OPTICAL MAPPING OF COMPLEX ELECTRICAL ACTIVITY IN ENGINEERED CARDIAC TISSUE CONSTRUCTS

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INTRODUCTION

Ensuring normal synchronized electrical behavior is a unique and critical requirement in the design of cardiac muscle *in vitro*. Even though tissue replacement is the ultimate goal, there are other, more immediate applications that could benefit from pertinent efforts – arrhythmia and defibrillation research, for example [1]. Previously, we have shown that cardiac cells grown on 3D deeply microgrooved elastic scaffolds self-organize to exhibit different electromechanical properties (calcium transients and nuclear deformation) than cells grown on flat substrates [2]. In this study, we set out to explore the excitability of these cardiac constructs and their capacity to support normal propagation, as well as signature arrhythmogenic activities.

METHODS

Scaffolds and Cardiac Myocytes Culture

3D scaffolds for cell growth were made from poly-dimethylsiloxane (PDMS), cast onto micro-grooved masters with depths in the range (7-50μm) and groove to groove spacing of (60-500μm). Neonatal rat ventricular cardiomyocytes were cultured as described previously [3]. Briefly, cardiomyocytes were isolated from the ventricles of 3-day old rats by enzymatic digestion with trypsin and collagenase. Cells were plated at high density onto fibronectin-coated scaffolds to allow reconstitution of cell-to-cell contacts and establishment of a functional syncytium.

Optical Mapping of Electrical Activity

For fluorescence measurements, cells were stained with either Fura-2 for ratiometric intra-cellular calcium ($[Ca^{2+}]_i$) determination or with di-8-ANEPPS for transmembrane potentials (V_m). Recordings were made with a photomultiplier tube for microscopic measurements and a macroscope-based CCD system for propagation studies.

Image Processing of Video Sequences

Specialized automated analysis software was developed to handle the large amount of data. The intensity image sequences from the CCD system (320x240pixels) were cropped (if needed), normalized on a pixel-by-pixel basis to fit within two standard deviations of the mean pixel intensity over the entire recording time. After baseline correction, each image was binned in up to 10x10 pixel sub-regions and activation times determined by locating the minimum of a Savitzky-Golay filtered first derivative. Conduction velocity calculations were performed from the activation maps, taking into account the main direction of propagation.

Short-Time Fourier Analysis

Time-variant electrical behavior, including self-organized activity, dynamic response to pacing, and alternans development, was analyzed in the time-frequency domain using a Short-Time Fourier Transform (STFT) with a Hamming window of appropriate size.

RESULTS

Cellular constructs were tested after 4-7 days in culture. All tested scaffolds were electrically excitable at field strengths 2-10V/cm. Our STFT analysis of experimentally obtained optical recordings revealed electrical temporal behavior consistent with tissue and whole heart measurements (**Fig 1**). At high enough frequencies (4-8Hz), external pacing was able to either invoke alternans, or to capture and drive a construct with inherent oscillatory behavior. As described before [2], the topographically modified scaffolds demonstrated increased propensity to self-organized activity.

To resolve if the complex behavior is determined by poor cell connectivity and lack of synchronization, we mapped electrical activity in more than 20 topographically modified scaffolds. The activation map, shown in **Fig 2**, is representative for the propagation observed under normal pacing conditions (local point stimulation). Conduction velocities in these constructs with introduced out-of-plane features were in the lower range (2-12cm/s) reported for cell monolayers [3], yet propagation was macroscopically uniform.

Furthermore, we tried to resolve whether the complexity of activation, seen in the STFT, could be a result of complex spatial events. Rapid point stimulation was able to effectively induce non-trivial patterns of propagation in some of the constructs, including the multi-armed spiral waves shown in **Fig 3**.

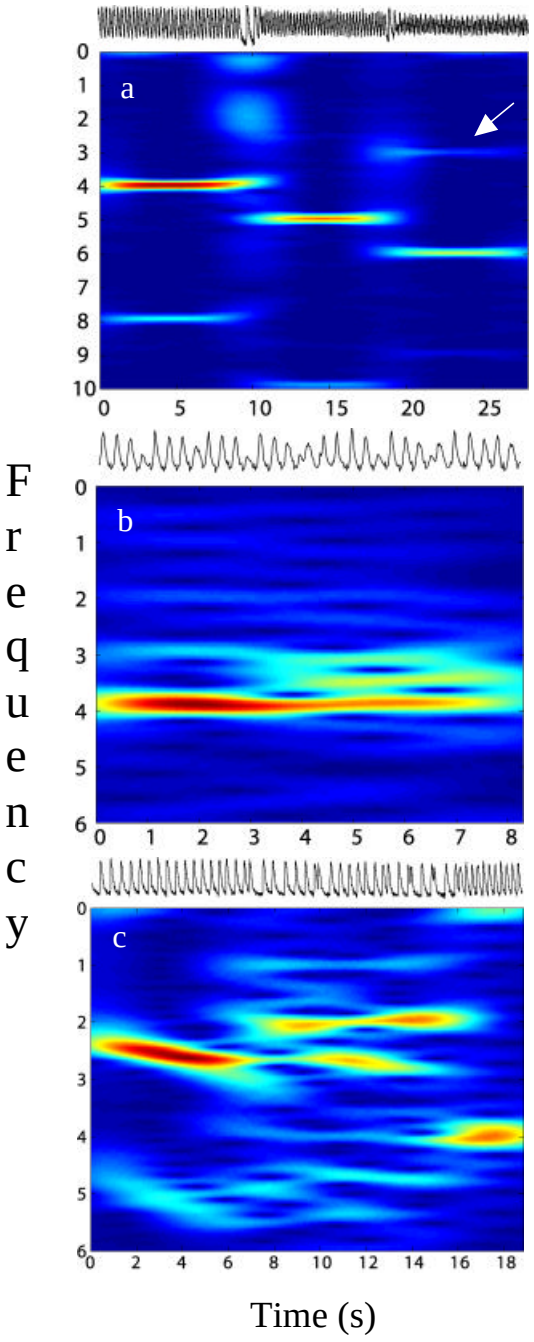

Fig 1. Time-frequency analysis (STFT). (a) Increasing pacing frequency (4, 5, 6Hz) leads to the development of alternans (arrow) and failure to capture; **(b)** Inherent oscillations might interact with the external pacing (4Hz here) to produce rich time-variant frequency content at lower frequencies; **(c)** Constructs with self-organized activity (roughly first 30% of the trace) normally could not be captured at lower (1Hz) frequencies, but are controlled by higher external frequencies (6Hz here).

DISCUSSION AND CONCLUSIONS

In this study we demonstrate that cardiac cell networks grown on topographically modified scaffolds: 1) develop good inter-cellular communication, are fully excitable and support tissue-like macroscopically-uniform propagation of electrical signals; 2) mimic classical tissue response to pacing, and can be driven to exhibit complex spatio-temporal patterns of electrical activity, normally linked to cardiac arrhythmias.

Our results suggest the engineered cardiac cell networks as versatile *in vitro* tissue equivalents for basic studies of cardiac electromechanics, with implications and practical value for arrhythmia research, biomaterials testing and tissue engineering efforts in the field.

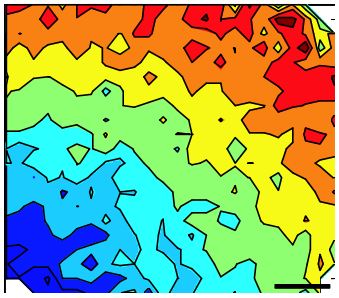

Fig 2. Macroscopic propagation at 1Hz external pacing in a microgrooved construct. A functional syncytium is formed across the grooves (23µm deep, 213 µm apart, running horizontally), as evidenced by the activation map, depicting relative times of depolarization. Contours are 20ms apart, with the earliest activation closest to the stimulating point electrode, bottom left corner. The conduction velocity along the diagonal measures 4.8cm/s. Scale bar is 1mm.

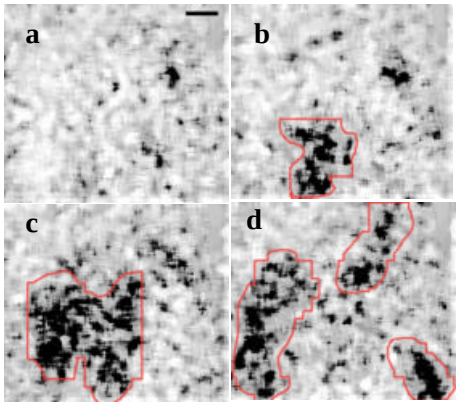

Fig 3. Self-organized multi-armed spirals in engineered constructs. An example of complex propagation, indicated here by four consecutive frames, 68ms apart. Scaffold grooves were 10µm deep, 75 µm apart, running vertically. Depolarization shows up in dark, outline drawn for clarity. Scale bar is 1mm.

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