Biochemical and Biophysical Research Communications 435 (2013) 520–525

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Acoustical sensing of cardiomyocyte cluster beating ☆

Nina Tymchenko [a], Angelika Kunze [a], Kerstin Dahlenborg [b], Sofia Svedhem [a,*], Daniella Steel [b]

[a] Dept. of Applied Physics, Chalmers University of Technology, 412 96 Göteborg, Sweden
[b] Cellectis, 413 46 Göteborg, Sweden

ARTICLE INFO

Article history:
Received 5 April 2013
Available online 3 May 2013

Keywords:
Cardiomyocyte
Cell cluster
QCM-D
Beating

ABSTRACT

Spontaneously beating human pluripotent stem cell-derived cardiomyocytes clusters (CMCs) represent an excellent *in vitro* tool for studies of human cardiomyocyte function and for pharmacological cardiac safety assessment. Such testing typically requires highly trained operators, precision plating, or large cell quantities, and there is a demand for real-time, label-free monitoring of small cell quantities, especially rare cells and tissue-like structures. Array formats based on sensing of electrical or optical properties of cells are being developed and in use by the pharmaceutical industry. A potential alternative to these techniques is represented by the quartz crystal microbalance with dissipation monitoring (QCM-D) technique, which is an acoustic surface sensitive technique that measures changes in mass and viscoelastic properties close to the sensor surface (from nm to μm). There is an increasing number of studies where QCM-D has successfully been applied to monitor properties of cells and cellular processes. In the present study, we show that spontaneous beating of CMCs on QCM-D sensors can be clearly detected, both in the frequency and the dissipation signals. Beating rates in the range of 66–168 bpm for CMCs were detected and confirmed by simultaneous light microscopy. The QCM-D beating profile was found to provide individual fingerprints of the hPS-CMCs. The presented results point towards acoustical assays for evaluation cardiotoxicity.

1. Introduction

There is increasing demand to access human material for evaluating existing and new chemical entities. In cardiotoxicity research, cells should be species relevant, have high reproducibility, and exhibit specific markers and functional similarities to adult human cardiac myocytes. Human pluripotent stem cells (hPS) have the potential to provide derivatives such as cardiomyocyte cells in large volumes [1,2]. Specifically, spontaneously beating hPS-derived cardiomyocytes in a cluster format (CMC) have become interesting for toxicity research. These cell clusters range in size from 200 to 300 μm in diameter and exhibit specific markers and functional similarities to adult human cardiac myocytes [3]. They are considered to be an excellent *in vitro* tool for studies of human cardiomyocyte function and are applied for cardiac safety pharmacology assays [4–8]. Cardiotoxicity assay development towards real-time, label-free monitoring of rare cell function using array formats, and monitoring of changes in optical or electrochemical properties of cells, is in progress [4,9], whereas techniques directly measuring changes in the mechanical properties of cells are largely lacking. Atomic force microscopy assays are under development, and forces exerted by individual CMCs have recently been measured [10].

The tight link between mechanical properties of cells and important cell processes (e.g. chronotropic events) suggests that acoustical sensing methods might have potential, alone or in combination with other techniques, in cell-based drug screening platforms, as well as to increase the fundamental understanding of cell properties. One such technique, the quartz crystal microbalance (QCM) technique, has already been successfully applied to studies of cells [11–13]; e.g., attachment and subsequent spreading of cells to the sensor surface [14,15], changes in cells exposed to cytomorphic agents [15,16], exocytotic events in neural cells on the sensor surface [17], pigment redistribution in melanophores [18], and cell responses when exposed to nanoparticles and nanotubes [19]. Furthermore, QCM has been applied to detect beating of cardiac myocytes, grown in a monolayer on the sensor surface [20]. This finding shows the potential of the QCM technology as a platform for non-invasive monitoring of chronotropic characteristics in a label free and real-time manner, aiming not only for the detection of, e.g., arrhythmias, but likely also properties of the cardiomyocyte contractile machinery.

This study addresses the monitoring of mechanical (viscoelastic) properties of single cell clusters by acoustical sensing using QCM with dissipation monitoring (QCM-D). A windowed QCM-D module was used for the detection of spontaneous hPS CMCs beating to allow for simultaneous live imaging by light microscopy (Fig. 1). Based on these results, we support the idea of QCM-D alone

* Corresponding author.
E-mail address: sofia.svedhem@chalmers.se (S. Svedhem).

0006-291X/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.04.070

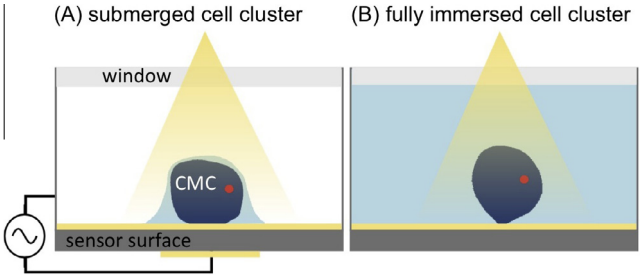

Fig. 1. Illustration of the experimental design. The CMCs are seeded *ex-situ* on either gelatin coated, or uncoated QCM-D sensors, and cultured for several days prior to measurement. Two measurement modes are shown, (A) under a liquid film where the CMC rests at the surface, and (B) in a chamber, filled with liquid, where the same CMC is tethered to the surface. The dot to the right in the cluster indicates the pacemaker cell.

or in combination with another technique (e.g., microscopy or impedance spectroscopy) as an attractive alternative to existing cardiotoxicity platforms.

2. Materials and methods

Unless otherwise stated chemicals were from commercial suppliers and used as received. Water was purified (filtered and deionized until a resistivity of 18 MΩ cm) using a MilliQ unit (Millipore, France).

2.1. Surface preparation

Surface preparation steps of SiO_2-coated QCM-D sensors (Q-Sense AB, Sweden) were as follows. QCM-D sensors were cleaned by a UV-O_3 treatment for 15 min followed by sterilization in 70% ethanol and rinsing with sterile water. For experiments using gelatin coatings, the cleaned and sterilized sensors were soaked for 30 min in aqueous gelatin solution (0.1%) at room temperature. Excess gelatin was removed followed by addition of a droplet of medium onto the sensor surface.

2.2. Cell culture

hPS-CMCs were obtained from Cellectis Stem Cells (Cellectis AB, Göteborg, Sweden). Briefly, CMCs were routinely cultured in knockout Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 20% fetal bovine serum (FBS), 1 mM GlutaMAX, 0.1 mM β-mercaptoethanol, 1% Minimal Essential medium (MEM) non essential amino acids, and 1% penicillin as described previously [3]. For QCM-D experiments, the sensors were placed in the central well of a humidified IVF culture dish, to keep the underside as clean and dry as possible during the cell attachment phase. Single clusters (~300 μm in diameter) were seeded centrally on sensors (gelatin pre-coated or plain) in a droplet of medium and incubated without further interference for 4–5 days in standard cell culture conditions (5% CO_2 and 95% humidity).

2.3. QCM-D experiments

The QCM-D experiments were performed using a Q-Sense E1 window module and QCM-D sensors with a fundamental frequency, f_0, of 5 MHz (Q-Sense AB, Sweden). Prior to mounting, cluster beating was verified by microscopic evaluation. The sensors were carefully taken from the culture dishes, excess medium was removed from the upper surface, and the under surface (and electrode) were gently blotted and dried with tissue. Care was taken not to perturb the attached cluster during the sensor mounting steps. All experiments were performed at 37 °C, and CMCs were either covered by a film of liquid only, or fully immersed in liquid carefully added on top (Fig. 1). Added solutions were pre-heated to 37 °C (to avoid formation of gas bubbles in the measurement chamber) and flowed at a slow rate (~25 μl/min) to fill the chamber without dislodging the cluster. Measurements were recorded at a single overtone (3rd) with the highest rate of sampling in order to ensure detection of rhythmic cell signals occurring in the range of 60–200 bpm. Frequency shifts were normalized to the fundamental frequency by dividing the values by three. Cluster beating (and continued attachment) was verified periodically throughout the QCM-D measurement.

2.4. Fourier transformation

The QCM-D signals were analyzed by fast Fourier transformation (FFT). FFT is particularly useful to look for periodicities of a signal. FFT of a periodic time series provides the corresponding power spectral density (PSD) function (or power spectrum), where any periodicity within the experimental data is identified as a peak at the corresponding frequency (here beating rate of the cell cluster). The absence of peaks in the PSD plot is indicative for a time series without any periodicity. In other words, FFT analysis of the recorded QCM-D data provides a frequency-resolved view on the composition of the measured time series. Note that, in order to avoid confusion with the frequency shifts recorded during the QCM-D measurement (Δf), the term beating rate will be used when the PSD and the clusters are described.

3. Results and discussion

The overall aim of the present study is to investigate the applicability of QCM-D for the detection of changes in spontaneously beating single cell clusters, hPS-CMCs, as a platform for studies on human cardiomyocytes based on changes in viscoelastic properties near the sensor surface. We will first describe a typical experiment where CMC beating is detected and analyzed, followed by discussing the origin of these signals and potential application areas of this new platform.

3.1. Analysis of QCM-D signals recorded during CMC beating

Cell clusters were added to the surface of QCM-D sensors *ex situ*, where the CMCs readily attached and grew under common cell culture conditions. Immediately after mounting of the sensor in the QCM-D instrument, spontaneous beating of single CMCs submerged in a thin liquid film on the sensor surface was observed in the registered signals, as verified by light microscopy using a windowed module. Typical frequency and dissipation signals (3rd overtone) as a function of time under such conditions are shown in Fig. 2A. The QCM-D signals (typical shifts from peak to peak were $\Delta f \sim 3$ Hz and $\Delta D \sim 1{\cdot}10^{-6}$) exhibit a clear periodicity in agreement with the visually observed beating. Direct information about the periodicity of the signal is obtained by transforming the measured time-dependent QCM-D signal to a power spectrum in the frequency domain using fast Fourier transformation (FFT). The FFT analysis of the QCM-D frequency and dissipation signals shows clear peaks corresponding to a beating rate of 168 bpm (Fig. 3A, and Table 1 "CMC 04"). Peaks appearing at higher beating rates are integers of the beating rate, so called harmonics, that occur for signals which differ from a sinusoidal shape (at an extreme, a rectangular signal would give an infinite number of harmonics which rates are an integer of the main peak). The observed beating rate is in agreement with previously reported rates, such as 94 ± 33 bpm [21], 12–120 bpm [6], 81 ± 31 bpm, 151 ± 40 bpm,