Original Paper

Mechano-Pharmacological Characterization of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

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Key Words

Cardiac myocytes • Induced pluripotent stem cells • Heart tissue culture • CellDrum • Ion channels • Pharmacology • Inotropic compounds

Abstract

Background/Aims: Common systems for the quantification of cellular contraction rely on animal-based models, complex experimental setups or indirect approaches. The herein presented CellDrum technology for testing mechanical tension of cellular monolayers and thin tissue constructs has the potential to scale-up mechanical testing towards medium-throughput analyses. Using hiPS-Cardiac Myocytes (hiPS-CMs) it represents a new perspective of drug testing and brings us closer to personalized drug medication. **Methods:** In the present study, monolayers of self-beating hiPS-CMs were grown on ultra-thin circular silicone membranes and deflect under the weight of the culture medium. Rhythmic contractions of the hiPS-CMs induced variations of the membrane deflection. The recorded contraction-relaxation-cycles were analyzed with respect to their amplitudes, durations, time integrals and frequencies. Besides unstimulated force and tensile stress, we investigated the effects of agonists and antagonists acting on Ca²⁺ channels (S-Bay K8644/verapamil) and Na⁺ channels (veratridine/lidocaine). **Results:** The measured data and simulations for pharmacologically unstimulated contraction resembled findings in native human heart tissue, while the pharmacological dose-response curves were highly accurate and consistent with reference data. **Conclusion:** We conclude that the combination of the CellDrum with hiPS-CMs offers a fast, facile and precise system for pharmacological, toxicological studies and offers new preclinical basic research potential.

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Introduction

Considering the fact that the major function of the heart muscle is the generation of force and tensile stress, there is a remarkable lack of methods that can be routinely used for mechanical testing of cardiac myocytes *in vitro*. This shortcoming arises primarily from the fact that setups for mechanical force or stress measurements on that scale tend to be complex, poorly controllable and labor-intensive. In particular, common mechanical measurements highly depend on the geometry of the test specimen and its connection to the measurement equipment. Thus, living samples of soft ultra-thin tissues are particularly difficult to characterize in comparison to other biogenic and non-biogenic materials. Previously developed mechanical techniques range from *ex vivo* methods, which make use of whole organs or partial explants to *in vitro* methods, commonly applied to isolated and/or cultured cells.

The Langendorff heart [1] developed in the late 19th century is still the most established technique to test cardio-active drugs [2]. It uses explanted mammalian hearts, kept in organ baths and perfused retrogradely with nutrient solutions containing cardio-active compounds. Despite its benefits in maintaining the structure of the organ, the Langendorff heart entails a list of significant drawbacks: Besides a huge demand for test animals and ethical concerns, the use of animal models itself may render the pharmacological results dubious due to their debatable translatability to human physiology. Additionally, the setup is fairly complex, making it unsuitable for high throughput analyses, which have become a rising and vital demand of the pharmaceutical industry.

Yet, the development of an adequate alternative has been an ongoing process for more than thirty years. In the meantime, various approaches for the quantification of cardiac contractility arose, each of which entailing assets and drawbacks regarding their accuracy, physiological relevance and scalability.

In 1986, Shepherd et al. mounted single bullfrog atrial myocytes to a pair of glass rods of known compliance [3]. The contractile force was calculated by means of optical displacement recording of the glass rods. Later, optical analysis was replaced by isometric force transduction [4, 5].

Harris et al. found that cells cultured on thin silicone substrates were able to produce wrinkles in the substrate [6]. They proposed to measure the magnitude of the cellular force by micromanipulation of the silicone sheets with microneedles of calibrated flexibility. A related concept uses elastic micropillars as a cell culture substrate [7]. Here, the deflection of each pillar is optically monitored. By calibrating the stiffness of the micropillars, the cellular force for each pillar can be calculated and summated.

All methods mentioned above have in common that a massive scale-up towards high-throughput analyses (e.g. for mid-stage preclinical studies) seems unfeasible. In analogy to manual patch-clamp experiments, their high accuracy and precision in the recorded data opposes the possibility to automatize and parallelize experiments.

In a different concept, Yin et al. used magnetic beads for measuring contractile forces of cardiac myocytes [8]. After attaching the beads to the cell, the displacement of the bead is optically recorded while a variable magnetic field is applied. Here, the cells are cultured on stiff substrates like glass or plastic in order to provide a stationary reference frame. The culture of cells on substrates of very high rigidity is suspected to significantly change mechanical characteristics [9, 10]. The same restriction applies to atomic force measurements (AFM) [11] of cellular monolayers, where the stiff substrate is a fundamental prerequisite for accurate force transduction. Van Vliet et al. provide an extensive overview about the commonly used means for mechanical measurements of live tissue *in vitro* [12].

In 2010 Kevin Kit Parker et al. published a study on thin film biohybrids to measure contractile forces of cardiac myocytes. These biohybrids consist of a cantilever like thin flexible polymer layer and a monolayer of so-called *cardiac engineered tissue*. The cantilever acts like a bimetallic strip. Contraction of the cardiac myocytes results in a coiling of the construct, which is optically recorded and used to calculate the cellular stress [13, 14]. As

this system relies on uniaxial deformation of the construct, the orientation of the cells on the surface plays a crucial role. If the cells were allowed to settle with random orientation on the substrate, only a portion of the exerted force would contribute to deformation. In view of this issue, surface micro structuring was applied to enforce alignment of the cells in the desired direction [15]. In this way, the natural alignment in the native heart tissue is modeled as a highly desirable byproduct. In additional studies, the optimal aspect ratio for maximal force transduction was examined [16].

In a different concept, Eschenhagen et al. developed so-called *engineered heart tissues* to examine contractile forces. These are ring-shaped gels formed from extracellular matrix proteins and seeded with cardiac myocytes. The rings are placed around two flexible polymer posts, which are deflected due to cellular forces. The deformation is analyzed indirectly by video observation or directly via a force transducer [17, 18]. In subsequent studies the rings were replaced with dumbbell-shaped constructs between the posts [19]. For contractile property determination of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) another method was used. Autonomously beating hESC-CMs clusters were co-cultured in irreversibly ischemically damaged slices of neonatal murine ventricles. After cluster integration isometric force measurements were performed. The cavity of the left ventricle provided a preformed hole allowing tissue mounting onto the tips of two adjacent steel needles. The hESC-containing spontaneously beating rings were mounted on an isometric force transducer and the length was increased stepwise until the maximal force development was reached [20].

Besides force measurements, other approaches make use of indirect methods to quantify cellular mechanics. One common application uses fluorescent dyes to quantify Ca^{2+} concentrations in the cytoplasm as an indicator for contractility [21]. As this approach relies on optical components, which could be miniaturized in the past decades by orders of magnitude by digital technologies, it enables real high-throughput analyses with up to 1536 samples at a time. This renders indirect contractility measurements available for preclinical trials for the first time. The major drawback of this technology is its underlying assumption of a direct correlation between the concentration of cytoplasmic Ca^{2+} and the contractility of a myocyte. A known issue of this approach is the interruption of the excitation-contraction coupling [22]. While those interruptions may have pathologic reasons, recently developed cardio-active drugs like the Ca^{2+} -sensitizer levosimendan [23], a cardiac myosin activator called omecamtiv mecarbil [24] and the myosin II ATPase antagonist blebbistatin [25] even make use of this effect.

Finally, electric cell-substrate impedance sensing is used to evaluate the shape of cells cultured on gold electrodes. First described in 1986 [26], it was used for measuring kinetics of cell spreading [27] and cytotoxicity [28]. In recent years, advances in data transfer rates allowed the measurement of cardiac beating with a high resolution. Like Ca^{2+} measurements, this system provides indirect information about the contractile forces, implying a direct relationship between force or tension and shape.

In the present study we intended to overcome previous technological drawbacks by using the CellDrum technology [29-33] to characterize cardiac myocytes, derived from human induced pluripotent stem cells (hiPS cells), with respect to their response to well-characterized positively and negatively acting inotropes. Previously, this technology has been applied to neonatal rat cardiac myocytes [31] and endothelial cells [32].

The key factor of the CellDrum technology is a well-defined biomechanical environment for cells, combined with a high degree of experimental handling simplicity. The cells are cultured on a physically defined reference material consisting of an ultra-thin silicone membrane sealing the bottom of a cell culture well. In the present design, the well has a diameter of 16 mm which corresponds to a standard 24-well-plate. While being deflected by the weight of the culture medium, a rhythmic contraction of the auto-contractile cardiomyocytes lifts the membrane upwards.

The deflection is monitored by a laser triangulation sensor. Simultaneously, the pressure beneath the membrane is recorded. The tension of the membrane is calculated from the

recorded values by application of Laplace's law. The utilization of self-beating hiPS-derived cardiac myocytes promises to avoid present-day issues regarding cross-species translation and ethical concerns while enabling the production of an essentially infinite amount of human material for examination.

We have investigated the effects of inotropes acting on Ca^{2+} channels (S-Bay K8644/verapamil) and Na^{+} channels (veratridine/lidocaine) in commercially available hiPS-derived cardiomyocytes. The inotropes were selected for their action on the contractility of heart tissue rather than their common clinical application.

In particular, we intended to examine the contraction-relaxation-cycles (CR-cycles) regarding their morphology changes upon drug application. Beyond their amplitude, we examined the durations of the CR-cycles as well as their time integral as an additional potential measure for inotropy. Finally, the beating frequency was monitored.

Materials and Methods

CellDrum Technology

Key factors of the CellDrum technology [29-35] (Fig. 1) are a well-defined, approximately isotropic and homogeneous biomechanical cell tension environment, combined with a good degree of experimental handling simplicity. The cells are cultured on a soft, 3 μm thin silicone membrane sealing the bottom of a plastic cylinder. This set-up, membrane and cylinder, resembles one CellDrum (Fig. 1). Each individual CellDrum (well) has a diameter of 16 mm and a height of 10 mm. It is placed in a *Single Well Tissue Tension Analyzer*. Due to the weight of the cell culture medium the membrane deflects downwards. The inner side of the membrane is covered with a monolayer of hiPS derived cardiac myocytes (Cor.4U, Axiogenesis AG, Köln). Mechanical rhythmic cell contraction and relaxation of the auto-contractile cardiomyocytes lifts the membrane up and down, respectively. The deflection is monitored by a laser triangulation sensor (model LK-G31, KEYENCE Deutschland GmbH, Neu-Isenburg, Germany). Simultaneously, the pressure beneath the membrane is recorded by a pressure sensor (model AP47, KEYENCE Deutschland GmbH, Neu-Isenburg, Germany). The tension of the membrane is calculated from the deflection by applying Laplace's law [36].

CellDrum membrane manufacturing

Membranes were manufactured at the IfB laboratories using poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning, Midland, MI, USA). The inner CellDrum surface was chemically functionalized to achieve long-term cardiac myocyte attachment. CellDrums were manually produced and tested for

Fig. 1. Schematic drawing of single CellDrum Tissue Tension Analyzer: 1) cylindrical CellDrum culture medium container, 2) ring heater, 3) air pump, 4) laser triangulation sensor 5) culture medium, 6) membrane and cell monolayer, each 3 μm thick, 7) Insert: CellDrum, enlarged: cell monolayer.

constant thickness by thin-film interferometry. Membranes of $3.0 \pm 0.3 \mu\text{m}$ thicknesses were selected for pharmacological experiments.

Buffers

Ingredients were purchased from: 1) Phosphate buffered saline (PBS) and trypsin/ethylenediaminetetraacetic acid (EDTA) (Life Technologies, Karlsruhe, Germany); 2) Isopropyl-alcohol and hydrochloric acid (37%) (Carl Roth, Karlsruhe, Germany), and 3) 2-(N-morpholino)ethanesulfonic acid (MES) (USB Corporation, Cleveland, OH, USA).

Membrane functionalization

“Classical” fibronectin deposition as usually used [32] was not sufficient to enable long-term adhesion of hiPS-derived cardiac myocytes. Thus, a three-step functionalization protocol consisting of a wet-chemical oxidation step [37], epoxy-silane conjugation from alcohol solution [38] and covalent binding of fibronectin was adopted [39].

For membrane oxidation, the CellDrum membranes were exposed to 500 μL oxidation solution for 30 min at room temperature. The oxidation solution consisted of a mixture of $\text{H}_2\text{O}/\text{H}_2\text{O}_2$ (20%)/HCl (37%) (5:1:1 volume ratio) and was prepared immediately before application. For silane deposition isopropyl alcohol and water were mixed at a volume ratio of 95/5 and adjusted to pH 5.0 with acetic acid. For fibronectin coating, a 50 mM MES buffer was prepared, adjusted to pH 6.1 with 1 M NaOH, and sterilized with a 0.22 μm filter. After thorough washing with deionized water, 200 μL of a 2 % solution of trimethoxy[2-(7-oxabicyclo[4.1.0]hept-3-yl)ethyl]silane (3MOBS) in silane deposition solution were added and left to incubate for 5 min. After another washing step, the freshly prepared silane layer was cured by autoclaving (121 $^{\circ}\text{C}$, 0.2 MPa, 20 min). Finally, fibronectin from bovine blood plasma was diluted to a final concentration of 10 $\mu\text{g mL}^{-1}$ with MES coating buffer. Each CellDrum membrane was incubated with 500 μL of this solution overnight. The solution was removed shortly before seeding the cells. Functionalization did not affect the mechanical properties of the membranes significantly (data not shown).

Cell Culturing

Autonomously beating human hiPS-derived cardiac myocytes were chosen for experiments avoiding present-day discussions regarding cross-species translation as well as ethical concerns. Cells originate from human skin fibroblasts reprogrammed by the application of the *Yamanaka factors* [40]. They were terminally differentiated to cardiac myocytes and selected using the αMHC promoter driving a puromycin resistance gene. Electrophysiological analyses indicate that these cardiac myocytes are a pan-organ population of ventricular, atrial, and nodal cells [41]. According to manufacturer data, cells consist of approximately 60% ventricular, 35% atrial, and 5% nodal cells. Cells were delivered live in standard cell culture flasks. After trypsinization, they were seeded on CellDrum membranes at a density of 150,000 cells cm^{-2} (a representative figure in [42] shows the irregular assembly of the cells in the confluent monolayer on a CellDrum membrane). The culture medium was replaced every day. Within 6 to 8 days of culture, cell synchronization occurred spontaneously in all CellDrums. Experiments were carried out after seven more days of culture.

Pharmacological agents

In these experiments S-Bay K8644 a selective calcium channel opener was used at concentrations up to 150 nM. As antagonist the selective calcium channel blocker verapamil up to 300 nM was chosen. As potassium channel modulators veratridin up to 2500 nM was applied as agonist, and lidocaine up to 2500 nM as potassium channel antagonist. Agents were purchased from Sigma Aldrich.

The optimal concentration range for each drug was estimated in preliminary experiments.

Cell Monolayer Mechanical Tension Determination

Under the pressure p the CellDrum membrane deflects in a spherical cap of a ball with radius (of curvature) R . The tensile stress of the membrane is calculated using Laplace’s formula for a spherical thin-walled pressure vessel with thickness t :

$$\sigma = \frac{p \cdot R}{2 \cdot t} \quad (1)$$

The radius R can be obtained from the deflection h by the Pythagorean Theorem:

$$R^2 = (R - h)^2 + r^2 \quad (2)$$

where r is the radius of the CellDrum ($r=8$ mm). This can be solved for:

$$R = \frac{r^2}{2 \cdot h} \cdot \left(1 + \frac{h^2}{r^2}\right) \quad (3)$$

After combining equations (1) and (3), the outcome is the general dependency between the stress σ , the differential pressure p , the resulting deflection h of the membrane, and the dimensions of the CellDrum:

$$\sigma = \frac{p}{h} \cdot \frac{r^2}{4 \cdot t} \cdot \left(1 + \frac{h^2}{r^2}\right) \quad (4)$$

For cell tension measurement, the CellDrums were integrated into a temperature-controlled measurement chamber and equilibrated for 500 s. After this period, the strains of the membranes were adjusted to 0.3-0.5% by application of air pressure. Subsequently, the cells were incubated with six increasing concentrations of the respective cardio-active compound for 5 min, starting with the control (no compound). The compounds were added cumulatively by replacing 20% of the culture medium containing the necessary amount to achieve the desired concentration. In order to exclude compound-independent effects like temperature, media pH or mechanical influences during media exchange, baseline measurements without compounds were recorded as stated above. Compound effects were normalized to controls.

Data Analysis

Data were recorded at a sample rate of 1 kHz (LabView 2010, National Instruments, Austin, Texas, USA). For each data point, 10 contraction-relaxation-cycles (CR-cycles) were matched and filtered with a moving averaging data filter over one hundred beats. Individual beats were analyzed for: a) contraction amplitude and b) beat duration, c) the contraction vs. time curve integral (area between curve and baseline). In addition, the beating frequency was analyzed (Fig. 2).

Mathematical Modeling and Computer Simulations

With this part of the paper the intention was to outline that computer simulations may very well complement the experimental data shown here. Detailed cell physiological models have been programmed for atrial, sinoatrial and ventricular cells. Each of these Hodgkin-Huxley type models consists of ordinary differential equations describing the kinetics of up to 20 ion channel gates, of sodium, calcium and potassium ions and of the membrane potential of an individual cell. In sum, the resulting individual ion channel currents give the total electrical current across the membrane.

The macroscopic electromechanically coupled model has been implemented and the simulations have been performed in the open source Finite Element software Code_Aster provided by the French electric utility company EDF (Électricité de France SA, Paris, France). Details including basic equations and additional simulations are presented in [42-44]. Parameter fittings and other statistical assessments related

Fig. 2. Parameters of an individual cell contraction-relaxation-cycle: a) Contraction amplitude, b) duration of the contraction-relaxation-cycle, c) time-integral of the contraction-relaxation-cycle.

to the simulations have been performed in the statistics software R and in Scilab. Due to the fine time and spatial resolutions we employed eight processors and provide up to eight GB memory on a Dell Precision workstation containing 16 CPUs and 32 GB memory.

Statistics

Each experiment was repeated 4 to 6 times. Values are given as mean values $\pm$ 1 standard deviation. Mann-Whitney U test was performed using the statistical software platform GNU R.

Results

Unstimulated Contractile Force and Stress

Table 1 summarizes the mechanical properties found in the literature. The results of the present study are listed in the last row. Contraction forces measured with the various setups range from 0.01 to 40 μ N for single cells and from 110 to 340 μ N for three-dimensional constructs, while the stresses range between 5.3 and 51 kPa.

Inotropic Drugs

Four different cardio-active drugs which target Ca^{2+} channels (S-Bay K8644/verapamil) and Na^+ channels (veratridine/lidocaine) were applied to hiPS-derived cardiac myocytes.

Table 1. Contractile force and tensile stress of cardiomyocytes reported in the literature, compared with the results of this study (last row) * IFT=isometric force transduction, AFM=atomic force microscopy, EM=elastic micropillars, CD=CellDrum, MTF = Muscular Thin Film, ** depending on substrate elasticity, *** equibiaxial

Species	Tissue	Maturity	Preparation	Setup*	Force [μ N]	Stress [kPa]	Source
Frog	Atrial	adult	single cell	Optical	0.2	-/-	[3]
Guinea pig	Ventricular	adult	single cell	Optical	1.23 $\pm$ 0.44	5.3 $\pm$ 2.6	[45]
Mouse	pan-organ	iPS	cell cluster	IFT	2.7	-/-	[5]
Rat	-/-	newborn	single cell	IFT	20 – 40	9.05	[46]
Rat	Ventricular	adult	single cell	IFT	-/-	41.1	[47]
Rat	Ventricular	adult	single cell	IFT	-/-	17.7 $\pm$ 1.7	[48]
Rat	Ventricular	embryonic	single cell	AFM	0.012	-/-	[11]
Rat	-/-	embryonic	single cell	EM	0.01 – 0.8**	-/-	[7]
Rat	Ventricular	newborn	3D matrix	IFT	340 $\pm$ 30	-/-	[49]
Rat	Ventricular	newborn	3D matrix	Optical	110 – 220	-/-	[19]
Rat	Ventricular	newborn	Monolayer	MTF	-/-	15.4 $\pm$ 1.4	[14]
Rat	Ventricular	newborn	Monolayer	MTF	-/-	9.2 $\pm$ 3.5	[13]
Rat	Ventricular	newborn	Monolayer	MTF	-/-	20.7 $\pm$ 5.6	[50]
Human	Ventricular	adult	single cell	IFT	-/-	51 $\pm$ 8	[51]
Human	Ventricular	adult	single cell	IFT	-/-	29.6 $\pm$ 4.5	[4]
Human	pan-organ	iPS	Monolayer	CD	-/-	43.1 $\pm$ 7.5***	this work

Table 2. Overview on global inotropic and chronotropic effects on hiPS-derived cardiomyocytes

	S-Bay K8644	Verapamil	Veratridine	Lidocaine
Amplitude	↑	↓	↑	↓
Integral	↑	↓	↑	↓
Duration	↑	→	→	→
Frequency	↓	↑	↓	↓

Fig. 3. Representative recordings of the dose-dependent effects of S-Bay K8644 (upper panel) and verapamil (lower panel) on the contractility of hiPS-derived cardiomyocytes.

Fig. 4. Effect of calcium channel modulators S-Bay K8644 (left) and verapamil (right) on the amplitude, time-integral, duration and frequency of the contraction-relaxation-cycles of hiPS-derived cardiomyocytes. * $p < 0.05$.

Table 2 summarizes the effects of the compounds on the amplitude, time integral, duration and frequency of the recorded CR-cycles which are explained in Fig. 2.

Ca²⁺ Channel Modulators

Representative recordings of the Ca²⁺ channel agonist S-Bay K8644 and antagonist verapamil are shown in Fig. 3. S-Bay K8644 (Fig. 4 left) raised the amplitude of the CR-cycle to 160% up to a concentration of 90 nM. The same applied for the duration. Above 90 nM, both values were not changed significantly. As result from the increase in amplitude and duration, the time integral was raised to 250% at 90 nM. Concurrently, the beating frequency was reduced above 60 nM to 80% at 150 nM.

Fig. 5. Effect of sodium channel modulators veratridine (left) and lidocaine (right) on the amplitude, time-integral, duration and frequency of the contraction-relaxation-cycles of hiPS-derived cardiomyocytes. * $p < 0.05$.

It reduced the amplitude to 25% at 300 nM (Fig. 4 right). While the duration was only decreased at the highest concentration of 300 nM, the time integral was reduced in accordance to the amplitude to 20% at the highest dose. The frequency was increased in a linear manner to 160% at 300 nM and did not reach saturation.

Na⁺ Channel Modulators

The Na⁺ channel agonist veratridine (Fig. 5 left) increased both the amplitude and time integral to 140% at a concentration of 1 μM, while the duration was not changed up to 2.5 μM. The frequency was reduced to 60% in a linear dose-dependent manner. Above this concentration, the compound induced pro-arrhythmic events (Fig. 6) and finally massive fibrillations.

The Na⁺ channel antagonist lidocaine (Fig. 5 right) reduced the amplitude to 40% at 100 μM. Beyond this concentration, rhythmic contraction ceased. The duration of the CR-cycle was unchanged. As a result, the time integral was decreased in accordance with the amplitude to 40% at the highest dose. The frequency was decreased to 50% at 60 nM.

Computer Simulations

The herein and in other papers [31, 34] presented experimental results provide a fundamental basis for the validation and parameterization of the computational model previously presented in [42] where theoretical details and the simulation results in Fig. 7 and Fig. 8, respectively can be found. The electromechanically coupled finite element model is mainly based on electrophysiological measurements published in the literature. Within the model it is possible to modify the microscopic cellular models that represent the cellular electrophysiology via systems of ordinary differential equations, in terms of drug application. Inotropic and chronotropic effects of the calcium channel modulators S-Bay K8644 and verapamil as well as of the sodium channel modulators veratridine and lidocaine have been simulated in [43]. Therein the different modes of action of a drug and the sensitivity of the ion channels with respect to that drug are taken into account. In [44] the authors describe a suitable way how to modify the cellular conductivity in the employed models of cellular electrophysiology in order to match the phenomenologically observed beating frequencies.

Figure 7 shows experimentally determined vs. simulated inotropic effects (relative membrane deflection) of lidocaine. The normalized curve in Fig. 7 shows a good qualitative agreement between experiment and simulation in terms of the inotropic effect of lidocaine although there is a quantitative disagreement that is hidden by the normalization but is thoroughly discussed in [42]. Nevertheless, even the seemingly nonlinear relationship between lidocaine concentration and membrane deflection in the range of 0 to 100 μM can be captured quite well.

Figure 8 represents the mean experimental results which have been obtained with two different cell cultures exposed to S-Bay K8644. The computational model could be adapted to the respective, phenomenologically observed beating frequency, keeping the ability to simulate the drug effect. With the experiment-specific modifications of the conductance g_{K1} of the inward-directed rectifier potassium current

Fig. 6. Pro-arrhythmic events (early afterdepolarizations) for 2 μM veratridine.

Fig. 7. Experimentally determined vs. simulated inotropic effects (relative membrane deflection) of lidocaine.

Fig. 8. Experimentally vs. simulated beating frequencies using S-Bay K8644 for two cell cultures.

in the cellular model we were able to capture the beating frequency of the monolayer under control conditions while keeping the ability to appropriately simulate the drug effect. Results like these indicate that the computational model is able to support the interpretation of experimental results by means of simulating hypotheses concerning differently expressed ion channels in different cell cultures.

From these two examples it can be concluded that even in this preliminary modeling the comparison between experimental and simulated data, respectively, are surprisingly good. Further computational results, including the chronotropic effect of lidocaine and the inotropic effect of S-Bay K8644 can be found in [42-44].

Discussion

Unstimulated Contractile Force and Stress

Literature data (Table 1) show broad ranges of cell layer force and tensile stress, respectively. Even the data based on the most commonly used and established *isometric force transduction* setup vary greatly. Possible (biological) reasons might result from donor varieties or the developmental stage of the tissue. Furthermore, force itself does not intrinsically contain any information about the geometry of the test specimen, making results incomparable. The deviations of the geometrically normalized stress values are substantially smaller (Table 1). Hence, it seems reasonable to restrict the comparison of our findings to only the stress data. In this context we conclude that the results from this study with hiPS-derived cardiac myocytes (43.1 ± 7.5 kPa unstimulated stress amplitude) compare well to the findings of van der Velden et al. with adult human cardiac myocytes from healthy biopsy material [4, 51].

Computer simulation

The developed software and contemporary hardware availability allows simulations of the contractile behavior of the cell layer and estimation of drug effects (Fig. 7 and Fig. 8). The comparison of experimental results with simulation results revealed that a phenomenological adjustment in the absence of drugs could lead to a quantitatively quite good agreement between experiment and simulation [43]. Our qualitative simulations of drug action (Fig. 7) indicate necessary model improvements to quantitatively capture the beating force of the cell layer. Promising mechanisms to adopt the beating frequency of the models to the experimentally determined ones have been selected and are under current investigation. The ability to adjust the models to certain beating frequencies while keeping the potential to model drug action can be seen in Fig. 8. It can be concluded that the CellDrum experiments enroll the capability to test the ability of computational models to simulate both, chronotropic and inotropic drug action on cardiac monolayers and (in the future) also 3D tissue.

Ca²⁺ Channel Modulators

The influence of S-Bay K8644 on the contractility of cardiac myocytes is consistent with its effect on their action potential [52]. Through the increase of Ca²⁺ influx, the transient Ca²⁺ concentration in the cell is raised, followed by an increased *Calcium-Induced Calcium Release* from the sarcoplasmic reticulum [53].

The prolonged activation of Ca²⁺ channels is the reason of increased influx of positive ions into the cell. This corresponds to the prolongation of the CR-cycle. In respect to the contraction amplitude, the effect of S-Bay K8644 measured with the CellDrum technology is in accordance with atrial preparations [52] and ventricular slices [54] of the guinea pig as well as with the results obtained with preparations from pig coronary artery [55].

The Ca²⁺ channel antagonist verapamil was selected instead of the clinical standard nifedipine due to its selective action on the heart tissue instead of the vascular system [56, 57]. In contrast to S-Bay K8644, it does not modify the duration of the CR-cycle. This confirms the findings of Singh et al. that verapamil does not modify the duration of the ventricular action potential [58]. However, this is only correct at the concentrations investigated here.

Verapamil is known to inhibit both Ca and hERG channels at higher concentrations. The time integral and amplitude both respond to increasing concentrations of the drug.

The IC₅₀ value of approximately 150 nM for the contraction amplitude measured in this study was comparable to the findings of Singh et al. [58] (300 nM) and an order of magnitude lower than literature data obtained with papillary muscle preparations of rabbit [59], cat [60] and dog [61].

In the current study, both compounds had an inverse effect on the beating frequency as compared to native heart tissue. This effect was reported for Ca²⁺ channel modulators on hiPS-derived cardiomyocytes in electrophysiological studies, where different hiPS-derived cell lines displayed different responses to those agents but their pharmacology was different from that observed in primary human heart cells [62, 63].

Na⁺ Channel Modulators

Like verapamil, veratridine and lidocaine have no effect on the duration of the CR-cycle. As a result, their amplitudes and time integrals increase/decrease concurrently. Veratridine induced proarrhythmic events at concentrations above 2.5 µM. For lidocaine the concentrations could be increased up to 100 µM until the cells stopped beating. In accordance with literature data, application of lidocaine did not result in an increase of frequency but rather in a decrease [64, 65].

In respect to the contraction amplitude, the EC₅₀ value of approximately 700 nM was slightly higher than reported in the literature (400 nM) [66]. In contrast, the IC₅₀ value of veratridine (1.5 µM) was two orders of magnitude smaller than measured on guinea-pig papillary muscle preparations [67] or with rabbit Langendorff setups [68].

In view of the reciprocal action of veratridine and lidocaine on sodium channels, their identical negative chronotropic effects seem remarkable at first sight. Yet, the observed negative effect of veratridine on the heart rate is in accordance with findings in animal *in vivo* experiments [69].

Can the CellDrum replace animal whole organ experiments?

The answer is “yes”, whenever a defined cell type and defined biomechanical/biophysical conditions or functional (mechanical) in near future high-throughput tests are necessary for direct comparisons of drug or experimental effects on cells. This avoids many organ specific, circulatory, metabolic and stress effects as present in whole organ studies. On the other hand whole organ studies cannot be replaced by the CellDrum whenever the whole organ matters e.g. in infarct size studies.

More detailed, the CellDrum is an idealized high precision experimental setup to test cultured cells for their ability to generate mechanical tension at various biochemical and -physical conditions. It enables precise measurements of this parameter using various (heart) cell types and at different conditions. It has the potential to simulate elevated blood pressure, oxygen partial pressure, and drug supply as well as to be upgraded to at least medium high-throughput testing. The CellDrum did never aim at heart infarct research studying effects of coronary blood flow nor at left ventricular pressure and infarct size in living explanted hearts. Circulating blood/energy supply in animal heart explants definitively changes contractile force but that is an issue far away from what the CellDrum aims at. However, at standardized conditions the CellDrum can simulate to certain extend conditions like ischemia/reperfusion by changing the oxygen partial pressure. As detailed in “methods” hiPS cells used here are a mixture of different heart cells. Future experiments may aim at separating those cells and perform individual cell type testing. In the adult heart especially for cardiomyocytes there is no typical cell division.

Conclusions

In summary, we conclude that the CellDrum technology is an accurate high precision method to quantify mechanical properties of cardiac myocyte cell layers *in vitro*. It combines

a high degree of handling simplicity and allows for precise conclusions on cellular mechanics. The major advantage of this approach is its potential scalability towards medium- and high-throughput-screening systems. The application of Laplace's law on circular tissue constructs enables biaxial measurement of cellular tensile stress. Hence, cellular alignment is not necessary for the acquisition of the exerted stress. As a result, this setup eliminates common deviations caused by improper control of the geometry of the specimen. As a research tool with well-defined biomechanical boundary conditions and its potential for upgrading the technology to high throughput tests the CellDrum satisfies many needs for drug testing of cardiomyocytes as well as the mechanical characterization of other cells and tissues. The experimental setup offers the possibility to simulate acute and chronic cardiovascular hypertension by adjustment of the membrane deflection through application of pneumatic pressure. Adopting the current 2D CellDrum technology to a 3D model may bring it closer to the three-dimensional environment in ventricular tissue. In addition, the CellDrum benefits from the fact that myocytes are not grown on a stiff substrate but on highly flexible membranes mimicking to some extent the natural compliance of soft tissue [70]. Yet, the highly anisotropic three dimensional environment and several other factors still rely on full organ tests suffering from the drawback of a non-scientific experimental setup – the heart of an animal. However, the parallel mathematical modeling and simulation allows in principle a transfer to different types of cardiac myocytes as well as to the anisotropic behavior of oriented tissues.

At present, human induced pluripotent stem cells promise to bring about a turning point affecting numerous applications from pharmacology to toxicology. Besides ethical advantages over animal models and the prevention of concerns regarding the translatability of the pharmacological results, hiPS-CMs can be produced in an infinite amount from individuals with known medical and clinical history. Yet, the promises of the new technology bear a certain number of unanswered questions to date. In particular, iPS-derived cardiac cells are suspected to not reflect the maturity of adult somatic cells, especially concerning the functionality of ion channel and expression of specific receptors [71, 72]. As a result, stem cell-derived cardiomyocytes show deviations in their electrophysiological characteristics depending on the respective cell line [73]. Despite these issues, we have shown consistent and physiological responses of hiPS-derived cardiomyocytes on well-characterized inotropic compounds. Hence, the findings of this study support the hypothesis that iPS-derived cardiac myocytes can provide an adequate model for pharmacological and toxicological studies. New strategies for the differentiation of stem cells promise to improve the functional consistency of hiPS-derived cardiomyocytes [74, 75].

During stem cell proliferation and differentiation, oxygen levels play an important role for maintaining their potency and enabling or restricting differentiation, respectively. Tetramethylpyrazine was shown to anticipate hypoxia-induced myocardial cell apoptosis and hence might become an important tool during stem cell cultivation and differentiation [76].

Beyond improvements in the differentiation process, the maturation from embryonic to adult phenotype plays an emerging role. Recent findings disclose new putative drugs that might facilitate accelerated maturation *in-vitro* like puerarin, a compound found in traditional Chinese medicine [77]. Future studies with hiPS-derived electrocompetent cardiac fibroblasts and biomimetic three-dimensional connective tissue scaffolds may improve the physiological conditions towards a more concise cardiac tissue model. Sufficient supply with oxygen and nutrients remains the fundamental hurdle in three-dimensional tissue construction. One approach to overcome this obstacle could be the induction of self-vascularization of the tissue. Recently it was shown that placental growth factor plays an important role in this process *in vivo* [78]. In respect to *in vitro* three-dimensional models of cardiomyocytes and cardiac fibroblasts, the parallel mathematical modeling and numerical simulation may be further developed to understand and predict maturation of iPS-derived cells and predict the transfer to adult cells.

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Disclosure Statement

The authors declare no competing interests.

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