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# SIMULTANEOUS IMPEDANCE SPECTROSCOPY AND STIMULATION OF HUMAN IPS-DERIVED CARDIAC 3D SPHEROIDS IN HANGING-DROP NETWORKS

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## ABSTRACT

Here, we present electrical impedance spectroscopy (EIS) data of human iPS-derived cardiac 3D spheroids with electric stimulation integrated in a hanging drop network. Microscopy videos of the beating spheroids were correlated with synchronously obtained EIS recordings. For stimulation, the spheroid was exposed to a continuous sinusoidal electric field – in contrast to traditional pulse trains. This stimulating field was supplied via the same electrodes that were used for the EIS recordings. Our measurements revealed a beating frequency modulation upon tuning the stimulation signal amplitude.

## INTRODUCTION

Cardiac dysrhythmia and cardiotoxicity are the most common causes for drug withdrawals from the market and termination of drug development at late stages [1]. Earlier identification of these potentially fatal side effects requires new in-vitro toxicity screening methods. It has been shown that cells arranged in 3D spheroids mimic in-vivo conditions more closely than traditional 2D cell cultures; spheroids, however, require novel systems for culturing and analysis [2]. Reconfigurable hanging drop networks can be used to combine spheroid formation and microfluidic culturing in a single platform [3]. Here, we add an exchangeable EIS plug-in to a similar hanging drop network, we characterize the beating of human iPS-derived cardiac spheroids by EIS as well as optical means, and we monitor the beating frequency upon electrical stimulation via the same integrated microelectrodes.

## MATERIALS AND METHODS

### Hanging Drop Network

The hanging drop network depicted in Figure 1 accommodates up to eight spheroids in separate hanging drops that are interconnected by microchannels. The perimeter of the hanging drops is confined by hydrophobic PDMS rims. The PDMS piece is bonded onto a glass substrate, and the entire chip is then flipped with the PDMS structure facing downwards. The chip is therefore open at the bottom, so that drops are suspended in air hanging from the chip. The substrate can then be placed on top of an inverted microscope. The EIS plug-in comprising the platinum electrodes is inserted into a rectangular recess in the PDMS matrix that has been realized for one of the drops. By using this plug-in approach, most of the droplet system consists of PDMS, which is bio-compatible, and the surface and wetting properties of which can be easily modified through O<sub>2</sub>-plasma treatment.

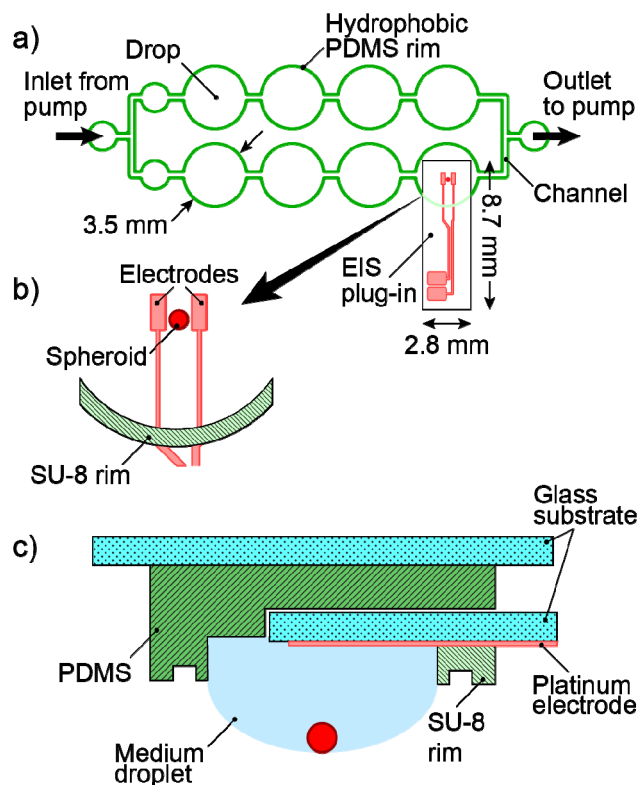


Figure 1: **Schematic** overview of the device in a), close-up of the measurement region in b) and side view of the drop with the EIS plug-in in c). The removable EIS unit features a set of coplanar electrodes for readout and stimulation of the spheroid. Inlet and outlet are connected to a syringe pump, the electrode pads are connected to an impedance spectroscopy and a transimpedance amplifier for EIS readout and stimulation.

The hanging drop network was fabricated by using standard soft lithography methods as described previously [3]. The EIS plug-in was fabricated on a glass substrate with integrated platinum microelectrodes patterned by lift-off. The plug-in also featured an SU-8 wall, which sealed tightly to the sidewalls of the drop rim to yield a leakage-free overall droplet compartment and overall microfluidic network. After SU-8 development, the glass wafer was diced into separate plug-ins.

### Electrical Setup

The EIS plug-in of the chip was wire-bonded to a custom printed circuit board facilitating the electrical interfacing of the platform. An HF2 impedance spectroscopy was used to provide the input voltages for the EIS measurements, as well as delivering the

stimulation signals to the spheroid. The output current was transformed into a voltage by using an HF2TA trans-impedance amplifier and then fed back to the impedance spectroscopy (both from Zurich Instruments AG, Switzerland). The applied signal amplitudes for EIS measurements were 100 mV per frequency while simultaneously using up to eight individual frequencies between 10 kHz and 15 MHz. For stimulation, a 1 kHz sine-wave of 4.5 V - 8 V amplitude was applied in parallel to the EIS measurement via the same electrode pair. Data were recorded on a PC and later analyzed using a custom Matlab tool (Mathworks Inc, USA). For visualization of the signal magnitude and phase spectra, the baseline has been subtracted.

### Microscopy

The microchip was placed in a holder frame on the stage of an inverted microscope (Leica DMI 6000B), which was inside a custom heating chamber and stage top incubator operating at 37°C in 5% CO<sub>2</sub> atmosphere at 95% humidity. Videos were acquired with a Leica DFR 320 camera (Leica Microsystems, Switzerland) at 30 fps, an exposure time of 1 ms, 2 x 2 binning and pixel depth of 8 bit to accommodate sufficiently high video frame rates, which can resolve temporal dynamics of the beating process. The inlet and outlet of the chip were connected to Nemesys syringe pumps (Cetoni GmbH, Germany) to perfuse the network with medium.

### Image Analysis

The recorded videos were analyzed by using a custom C# tool. For each frame, the preceding frame was subtracted, so that the motion of the spheroid could be extracted from this differential video. The number of pixels in the differential image with an intensity value above a defined threshold was then determined. Therefore, the optical signals have low values during periods of little or no spheroid motion and peaks during the beating of the spheroid. Visualization of the differential image sequence shows white pixels in areas with large motion of the spheroid tissue and black areas elsewhere.

### Spheroid Formation

Human iPS-derived cardiac microtissue spheroids and cardiac maintenance medium were obtained ready-to-use from InSphero AG, Zurich, Switzerland.

For experiments, a pre-formed spheroid was inserted into the hanging drop containing the EIS plug-in by manual pipetting.

## RESULTS AND DISCUSSION

### Optical and EIS Beating Analysis

The cardiac spheroids were beating spontaneously, as was observed optically and by EIS in parallel, and the results are shown in Figures 2 and 3. The differential optical signal in Figure 2 (bottom curve, left axis) showed a spike, whenever the spheroid was beating. The smaller secondary peak is an artefact of the differential image analysis method and pertains to the same beat as the larger primary peak. Each peak in the optical signal was

coinciding with a peak in the 5.3 MHz impedance magnitude signal (upper curve, right axis). The spheroids were beating regularly, when fresh medium was continuously supplied. The arrhythmic beating shown in Figure 2 was evoked upon stopping perfusion through subsequent nutrient depletion (about 10 minutes).

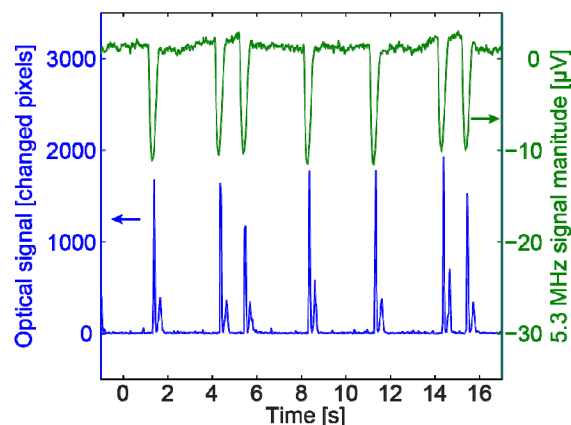


Figure 2: **Beating sequences** obtained by microscopic video recordings (blue, left axis) and EIS-recording (green, right axis) show a clear correlation, which indicates that EIS is a suitable tool to extract the beating frequency of the cardiac spheroid in the hanging droplet.

The change in the impedance signal due to the beating of the spheroid is analyzed in more detail in Figure 3: The image sequence (Figure 3a) and impedance magnitude and phase spectra (Figure 3b and c) show the very same beating cycle. The spectra were obtained from the time-domain signals, which were similar to the one shown in Figure 2, by subtracting the baseline level from the beating-induced spike. These peak-to-baseline signals were evaluated at each of the eight frequencies in terms of magnitude and phase component to obtain the spectra in Figures 3b and 3c.

During the maximal contraction of the spheroid ( $t=0$ , green frame and green line), the magnitude over the entire spectral range decreased, while the phase spectrum showed a change in slope. Furthermore, the EIS data showed – in agreement with optical analysis – that spheroid contraction is about 5 times faster than full relaxation: The differential images as well as the EIS results revealed that the contraction of the spheroid, accompanied by a corresponding decrease in the signal magnitude spectrum, took approximately 0.2 s. The full relaxation of the spheroid to its initial state, and the corresponding increase of the signal magnitude spectrum back to the original values, however, took more than 1 s.

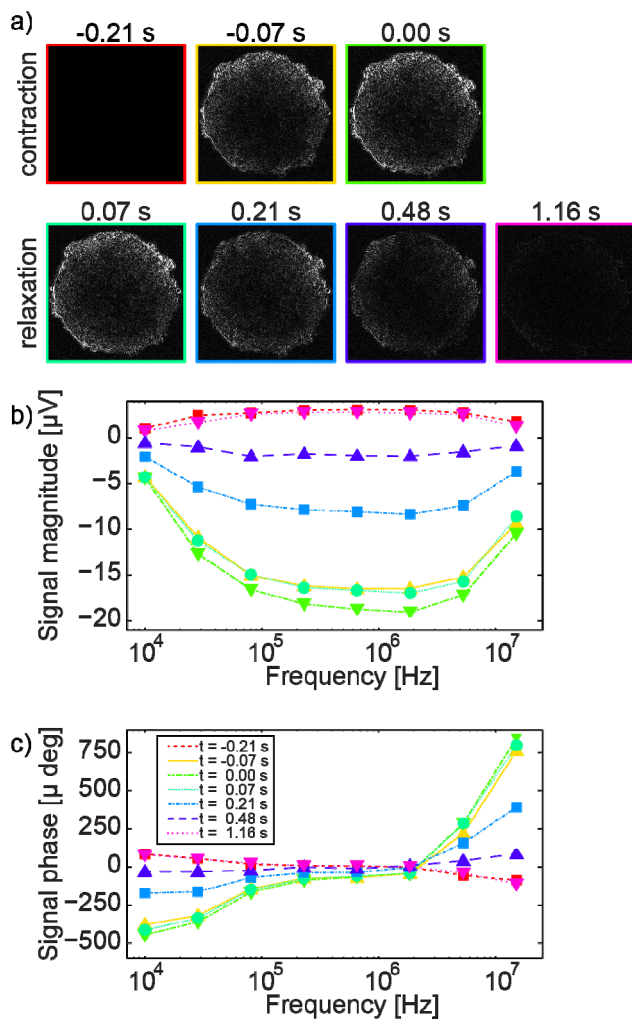


Figure 3: **Single beat characteristics** as observed optically in a) and through EIS in terms of signal magnitude in b) and phase spectra in c.). The timing of all three recorded signal is identical, and the times are displayed in a) and c).

### Stimulated Beating

Upon applying a 1 kHz sine wave of amplitudes between 4.5 V – 8 V to a regularly beating spheroid, the spheroid beating frequency could be tuned (Figure 4a, b). As observed optically and through EIS, the 1 kHz stimulation signal influenced the beating frequency of the spheroid that could be modulated in dependence of the stimulus amplitude. Larger stimulus amplitudes led to higher beating frequencies of up to 3 Hz at 8 V. The observed relationship between the evoked beating frequency and applied stimulation signal amplitude was almost linear and had a slope of 0.7 Hz/V (Figure 4c). Amplitudes above 8 V were not accessible due to hardware limitations; lower frequencies (below 4.5 V stimulus amplitude) produced arrhythmic beating. The amplitude-frequency modulation did not show hysteresis behavior upon ramping the stimulus amplitude up and down.

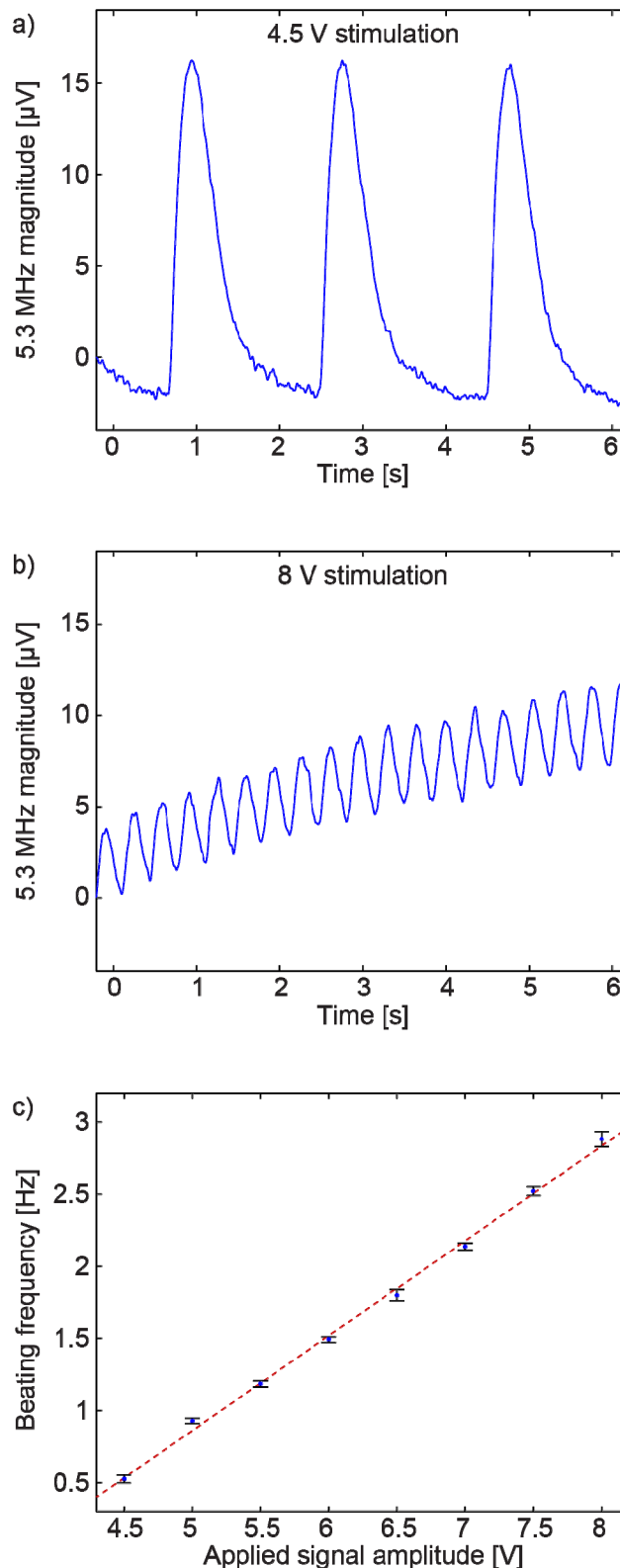


Figure 4: **Stimulated beating** with amplitude-frequency modulation and parallel EIS readout. Slow beating at an applied stimulation signal amplitude of 4.5 V is shown in a), fast beating upon applying 8 V in b), a stimulation voltage sweep and the resulting spheroid beating frequencies in c). Each data point in c) corresponds to at least 9 beating cycles from which the mean and standard deviations were obtained and displayed.

## CONCLUSION

We presented the successful integration of an EIS unit into a hanging drop network, and were able to simultaneously characterize the beating of human cardiac spheroids optically and by means of EIS. The detection of arrhythmias as shown in Figure 2 for the case of medium depletion or the detection of irregularities in contraction/relaxation duration are fundamentally important. Such features may also occur upon drug administration and may then be directly correlated to the nature of the applied different drugs and the respective dosages. We further observed an amplitude/frequency modulation upon varying the electrical stimulation signal that was applied to the cardiac spheroids. Advantages of EIS over optical detection methods include the possibility for device integration, the associated higher temporal resolution (typically better than 1 kHz) and label-free non-invasive multi-parameter analysis.

The system presented here holds great potential for addressing key questions of cardiotoxicity in the emerging field of 3D microtissues.

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