Introduction
Cardiotoxicity is one of the major causes of failed drug development and withdrawals. Development and implementation of sensitive in vitro assays that can evaluate potential adverse effects on human cardiac tissue is extremely important to the drug discovery process. Over-expressed HER2 and other cardiac ion channels screening assays are the major in vitro assays to predict cardiotoxicity. However, the predictability of ion channel screening assays for clinic cardiotoxicity outcomes is not satisfied. Stem cells provide a renewable and reproducible source of human cells for drug safety testing. Cardiomyocytes derived from human embryonic stem cells (hESC) provide a physiological relevant model system for drug toxicity testing. GE Healthcare’s CytoLine™ Plus cardiomyocytes, which were used in this study, are differentiated from the human embryonic hESCs and have been fully characterized for cardiac transcription factor expression, structural markers and individual ion channel activity. Three technologies were used in this study to develop comprehensive and predictive cardiotoxicity assays using human stem cell derived cardiomyocytes (hESC-CM): multi-electrode array (MEA), conventional patch clamp and image-based high content analysis (HCA). The MEA measuring cardiac field potential variations in the cultured cardiomyocytes that compare to the original condition. The conventional patch clamp measuring action potential parameters confirms MEA data. The HCA measuring cell health parameters can detect the compounds that cause structural damage, which may not be identified by electrophysiological methods. This study demonstrated that using hESC-CM by MEA and HCA can facilitate cardiotoxicity identification.

Methods
Field Potential Measurement by Multi-Electrode Array (MEA) in CytoLine™ Plus Cardiomyocytes

To determine the cardiotoxicity, MEA was used to record action potential parameters. The study was carried out using a 124-well MEA plate (Acell Biosystems). The 37°C buffered saline containing CO2 was made up and introduced to the MEA at a flow rate of 4 µL/min to 37°C. After 2 days, the culture media was replaced with a fresh medium at 37°C for 2 days. The experimental groups were treated with different concentrations of test compounds, and the control group was treated with the test compound and fresh medium only. The cell viability was measured using the MTT test.

Results

Conclusion
This study validated that extracellular field potential measurement assay by multi-electrode array (MEA) from human embryonic stem cell derived cardiomyocytes can correctly identify drugs that have known cardiac effect. The MEA assay can detect the compounds that interact with cardiac ion channels other than HER2. The manual patch clamp assay measuring cardiac action potential provides additional mechanistic information. Image-based HCA combined with MEA screening is a powerful tool for in vitro structural cardiotoxicity assessment. The Kd values generated demonstrated high concordance with published values. More adverse effects were observed with HER2 and the structural cardiotoxins (mesotansone, suramin, sorafenib and amiodarone) significantly modulating all assay parameters. Mitochondrial membrane potential (MMP) was the most sensitive and specific indicator of structural cardiotoxicity. Omax values demonstrated high correlation with published BNP expressions indicating toxicity detection at in vivo therapeutic levels. This study demonstrates the utility of MEA and HCA assays using hESC-derived CytoLine™ Plus cardiomyocytes to facilitate cardiotoxicity hazard identification and provide insight into the intracellular mechanisms mediating in cardiotoxicity.

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