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Atomic force microscopy combined with human pluripotent stem cell derived cardiomyocytes for biomechanical sensing

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ABSTRACT

Cardiomyocyte contraction and relaxation are important parameters of cardiac function altered in many heart pathologies. Biosensing of these parameters represents an important tool in drug development and disease modeling. Human embryonic stem cells and especially patient specific induced pluripotent stem cell-derived cardiomyocytes are well established as cardiac disease model.. Here, a live stem cell derived embryoid body (EB) based cardiac cell syncytium served as a biorecognition element coupled to the microcantilever probe from atomic force microscope thus providing reliable micromechanical cellular biosensor suitable for whole-day testing.

The biosensor was optimized regarding the type of cantilever, temperature and exchange of media; in combination with standardized protocol, it allowed testing of compounds and conditions affecting the biomechanical properties of EB. The studied effectors included calcium , drugs modulating the catecholaminergic fight-or-flight stress response such as the beta-adrenergic blocker metoprolol and the beta-adrenergic agonist isoproterenol. Arrhythmogenic effects were studied using caffeine. Furthermore, with EBs originating from patient's stem cells, this biosensor can help to characterize heart diseases such as dystrophies.

1. Introduction

Cell based biosensor (CBB) devices represent a novel tool to understand and treat various human diseases including heart diseases which are leading cause of death in developed countries (Pilkerton et al., 2015). Most of them have hidden cause at the cellular and genetic levels (Aistrup et al., 2009; Stienen, 2015) not easily accessible for diagnostics, as heart biopsy is invasive procedure with significant risk (Holzmann et al., 2008; Imamura et al., 2015). Since the discovery of pluripotent stem cells (PSCs), such as human embryonic stem cells (hESC) (Thomson et al., 1998) and especially disease specific induced pluripotent stem cells (hiPSC) (Takahashi et al., 2007) and their differentiation into cardiomyocytes (Mummery et al., 2002), these cell types represent an important cellular model in drug and disease screening (reviewed in Acimovic et al., 2014).

The electrophysiological phenotype of individual cardiac cells is often studied providing information representing pacemaker or messenger function. Despite most of studies use single cells, the CMs syncytium seems to be a critical parameter in biosensor assembly as unstable signals are obtained in case of individual or small cluster of cells (Kaneko et al., 2007, 2014). These methods follow mostly action potential, cell to cell conductivity or electrical cell-substrate impedance (Giaever and Keese, 1984). Recently, xCELLigence RTCA Cardio multiwell sensor combined with primary neonatal rat cells was used to monitor the effect of antiarrhythmic drugs on cell growth and contractions (Guo et al., 2011). However, the electrophysiological data cannot be fully separated from the mechanical triggers and consequences (Kelly et al., 2006) rendering the method not suitable for large group of diseases affecting electro-mechanical coupling. Thus CM contraction and relaxation biosensing represents an indispensable step for the in vitro disease modeling. Indirect methods of contraction measurement, such as optical ones based on optical fiber deformation (Fearn et al., 1993) and image analysis (Neagoe et al., 2003) have limited potential especially in case of in vitro differentiated CMs,

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which do not fully match the shape of the isolated CMs and often require dissociation of the syncytium and analyzing the individual cells in microposts (Rodriguez et al., 2014). Furthermore, the combination of optical analysis and the direct methods measuring contraction force on artificially assembled structures are significantly impacted by stiffness of the substrate, such as the twitch power in the case of dissociated cells plated on substrate with varying stiffness (Rodriguez et al., 2011). Further the increasing cell number negatively impacted the force-length relationship measured directly on neonatal cardiac cells self-assembled on fibrin fibers (Sondergaard et al., 2012), again rendering the syncytium analysis at least difficult. Thus it seems that the most promising structure for studying the contraction properties of human cardiac syncytium might be uniformly sized clusters of differentiated CMs in the form of PSCs-derived embryoid bodies (Pesl et al., 2014). Therefore we opted for a direct approach to assess the EBs' cardiac syncytium mechanical properties in real time and under the alternating biophysical and biochemical conditions using atomic force microscopy (AFM).

AFM is a three-dimensional high resolution topographic technique. It is suitable for biological applications in native conditions (Vahabi et al., 2013) with the ability to measure bending of the cantilever probe with extremely high precision (Sundararajan and Bhushan, 2002). It allows AFM to be used as a mechanical nanosensor (Wang et al., 2015), or as a micromechanical transducer for the construction of biosensors (Lavrik et al., 2004). Furthermore, modified AFM tip biosensor has been used in numerous studies, including the analysis of Alzheimer's disease process (Hane et al., 2014), as mechanical sensor in the study of cell penetration by nanoneedles (Obataya et al., 2005), in cell-based biosensing of drug effects (Wang et al., 2009) and in study of growth factor effects on epidermal cells (Zhang et al., 2014).

In this study, our previously developed uniformly sized EBs containing human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (Pesl et al., 2014) are integrated into the AFM force sensing platform to perform a high fidelity contraction pattern as a unique CBB for the measurement of absolute values of contractile forces together with frequency of the beat rate in complex conditions and cellular models. Development and optimization of the whole measurement setup is described with proposed application for characterization of cell clusters of cardiac cells and testing effects of model drugs.

2. Materials and methods

2.1. Preparation of hiPSC and hESC cardiomyocyte clusters

Measurements were performed using the hESC lines CCTL14 and CCTL12, characterized previously (Dvorak et al., 2005; International Stem Cell Initiative et al., 2007; Krutá et al., 2013). The hiPSC line cl1 was obtained from Dr. Majlinda Lako, Institute of Genetic Medicine, Newcastle University and the iPSC lines MDMD2Se were derived in our laboratory from human skin biopsies (Krutá et al. 2014). The hESCs and iPSC lines were propagated on mitotically inactivated mouse embryonic fibroblasts (Dvorak et al., 2005). Regular round shape of embryoid bodies was achieved by forced aggregation in silicone mold preformed microwells (1.5% agarose, VWR) (Dahlmann et al., 2013), their differentiation and maturation was achieved using our previously described method (Pesl et al., 2014). For statin samples, PSC were cultivated with pravastatin (20 μM, Sigma) for 4 days prior to EB formation.

2.2. Clusters seeding and measurement set-up

Beating EBs cultured for 28 days after initiation of differentiation were selected for round shape and adequate size and seeded onto adherent 30 mm Petri dishes (TPP Technoplastic Products, Trasadingen, Switzerland). The cultivation media was replaced by Tyrod solution (135 mM NaCl, 10 mM HEPES, 5.4 mM KCl, 0.9 mM $MgCl_2$, pH 7.4) and suplemented with 10 mM glucose and $CaCl_2$ (in desired concentration) and the EBs were maintained at 37 °C in a standard CO_2 incubator. Before experiment, a dish equipped with media exchange tubes was placed on the motorized stage of an inverted microscope (Olympus IX-81S1F-3, Tokyo, Japan) and the AFM recording head (BioAFM NanoWizard 3, JPK, Berlin, Germany) was finally placed on top of the sample. The AFM set-up was modified in order to provide real-time biomechanical characterization of CMs cell clusters (Fig. 1).

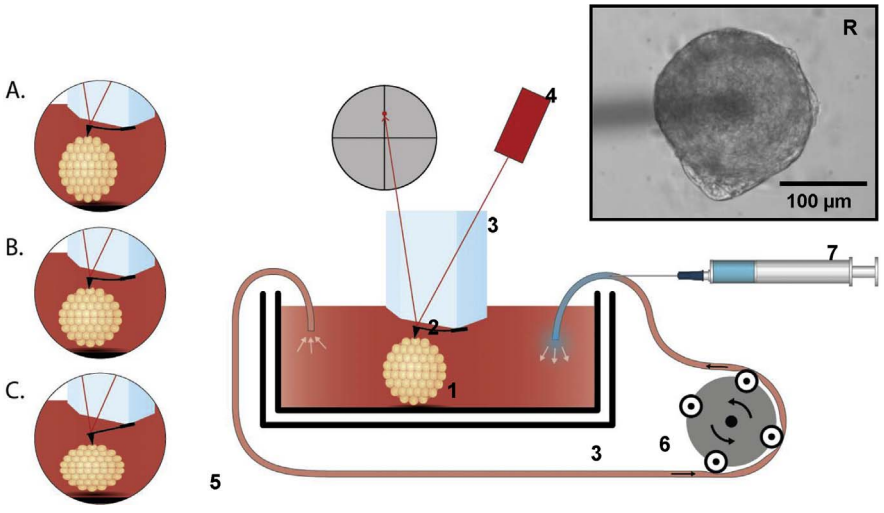

Fig. 1. Scheme of the biosensor setup used for biomechanical characterization of cardiomyocyte clusters. Most important parts are schematically shown and are labeled as follows: 1 – Embryonic body (EB, cluster of living cells) in plastic Petri dish, (3 cm in diameter), 2 – AFM cantilever, equipped with a sharp tip, 3 – glass cantilever holder block, 4 – AFM laser source (a) (left) and photodetector of laser (b), position, 5 – plastic (PP) tubing, i.d. 0.2 mm (an arrow shows the flow direction), 6 – peristaltic pump, driving the flow direction and rate (rotation direction is shown by an arrow), 7 – medical syringe (total volume of 1.0 ml) for drug injection, 8 – Petri dish heater (to keep constant temperature of 37.0 ± 0.1 °C). The little insets on the left (A, B and C) show the subsequent stages of the EB contractile movement as this is measured by the AFM cantilever. Image (R) in the top-right corner shows real image of EB cluster with cantilever on its surface (captured by optical microscope). The size of the EB cluster is 175 μm.