLN) expression7, which makes it impossible for hPSC-CM to respond to increased pacing frequency with enhanced contractile force, or to inotropically and lusitropically respond to β-adrenergic stimulation (although the chronotropic response is present).

In a study published in this issue of Circulation, Tiburcy and colleagues8 studied the maturation status of cardiomyocytes in engineered human myocardium (EHM). This was an impressive international collaboration, involving 31 researchers from Germany, Canada, Israel, the Netherlands and the United States. The EHM was generated by casting cardiomyocytes and non-myocytes in a hydrogel based on type I collagen, as described earlier9, but the authors painstakingly optimized the cellular and chemical components for EHM generation and also EHM culture medium to make the conditions readily adaptable to current good manufacturing practice (cGMP), a requirement for clinical translation.

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CEM is co-founder and equity holder in BEAT Biotherapeutics.
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Prior to this study, procedures to generate cardiac constructs had several aspects that needed refinement: (i) While we know that stromal/fibroblastic cells are needed for construct compaction, matrix remodeling and enhanced force production, it has not been carefully investigated what ratio of cardiomyocytes to stromal cells is optimal for cardiac construct force generation; 2) the usage of undefined additives such as basement membrane preparations (Matrigel or Geltrex) and serum makes it hard for the community to standardize the construct-making process and thus hard to compare the data from different laboratories. In this paper, Tiburcy and colleagues defined cell composition in EHM and showed that a 70%/30% cardiomyocyte/fibroblast input ratio was optimal for force generation, and also, the authors showed that this ratio yields cardiomyocytes with higher sarcomeric protein content and decreases the intra-line and inter-line variability. Tiburcy and colleagues also developed a defined, serum-free EHM construction protocol towards cGMP. Instead of the heavily used rat collagen and Matrigel, the researchers found that medical grade bovine collagen works equally well. In addition, to optimize either cell viability, cardiomyocyte size, non-myocyte cell size, cardiomyocyte and non-myocyte percentage, or enhanced cardiomyocyte α-actinin content, a factorial screening was performed. IGF-1, FGF-2, VEGF165, and TGF-β1 were chosen because this combination of growth factors leads to 1) neutral or enhanced cell viability, and 2) enhanced cardiomyocyte actinin content or cardiomyocyte size. Another important note from the paper is that the authors pointed out that the commonly used cardiomyocyte medium RPMI only contains 0.42 mmol/L of calcium, which is one-third of the physiological level (1.2 mmol/L). When one considers the essential role of calcium for cardiac development and function, we feel an urgent need for the cardiovascular community to realize this and appropriately adjust the RPMI medium.

The maturation status of the cardiomyocytes in the EHM goes significantly beyond the state of the art in several respects.

First, the human cardiac constructs displayed a positive force-frequency relationship (FFR), which is absent in human newborns but develops over the first year of life\textsuperscript{10}. The positive FFR is a complex trait that relates to increased calcium loading of the sarcoplasmic reticulum with increasing stimulation frequency. Multiple components of excitation-contraction coupling must be integrated for this phenomenon, including an increased calcium influx via the L-type calcium channel, increased levels of sarcoendoplasmic reticulum calcium ATPase (SERCA), increased PLB, and potentially, reduced Na/Ca exchange. While our group had recently matured engineered human heart muscle to the point where the FFR went from negative to flat\textsuperscript{11}, to our knowledge, this is the first demonstration of a positive FFR in engineered human myocardium.

Second, the authors observed both inotropic and lusitropic responses of the cardiac constructs to β-adrenergic stimulation, suggesting an upregulation of phospholamban, the calcium handling regulatory protein that is target by β-adrenergic stimulation. This may be the first report of both an inotropic and lusitropic response to β-adrenergic stimulation in hPSC-CM, although Bursac’s group had previously demonstrated a positive inotropic effect\textsuperscript{12}. 

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Third, the cardiomyocytes isolated from EHM are rod-shaped, and impressively, electron microscopy reveals M-bands. In electron micrographs of the sarcomere, the M-band appears as a series of parallel electron-dense lines in the central zone of the A-band. The M-band has been reported to play a role not only in mechanical stability in the activated sarcomere, but also in the biomechanical conditions in contracting muscle such as stress sensing. During human heart development, M-bands develop postnatally. Prior to this paper, there is only one paper showing the appearance of M-bands in the minority of 360-day-old embryoid body-derived cardiomyocytes.

Finally, the contractile force generated by EHM amounted to 6.2 mN/mm$^2$ after 8 weeks in culture and this exceeded the force by papillary muscle from 3–14 months human infants significantly. While this EHM force improved by ~5 times during optimization, they are short of the 23.2 mN/mm$^2$ achieved in EHMs built by Jackman et al, so other conditions remain to be optimized.

Where do we go from here? Obviously, there is still room for improvement. The overall degree of cardiac maturation, though better than previously reported and observed in standard 2D cultures, is still limited and needs to be further improved. The force generated by the EHM is ~6.2 mN/mm$^2$, which is about one-fourth of a normal adult heart muscle. Individual cardiomyocyte volume is less than one-third of their counterparts in adult human heart, mainly due to a significantly smaller cell width. In addition, unbiased global transcriptome profiling suggested that EHM largely similar to human fetal heart at 13 weeks of gestation. It is worth further investigating to assess whether combining multiple cues, e.g. enhancing EHM preload and/or afterload, electrical stimulation, growth hormonal treatment, or microRNA will lead to greater degree of EHM maturation.

Are the EHMs useful in their current state? With a record level of hPSC-CM maturation, one could reason that the EHM serves as a better model for drug screening and disease modeling. Tiburcy et al provide evidence for this in their paper. EHM responded to chronic catecholamine toxicity with some of the classical hallmarks of heart failure, including contractile dysfunction, cardiomyocyte hypertrophy, cardiomyocyte death, and NT-pro-BNP release.

Could the EHM be used for heart regeneration? In the paper, the authors scaled up the EHM patch production, reaching sizes relative to clinical translation and transplanted the EHM patches into normal nude rats through epicardial delivery. Grafts were detected 107 days after implantation, and encouragingly, the grafts were vascularized. However, it was not investigated whether the grafts are electromechanically coupled with the host rat hearts. Actually, histology showed a substantial gap (probably fibrous tissue) between grafts and host hearts (Fig. 5). This indicates that the grafts may not contract in synchrony with the host hearts. Indeed, by $ex$ $vivo$ fluorescent imaging of hPSC-derived cardiomyocytes expressing GCaMP3 calcium reporter, our group has shown that after implantation on the epicardial surface, cardiomyocytes in scaffold-free tissue patches were electromechanically active, but beat slowly and were not electrically coupled to the host infarcted hearts. Histologically, scar tissue physically separated the patch graft and host myocardium. Therefore, additional studies will be required to promote electrical and mechanical
integration of epicardially-implanted engineered tissues, which continues to be a promising
approach.

In conclusion, the present study by Tiburcy and colleagues is a nice piece of work that
advances the field of cardiac tissue engineering by establishing a defined protocol to
generate and culture functional human heart muscles from pluripotent stem cells. Many
questions including those formulated above need to be answered, but this current study made
significant strides in standardizing the production and maturation of engineered human
myocardium.

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