Characterization of a Physiologically Relevant Screening Platform for hiPSC-derived Cardiomyocytes

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Abstract
Cardiac toxicity plays an important role in the failure of therapeutic agents in late stages of clinical trials, as well as for the removal of approved drugs from the market. Current approaches on the study of acute cardiac safety and toxicity have been transformed with the availability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The Comprehensive in vitro Proliferation Assay (CIPA) constitutes a novel safety screening proposal intended to replace current regulatory strategies that have failed to predict the acute toxic effects of developing drugs. Through the CIPA initiative, researchers from diverse organizations such as the FDA, academic institutions, and pharmaceutical companies are evaluating hiPSC-CMs as an integral tool for the safety assessment of novel therapeutic compounds. Nonetheless, key challenges under consideration for the hiPSC-CM system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization and overall electro physiological maturity. As an example, hiPSC-CMs frequently display undefined or disarrayed sarcomeric organization when plated in standard cell cultureware. We have developed a novel high-density screening platform for hiPSC-CMs that is micro-engineered to emulate correct cardiac muscle fiber organization. This platform allows for passive self-alignment of hiPSC-CMs, leading to improved sarcomeric organization, as seen by readily identifiable, correctly patterned myofibrils along the cell body. Concomitantly, directionality of contraction of hiPSC-CM preparations were observed to be increased in our platform. We also observed increased gene expression of KCNJ5, KCNJ6, KCNJ7, as well as cardiac cell junction components plakoglobin and desmoplakin. In addition, we did a comprehensive analysis of calcium flux in hiPSC-CMs and observed that microHeart® screening platform influences cardiomyocyte physiology, which can be of great importance for the evaluation of compound cardiotoxicity. Altogether we describe a novel screening platform for hiPSC-derived cardiomyocytes amenable to high throughput screening that leads to greater physiological relevance.

Methods
hiPSC-CMs from two independent sources (StemonX® - STMX-CMs and Cellular Dynamics iCell® Cardiomyocytes™ - iCMs) were cultured on standard cell cultureware (Control) or microHeart® 384-well plates for 6 to 14 days prior to analyses. Cell cultures were analyzed by bright light microscopy optical flow and immunofluorescence in a ImageXpress Micro High Content Confocal (Molecular Devices). Gene expression was analyzed with TaqMan® Real-Time assays (Applied Biosystems®). Recordings of hiPSC-CM spontaneous beating for motion tracking studies were performed prior to analyses of dynamic changes in intracellular calcium flux was monitored in a High-Throughput Fluorescent Imaging Plate Reader (FLIPper®, Molecular Devices).

Results

**Figure 1:** Compared to standard cell cultureware (A, C) microHeart® promotes alignment of STMX-CMs with evident structural reorganization of the culture topography (B, D). Density histograms of light scattering angles caused by cells in regular cell cultureware show a wide distribution (E), while in microHeart® light is more tightly scattered near a 90° angle, validating cell alignment (F).

**Figure 2:** A: Representative cardiomyocyte motion tracking analysis of STMX-CMs plated on control plates or microHeart®. Arrows highlight vectors indicating direction of the contractions. On microHeart®, hiPSC-CMs contract mostly along one single direction, more closely resembling the physiological contractile behavior of human heart tissue. Optical flow analysis script obtained from Huebsch N. et al., Tissue Eng Part C Methods, 2015. B: Contraction vector maps obtained with Sony Biotech S8000 Motion Vector Software confirmed unidirectionality of contraction.

**Figure 3:** Representative immunofluorescence analysis of hiPSC-CM from two independent sources plated on control plates or microHeart®. After 6 days of culture on microHeart®, hiPSC-CMs display readily identifiable, aligned sarcomeres. cTNT: Cardiac Troponin T. SCAAct: Sarcomeric Alpha Actinin

**Figure 4:** Representative immunostaining highlighting the cardiac intercalated disc marker plakoglobin in iCMs plated on control or microHeart® plates. Arrows highlight distal intercalated discs. On microHeart®, Plakoglobin is more frequently restricted to distal intercalated discs, as opposed to being distributed throughout the cell perimeter, as in standard cell cultureware (arrows).

**Figure 5:** High Content Analysis of nuclear parameters changes indicates relevant and significant changes between iCMs cultured on control plates or microHeart®.

**Figure 6:** Expression analysis of key genes linked to different aspects of cardiac physiology. STMX-CMs plated on microHeart® show increased expression levels of genes implicated in calcium handling, ion channels, cardiac junction proteins and cardiac transcription factors.

**Figure 7:** STMX-CMs were assayed for baseline calcium flux. Peak amplitude, peak decay time and peak width at 10% amplitude showed significant differences in microHeart®, suggesting faster and possibly more efficient calcium cycling. Significance in unpaired t-test, **** p<0.0001.

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**Conclusion**
MicroHeart® is a novel high throughput screening platform that promotes hiPSC-CM alignment, leading to a more physiological cell geometry and a coherent directionality of mechanical contraction. In addition, microHeart® enables more defined sarcomeric organization, proper targeting of cardiac cell junction proteins to distal intercalated discs, nuclear re-organization and induces changes in the expression of genes that are critical for cardiac physiology, ultimately supporting a functional improvement in calcium cycling.