Beating heart-on-a-chip: Integration of electrodes to measure contractility of cardiac spheroids

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INTRODUCTION

Human cardiac spheroids hold great potential to study cardiac therapies in vitro. We are developing multi-organ microphysiological system (MPS) for cardiometabolic research using cardiac spheroids, liver and pancreas organ models[1,2]. However, there are no commercially available technologies to evaluate contractility, a key heart function, of 3D spheroids cultured on chip. Indeed, the lack of disease relevant readouts critically limits the use of cardiac spheroids to study efficacy of cardiac therapies. Therefore, we have embarked upon integrating electrodes on chip to follow effects of drugs on cardiac contractility.

SETUP OVERVIEW

In this work we used the HUMIMIC Chip2 featuring multiple electrodes integrated at the bottom of mini-wells, in which a single cardiac spheroid can be cultured. The chips are connected via a flat cable to the HUMIMIC ActSense, a device capable of measuring voltage signals down to microvolts.

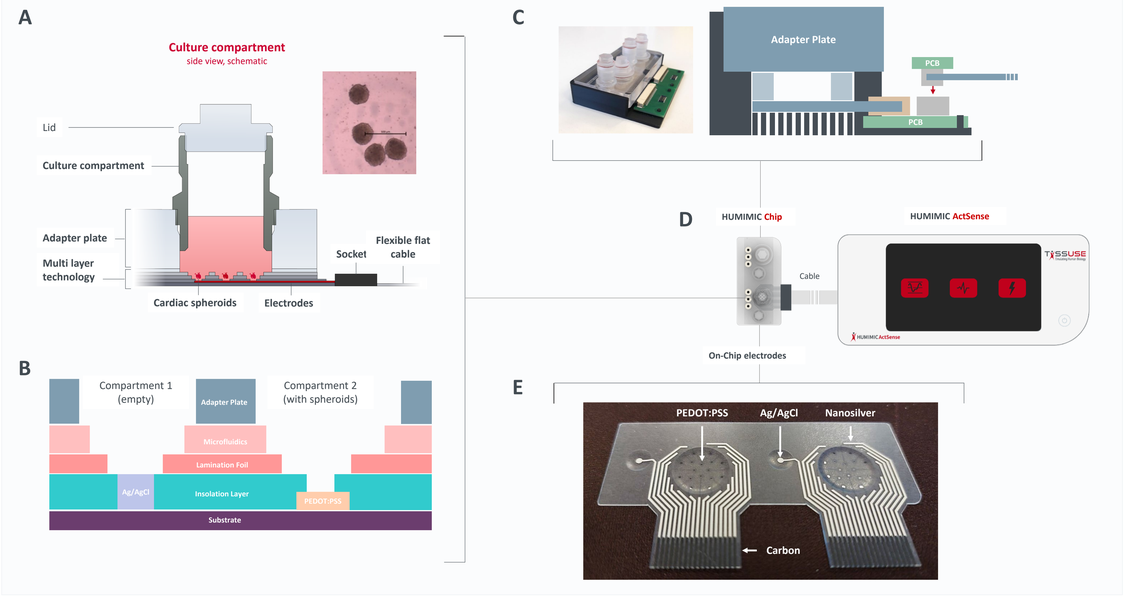

Fig. 1 Setup Overview. (A) Cross-section schematic representation of the culture compartment. (Insert) Brightfield microscopy of spheroids on top of transparent PEDOT:PSS electrodes (B) Cross-section of the different layers of the stack constituting the HUMIMIC Chip2. (C) Cross-section of the electrical connection between the HUMIMIC Chip2 and the external cable. The chip is mechanically stabilized by means of a 3D-printed case (black). (D) Rendering of the entire setup. (E) Picture of the sensing layer with annotation of the different materials.

SENSING LAYER

The material of the integrated electrodes is conductive poly (3,4-ethylenedioxythiophene): poly(sodium4-styrenesulfonate) (PEDOT:PSS), a transparent conductive polymer that can be easily ink-jet printed on plastic materials at a very high throughput. The material is characterized by low thermal noise and superior charge injection when compared to standard metal electrodes, thus resulting in higher signal-to-noise recordings. Moreover, the transparency of the electrodes allow a simultaneous monitoring of the culture by means of standard microscopy techniques.

In a revised design, an Ag/AgCl reference electrode was added to the second compartment of the HUMIMIC Chip2, which was left empty and without the cardiac spheroid culture. This was done to provide a more accurately maintained potential the measurements can be referenced to.

ELECTRODE CONFIGURATION

The measurement setup allows to measure up to 8 sensing sites in a referential montage configuration (employing a reference-electrode) or up to 16 sensing sites in a differential montage, thus resulting in 8 parallel data streams, recorded at a sampling rate of 250 samples per second.

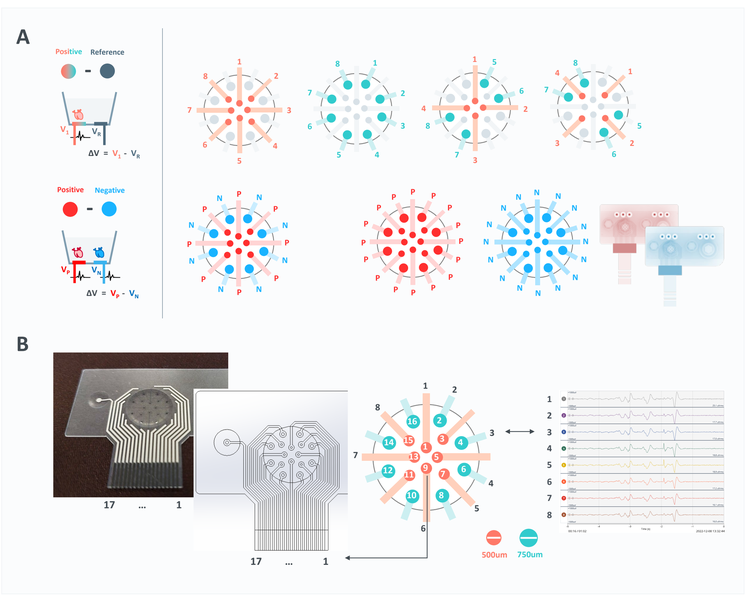

Fig. 2 Electrode configuration and data visualization.

(A) Measurement configuration: the sensing sites and the type of measurement (referential vs. differential) are selected.

(Top) Referential montage: the electrical signal of the sensing site (with spheroid) is measured against the voltage of a reference electrode (empty). The diameter of the sensing sites are 500 µm (orange) or 750 µm (turquoise).

(Bottom) Differential montage: the electrical signal of one sensing site (red) is measurement against another sensing site (blue).

(B) Electrode indexing and data stream visualization

RESULTS

First, we confirmed the biocompatibility of all the materials constituting the stack of the multilayered chip. 3D cardiac spheroids formed of human induced pluripotent stem cell (hiPSC) derived cardiomyocytes and human primary fibroblasts showed similar viability on electrode-chips as in the standard well plate culture. Next, we cultured hiPSC-derived cardiomyocytes on the electrode-chip as a monolayer and monitored their electrical activity for 14 days. Upon controlled administration of isoprenaline, a synthetic catecholamine that increases the heart rate and cardiac contractility, the spontaneously contracting cardiomyocytes increased their beat rate. This positive chronotropic effect did not only prove the expected in vivo like response[3] but also demonstrated functionality of the electrodes.

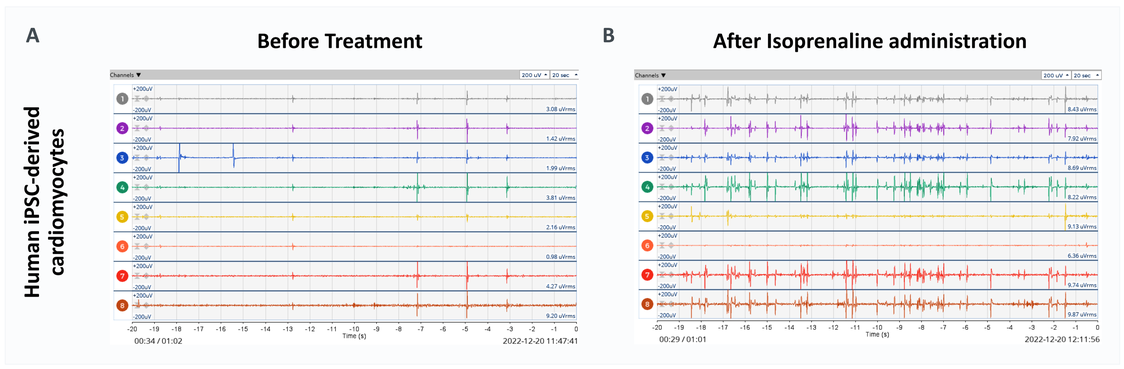

Fig. 3 Recorded electrical activity of the cardiomyocytes, (A) before and (B) after Isoprenaline administration.

DISCUSSION

The recorded electrical signal requires additional processing and filtering to highlight buried features and enable further analysis. A band-pass filter was used to filter out high-frequency noise and eliminate low-frequency signal drift. It is worth noting, however, that valuable information can be lost or data interpretation is compromised (e.g. the identification of the different beating phases), if the signal is not filtered properly. The shape of the recorded beating cycle differs from those of a standard field potential or clinical ECG possibly because of the relatively large size of the electrodes. The result is a hybrid measurement of the electrical activity at cellular level and of the impedance change generated by the mechano-electrical effect of the contraction of a cardiac spheroid (or cell monolayer) within the confined space of the mini-well.

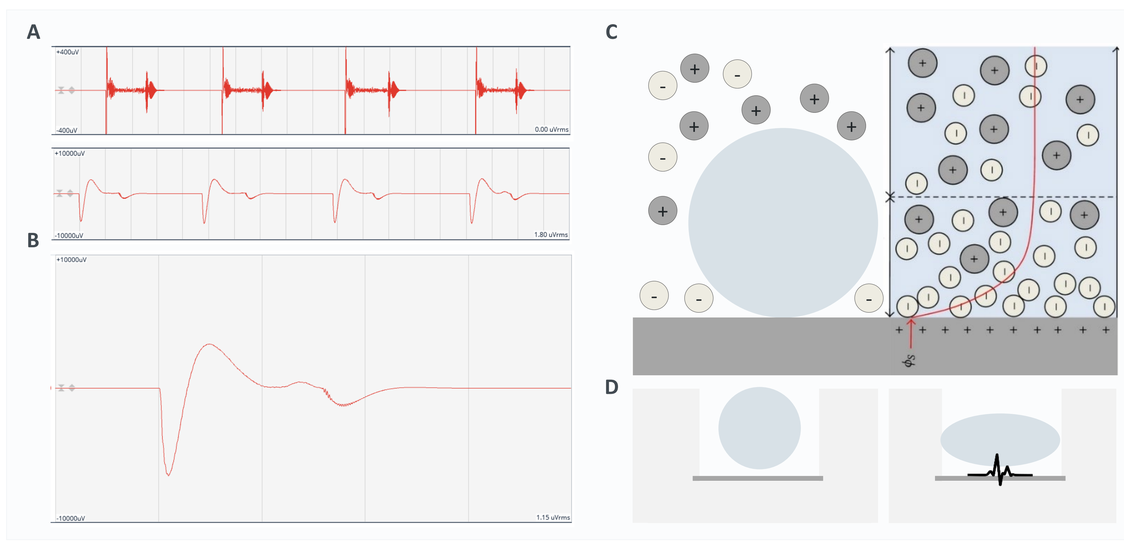

Fig. 4 Electrical signal filtering and data interpretation. (A) Effect of signal processing: (top) not optimized vs (bottom) optimized filtering. (B) Processed signal of one beating phase. (C) Representation of the electrical double layer and ions distribution in the compartment around the spheroid. (D) Visualization of the contraction of a cardiac spheroid or a monolayer.

SUMMARY AND OUTLOOK

Development of disease-relevant readouts, such as electrode-chip technology, can utilize the full potential of the MPS and shall aid the discovery of new therapies. The long-term probing of cells' electrical activity provides, in fact, fundamental information on dynamic tissue responses and organ-specific reactions. Consequently, the ability to simultaneously and continuously exploit different electrical sensing and actuation techniques in a single device will be an essential feature. Therefore, we will next combine the presented multi-channel voltage sensing with multi-frequency impedance spectroscopy analysis and electrical stimulation.

REFERENCES

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