ON-CHIP PRE-CLINICAL CARDIAC TOXICITY: TESTING COMPOUNDS BEYOND hERG AND QT USING hES/hiPS CARDIOMYOCYTE RE-ENTRY CELL NETWORK MODEL ON A CHIP

K. Yasuda*, T. Kaneko and F. Nomura
Tokyo Medical and Dental University, JAPAN

ABSTRACT

Limitation of conventional hERG assay and QT prolongation testing for accurate prediction of Torsades de Pointes (TdP) by compounds unvailed us the necesity of new approach to evaluate global cardiac safety. On-chip re-entry cell network model assay has the potential to measure the TdP probability as pre-clinical testing for cardiac safety by the single cell-based optical/electrical measurement of the heart pressure, Na, K, Ca ion channel conditions and their fluctuations. The application of ion channel inhibitors resulted in dose-dependent changes to the field potential waveform, and these changes were identical to those induced in the native cardiomyocytes. This study shows that on- chip model represent a promising *in vitro* assay for cardiac toxicity and drug screening.

KEYWORDS: Torsades de Pointes (TdP), QT prolongation, cardiac toxicity, on-chip cellomics, functional reconstitution of cardiac cell network model, re-entry model, microelectrode array, agarose microfabrication, community effect, field potential duration (FPD), hES/hiPS delivered cardiomyocyte cells, Poincaré plotting

INTRODUCTION

QT prolongation is a major safety concern for selecting and developing candidate compounds. The current integrated assay systems using hERG-transfected HEK-293/CHO-cells (hERG assay), isolated animal tissues (APD or MAP assay) and conscious and/or anesthetized whole animals (QT or MAP assay) may identify the QT prolongation, but still cannot fully predict the potential lethal arrhythmia including Torsades de Pointes (TdP) or ventricular fibrillation (VF) of drug candidates. Such limitations of conventional hERG assay and QT prolongation testing for accurate prediction of TdP and VF by compounds revealed us the necessity of new cell-network-based approach to evaluate global cardiac safety because these lethal arrhythmias are caused by the asynchronization of the signal propagation in heart tissue (Fig. 1) [1-3].

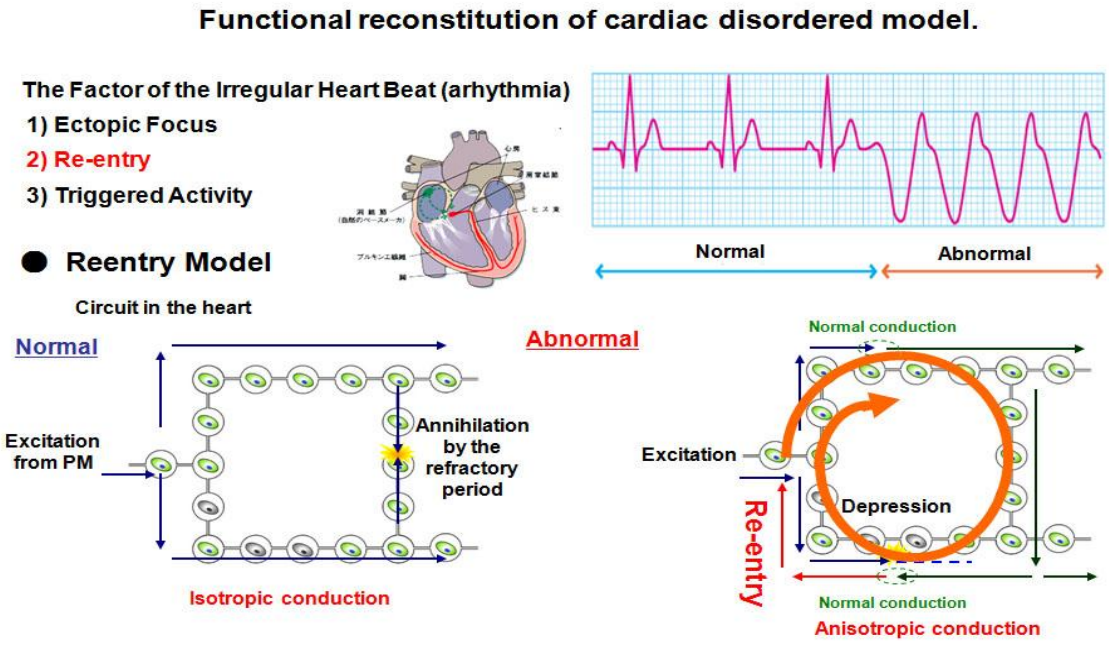

Figure 1. Concept of single-cell-based on-chip re-entry model for pre-clinical testing for TdP prediction. The network formation enables us to make a model of the signal propagation in the heart tissue.

Principle

Spatial and temporal regulation of cellular orientation, community size and shape, variety and interactions is one of the keys to resolve the mechanism of the higher complexity of cellular system like tissue and organ [4]. For example, the community size of cardiomyocyte cell group is important for the maintenance of stable beating intervals, and the difference of community size also gives us different results by same compounds (Fig. 2) [5-6]. As TdP is causedincreasing of the by the fluctuation of ion channel responses, exploiting “community effect” of cell network is essential.

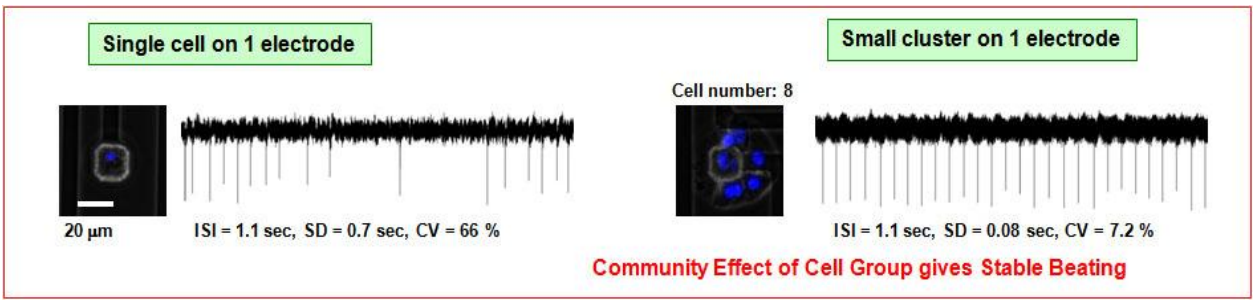

Figure 2. Stabilization of single cell response caused by community effect. Left, unstable beating of isolated single cell; right stable beating of single cell in the 8 cells community.

EXPERIMENTAL

To study the re-entry cardiomyocyte cell network assay, we have developed the on-chip cell network cultivation system, and extra-cellular signals (field potentials: FP) of human embryonic cardiomyocytes in geometrically patterning chambers have been recorded with on-chip multi electrode array (MEA) system. Then, we have functionally reconstructed the normal and abnormal re-entry model of cardiomyocytes network loop from the viewpoint of propagation of contractile signals to be able to include the characteristics of heart into the chip like the functional spiral re-entry model (Figs. 3 and 4). And we found that the on-chip cardiomyocyte cell network assay is expected to be one of the candidates having the potential to measure the TdP and VF probability as pre-clinical testing for cardiac safety.

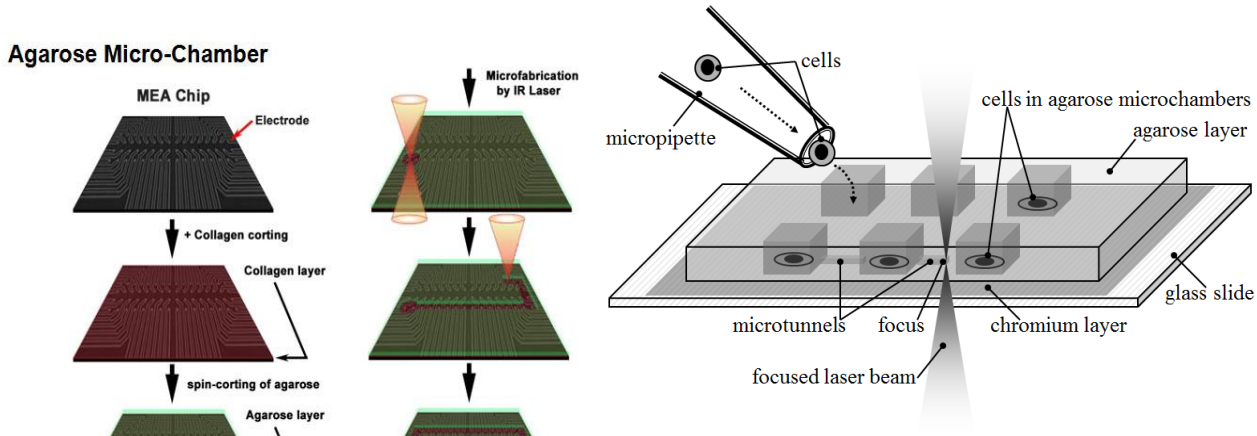

Figure 3. Formation of agarose microstructure to control the spatial arrangement of cells.

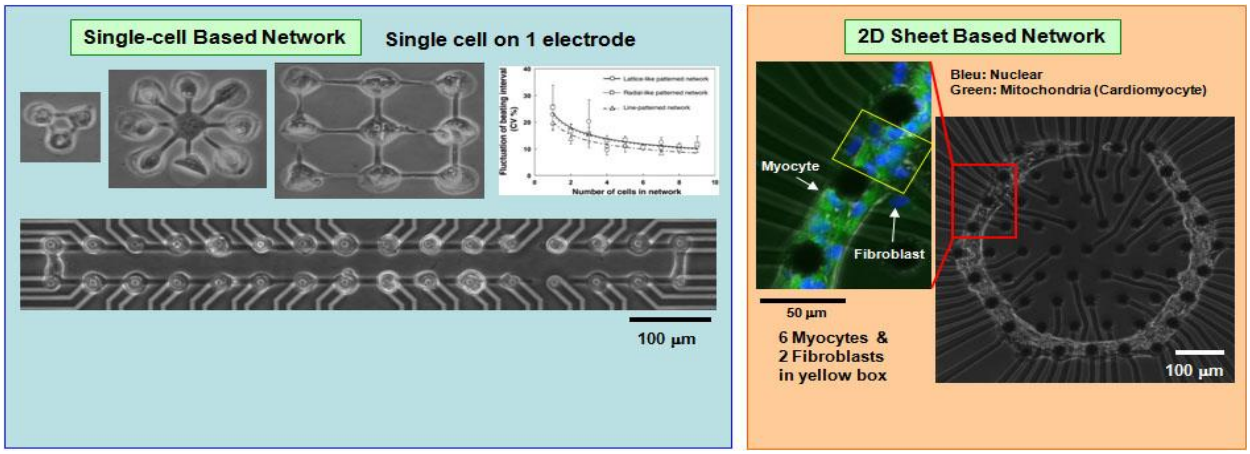

Figure 4. Formation of single-cell-based re-entry cell circuit in the agarose microstructures on a MEA chip.

RESULTS AND DISCUSSION

To characterize the electrophysiological properties of *in vitro* re-entry model, the FPs of the spontaneously beating cardiomyocyte cells were recorded using the On-Chip measurement system. First, we investigated the effects of ion chan-

nel-specific inhibitors on the FP waveform, to determine whether the expressed channels were functional. The sodium channel inhibitor quinidine dose-dependently and reversibly attenuated the length of time required for the decline in voltage from the baseline to the first negative peak (FP amplitude), which reflects the action potential (AP) upstroke, without significant changes in the spontaneous beating rate. Next, we tested the effect of the calcium channel blocker verapamil on the FP waveform. Verapamil dose-dependently shortened the length of time from the first negative peak to the first positive peak (field potential duration, FPD), which was consistent with the effect of calcium channel inhibition on AP duration. Disruption of potassium channels might lead to prolongation of cardiac repolarization, potentially leading to life-threatening arrhythmias, e.g., TdP and ventricular tachycardia. Therefore, we examined the effect of the I_{Kr} blocker E-4031 on the FP waveform. E-4031 dose-dependently and reversibly shortened prolonged the FPD (Fig. 5).

To study the response to adrenergic stimulation, isoproterenol was added to the cells. Isoproterenol dose-dependently increased the beating frequency of the cardiomyocytes, as compared with the baseline frequency.

The on-chip assay for providing further insight into the extrapolation of preclinical data to human clinical settings or for replacing existing *in vitro* and *in vivo* cardio-toxicity models has the following features; i) abnormal triggering (temporal dispersion) causing lethal arrhythmias is estimated by analyzing the time course field potential dispersion of single cells in loop network using Poincaré plotting (Fig. 5), ii) spatial dispersion of cells causing spiral re-entry is modeled by using wider width of cell network loop which can choose a different propagation pathways of cells among neighboring circulations, and iii) human ES / iPS cell-based cardiomyocyte is used for cell network formation [7].

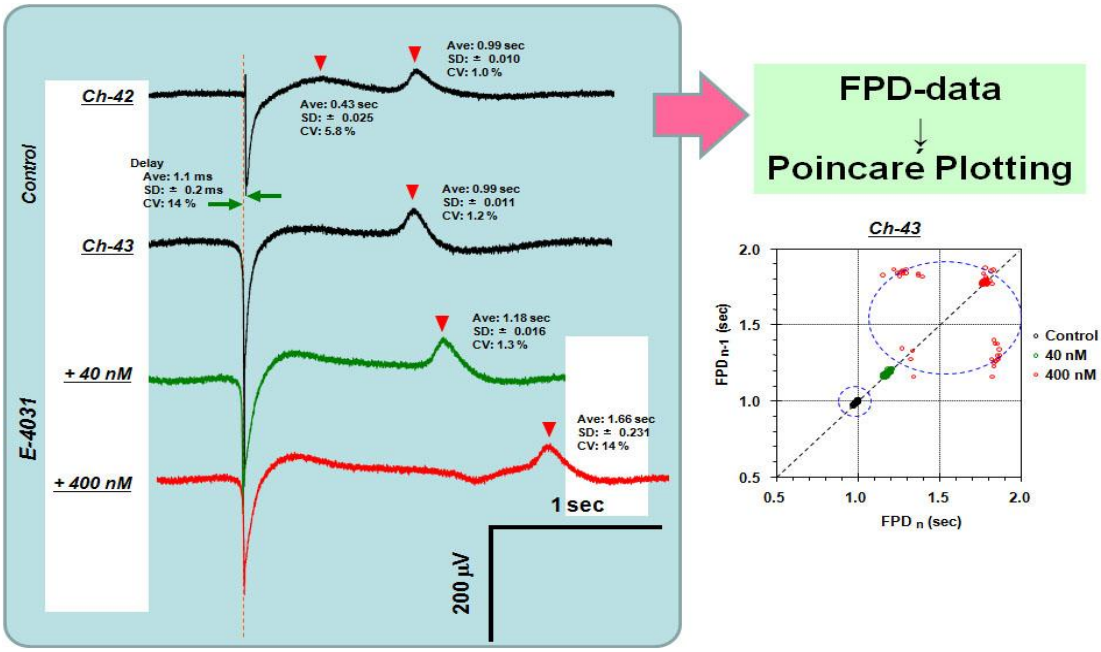

Figure 5. TdP prediction by measuring the fluctuation change of FPD duration among neighboring beatings (Poincare plotting).

CONCLUSION

On-chip cardiomyocyte cell network-based re-entry model assay has been developed and examined its response against the the potential to measure the TdP probability as pre-clinical testing for cardiac safety.

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CONTACT

*K. Yasuda, tel: +81-3-5280-8046; yasuda.bmi@tmd.ac.jp