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Original Paper

Mechano-Pharmacological Testing of L-Type Ca^{2+} Channel Modulators via Human Vascular Celldrum Model

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Key Words
Celldrum • Biomechanics • Mechanobiology • Human arterial smooth muscle cells (haSMC) • Vasoconstriction • Vasopressors • Vasoactive agents

Abstract
Background/Aims: This study aimed to establish a precise and well-defined working model, assessing pharmaceutical effects on vascular smooth muscle cell monolayer in-vitro. It describes various analysis techniques to determine the most suitable to measure the biomechanical impact of vasoactive agents by using CellDrum technology. ***Methods:*** The so-called CellDrum technology was applied to analyse the biomechanical properties of confluent human aorta muscle cells (haSMC) in monolayer. The cell generated tensions deviations in the range of a few N/m^2 are evaluated by the CellDrum technology. This study focuses on the dilative and contractive effects of L-type Ca^{2+} channel agonists and antagonists, respectively. We analyzed the effects of Bay K8644, nifedipine and verapamil. Three different measurement modes were developed and applied to determine the most appropriate analysis technique for the study purpose. These three operation modes are called, particular time mode" (PTM), "long term mode" (LTM) and "real-time mode" (RTM). ***Results:*** It was possible to quantify the biomechanical response of haSMCs to the addition of vasoactive agents using CellDrum technology. Due to the supplementation of 100nM Bay K8644, the tension increased approximately 10.6% from initial tension maximum, whereas, the treatment with nifedipine and verapamil caused a significant decrease in cellular tension: 10nM nifedipine decreased the biomechanical stress around 6,5% and 50nM verapamil by 2,8%, compared to the initial tension maximum. Additionally, all tested measurement modes provide similar results while focusing on different analysis parameters. ***Conclusion:*** The CellDrum technology allows highly sensitive biomechanical stress measurements of cultured haSMC monolayers. The mechanical

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stress responses evoked by the application of vasoactive calcium channel modulators were quantified functionally (N/m^2). All tested operation modes resulted in equal findings, whereas each mode features operation-related data analysis.

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Introduction

Increased blood pressure and cardiovascular diseases are major risk factors in the aging population. Due to the high prevalence rate of hypertension (1.13 billion worldwide, 2015 WHO) and cardiovascular diseases, the development of new medications requires methods that ensure a precise and personalized evaluation of active agents as well as their biological response [1]. Additionally, new combinations of already applied medications need to be determined to evaluate appropriate personalized dosages as well as to alleviate possible side effects.

The vascular smooth muscle cell tone is the major blood pressure regulation factor. To vary the blood pressure according to the organism's needs, the cellular tone can vary temporarly and also in the long term. Pathologically increased blood pressure is commonly associated with vascular wall stiffening, caused, for example by heart failure, vascular constriction and vascular wall injury [2-4].

The physiology and biochemistry of vasoconstriction, vasodilation as well as stiffening of blood vessels is largely understood. The stiffness of the smooth muscle cells, the cellular arrangement, anchoring and also the composition of the extracellular matrix (ECM) determine mainly the vascular tone [3, 5].

Smooth myocytes contract more slowly than skeletal and cardiac muscle cells, but they can remain contracted for longer periods [6]. Due to numerous intrinsic and extrinsic factors, the ECM and smooth muscle cells (SMCs) can undergo various adjustment mechanisms, leading to cellular stiffening, dedifferentiation, and proliferation inclusive of cellular hypertrophy. Pathological changes in vascular flexibility can be caused by hormonal signals, mechanical stress or imbalances of vasoactive substances, leading to severe cardiovascular diseases [2, 7, 8].

Technologies like microelectrode arrays, patch-clamp, and high-resolution imaging are helpful tools to acquire insights of electrophysiological, molecular mechanisms and gain a fundamental understanding of cellular physiology. Aside from basic research, cellular forces measurement gains great importance for clinical and pharmaceutical research. Current methods to analyze the biomechanical effect of pharmaceutics ranges from organ bathes to very specific and sophisticated in-vitro systems, requiring tissue dissections and organ equivalents [9]. Although tissue dissections and animal-testing offer ideal physiological conditions, the inconstancy of biological material might lead to data inhomogeneity and are ethically questionable.

Systems like traction force microscopy, atomic force microscopy, cell tweezer or cellular force transducers have been developed to evaluate in-vitro biomechanical effects in ranges of µN [9-14]. In sum, these methods are often limited to specific applications or single-cell experiments, which might bias the data due to none physiological culture conditions.

The herein presented CellDrum technology is an in-vitro based evaluation tool to be used for fundamental research as well as a possible clinical screening approach [15]. Besides the methodical development, this study presents the first human vascular CellDrum model. To evaluate the system and the model, we focused on the modulation of L-type Ca^{2+} channels by vasoactive agents, of which working principles are already well described [16].

Like all other muscular cells, the contraction and relaxation in SMCs depend on Ca^{2+}. Ca^{2+} ion influx is implemented by Ca^{2+} ion channels, which allow Ca^{2+} ions to enter the cytosol, leading to further signaling cascades within the cell and finally to a biomechanical response [17].

In modern medicine, Ca^{2+} ion channels are common pharmacological entry points to treat cardiovascular symptoms, like hypertension and cardiac arrhythmia. Hence, for clinical and research purposes, a wide variety of calcium ion channel agonists and antagonists are available to modulate Ca^{2+} flux [18, 19].

In this paper, we will use the CellDrum technology to establish a new methodical approach to elucidate the effect of Ca^{2+} channel targeting agents with the help of an in-vitro vascular wall model.

Materials and Methods

CellDrum Technology

The CellDrum is a round-shaped cell culture well with a highly flexible $2cm^2$ cultivation area, for adherent cells. The cultivation area made of a 4µm thick polydimethylsiloxane (PDMS) membrane, which enables slight displacements by cellular contraction and relaxation (Fig. 1A).

The CellDrum technology was developed to estimate biomechanical properties of standardized cellular monolayer and 3D tissue equivalents. The CellDrum is a methodologically important technology that, in contrast to known indirect electrophysiological methods, quantifies laterally generated cellular stress in N / m^2. Previous studies have shown that the CellDrum system is a useful tool to elucidate mechano-pharmacological characterizations of human-induced pluripotent cardiomyocytes [20, 21]. In this study, we are focusing on none autonomous contracting cells and evaluate the lateral forces induced by human aortic smooth muscle cells.

Before each test series, the CellDrums are manufactured in our laboratory and characterized with respect to their mechanical properties.

Solely standardized CellDrums, having a thickness of 4µm ± 0,4µm and an initial cell-free membrane tension around 100kPa were used for experimental evaluation.

The measurement is carried out by a so-called Tissue Tension Analyzer. A Tissue Tension Analyzer is composed of a pressure sensor, a deflection sensor and a syringe precision pump. For the deflection measurement, a patented capacitive sensor principle is used, which is described in detail in the FH Aachen patent" CellDrum Electrode arrangement for measuring mechanical stress" [22].

The system used in this study is a prototype that was developed and built in the Institute for Bioengineering at the University of Applied Sciences Aachen. Together with the company HiTec Zang GmbH a comparable device is under development (Hitec Zang GmbH, Herzogenrath, Germany).

For the measurement, the CellDrum is airtightly placed into the measurement socket. Subsequently, the pressure underneath the membrane is increased by a defined volume and flow rate of air. The Tissue Tension Analyzer records the membrane deflection in dependency on the pressure progression, resulting in a pressure-deflection-curve. From this pressure–deflection-curves the internal stress of the cell monolayer and the PDMS membrane can be calculated.

Data Analysis

The mathematical model to calculate the mechanical stress is described in Fig. 1B. The graphical user interface and evaluation software was programmed using LabView®, which uses the Barlow's formula to describe the mechanical properties in thin-walled kettle shaped geometries. The formula is tailored to the CellDrum geometries and recorded parameters (Fig. 1B).

From the calculation, cellular stress is read out in N/m^2. To normalize the results from multiple specimens, the biomechanical changes are expressed in percentage deviation to the initial tension. Hence, slight initial tension deviations are taken into account for the analysis.

Cell culture

Primary haSMC (delivered by Thermofisher) were cultured in T75 flasks until they reached appropriate confluency. Afterwards, the growth factors and supplements were changed to induce cellular differentiation. As the cells were fully differentiated, the proliferation has stopped and maximal contractility has developed. The quiescence proliferation behavior ensures, that the biomechanical analysis is not affected by either proliferation or cell migration. Fully differentiated cells were passaged to CellDrums. To maintain the

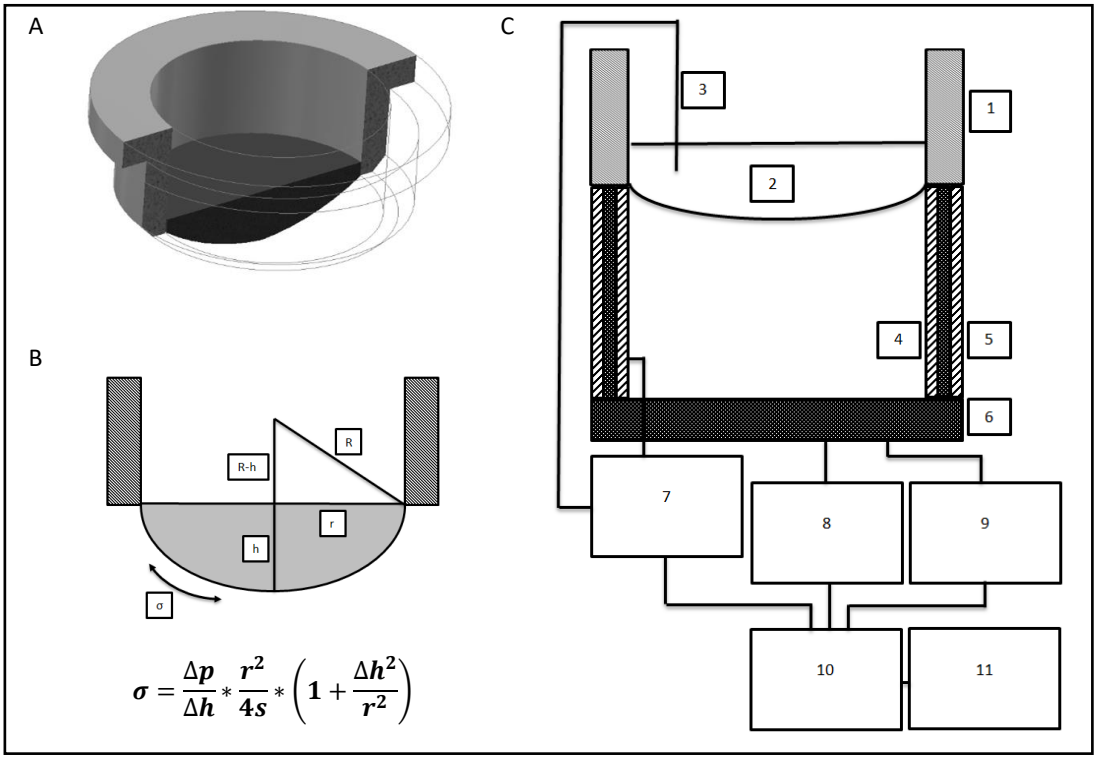

Fig. 1. A) Sliced computer-aided design scheme of a CellDrum with membrane and cell culture medium. The CellDrum offers a 2cm^2 cultivation area, consisting of an ultra-flexible poly-dimethylsiloxane (PDMS) membrane. Due to surface functionalization, cells can attach to the membrane. B) Key Fig. for the CellDrum physical model with CellDrum fitted Barlow´s formula to derive the cellular tension from recorded pressure deflection curves. r) Radius of the CellDrum (16mm). h) Indicates the deflection of the CellDrum membrane to the baseline (in µm). R) Radius of the theoretical hemisphere. The formula is used to derive the tension from the recorded pressure deflection curves. σ) Calculated axial stress (N/m^2). p) measured pressure (in Pa). s) CellDrum membrane thickness (in µm). C) Picture of the Tissue Tension Analyzer with detailed schematic arrangement of the measurement socket - 1) CellDrum; 2) Cells attached to the CellDrum membrane with cell culture medium (500µl) on top; 3) Ground electrode; 4) Counter electrode; 5) Electrical shielding; 6) Measurement socket; 7) Deflection sensor; 8) Pressure sensor; 9) Syringe pump; 10) Processing unit; 11) Peripheral computer and data storage.

cells within the T-flasks as well as in the CellDrums, they were kept in an incubator at 37°C and 5% CO_2 atmosphere. The medium was changed every second day.

For this study, cells only from the fourth and fifth passages were used. To seed the cells to the CellDrums, the cells were trypsinized, passaged and counted by an automated cell counter (Biorad-TC20^{Tm}). Every CellDrum was cultured with 1.5x10^5 cells.

Pharmacological Testing

Bay K8644 was tested to induce vasoconstriction [23-25]. In contrast, vasodilation was triggered by nifedipine and verapamil [26-28].

For all test compounds, concentrations of 1nM, 10nM, 50nM, 100nM and 1000nM were used, either as single concentration measurements or as a dose-response model.

All substances were kept as a stock solution of 10mM at -80°C. Immediately before the measurements, the stock solutions were diluted by the culture medium to the final concentrations and acclimated to 37°C in the water bath. Upon substance application in the CellDrum, 50µl of the medium was removed and replaced by the respective substance. Further dilution with the residual CellDrum medium reached the final concentrations mentioned above. By repeated pipetting, the test substances were evenly distributed in the medium. The control groups were exposed only to the corresponding solvents, without the test substances.

Biomechanical Analysis

For the biomechanical investigation, six CellDrums per test group were prepared and an additional number of six CellDrums were used for each control group. During the measurements, the CellDrums were kept in the Tissue Tension Analyzer socket, which prevents undesired shaking or temperature drop. The test substances were applied through a hole of the apparatus cap from above. Before the measurements, six successive measurements were carried out, purposing the normalization of each CellDrum and to describe the initial tension of the cells.

All in all, we tested three different analysis modes to determine the most appropriate method for our objective: "particular time mode" (PTM), "long term mode" (LTM) and "real-time mode" (RTM).

Table 1. Showing the number and viability of cells per CellDrum. The column called "pre" represents the number of seeded cells (1.5×10^5 /CellDrum), the column labeled "post" representing the mean of the number of cells(N=3) after substance incubation of 30 minutes. The last column shows the viability of the detached and trypan-blue stained cells after the substance incubation time of 30 minutes. Control = untreated; Pos. Control = Lysis Buffer; Bay K8644 1µM; verapamil 1µM; nifedipine 1µM

Treatment	Cells Pre	Cells Post Mean	Viability
Control	1.5×10^5	$1.5\times10^5 \pm 2\times10^3$	97±2%
Pos. Control	1.5×10^5	0 ± 0	0±0%
Bay K8644	1.5×10^5	$1.5\times10^5 \pm 6\times10^4$	93,5±6%
Verapamil	1.5×10^5	$1.5\times10^5 \pm 4\times10^4$	97±0%
Nifedipine	1.5×10^5	$1.5\times10^5 \pm 3\times10^4$	98,5±2%

The PTM describes the initial stress compared to five measurements, which were carried out five minutes after substance application and incubation. Within the incubation time, the sample was left alone. This mode was also used to measure the dose-response model.

The LTM demonstrates the recording of several measurements in a row. This mode focuses on the time progression of substance effectiveness, over 25 min.

The RTM focused on the exact observation of the membrane deflection without monitoring the generated pressure deviation. This mode is exclusively suitable for concentric muscle contraction, so isometric contraction stays undetected. In this mode, the membrane deflection is monitored with a sample rate of 1 kHz, to visualize membrane movement in the nanometer range.

Viability Test

Viability and cell numbers were examined by fluorescence microscopic observation, trypan blue staining and an automated cell counter (BioRad-TC20™ Automated Cell Counter).

To prove the non-toxicity of the used substances, three CellDrums for each substance have been exposed to the highest tested dose for 30 minutes. As a negative control group, three CellDrums were only treated with the corresponding agent solvent (Ultrapure Water/ DMSO). In contrast, a positive control group of another three CellDrums was treated with 500µl Qiagen RLT-lysis buffer (Table 1).

Additionally, the cells were stained by a LIVE/DEAD assay, to ensure a viable and fully attached monolayer (Thermofisher-LIVE/DEAD® Cell Imaging Kit). The kit bases on two-component staining with calcein AM and ethidium homodimer-1, allowing fluorescent distinguishing between viable and damaged cells. The kit was performed according to the manufacturers' protocol (Fig. 2).

Statistical Analysis

The statistical analysis was performed using IBM's SPSS Statistical Analysis. According to the trials, the data were analyzed using paired and/or unpaired Student's T-Test. The significance level is marked according to $P^*\leq0.05$ and $P^{**}\leq0.01$.

Results

Our experiments have shown that the biomechanical response of haSMCs to the addition of vasoactive agents can be precisely measured by CellDrum technology for different time scales. Fig. 3 shows the time courses of haSMCs reactions evoked by three test substances (Bay K8644, verapamil and nifedipine) acquired in the LTM. Due to the supplementation of the calcium agonist Bay K8644 (100nM), the stress of the cell model increased by 4.4±0.8%. Smooth muscle relaxing agents nifedipine (10nM) and verapamil (50nM) decreased the

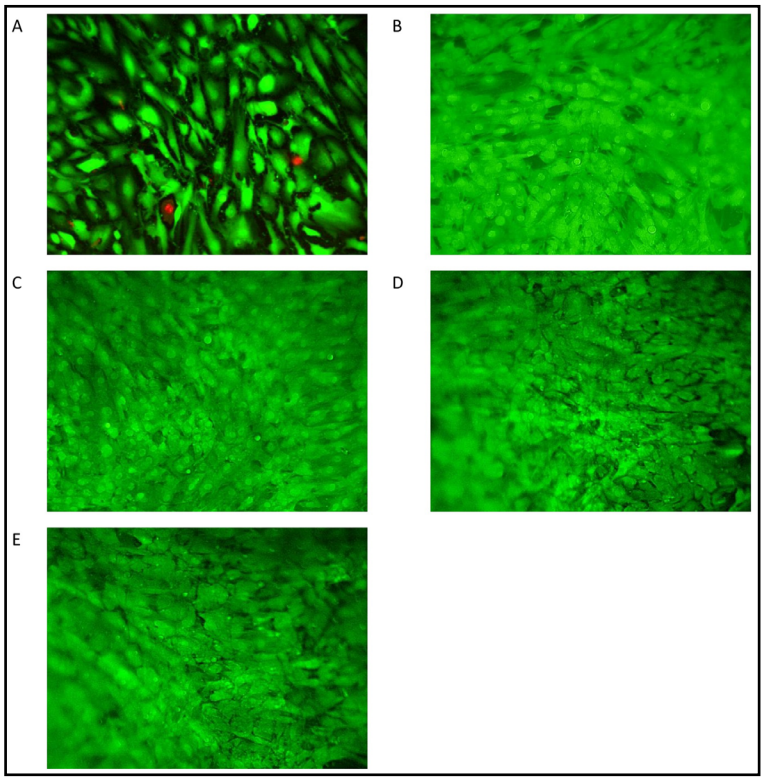

Fig. 2. Representative LIVE/DEAD staining, recorded with a Bio Zero 8100 fluorescence microscope from Keyence, 20x magnification, 1.5x105 haSMC were grown on all displayed CellDrums. Due to the curvature of the CellDrum membrane, only a small part of the specimen is in the focal plane. (A) Reference LIVE/DEAD staining of the haSMC monolayer on the CellDrum. Functional testing of the staining, cell damage was induced by excessive bi-axial mechanical stretching. Intact cells are shown in green and damaged cells in red fluorescence. (B) Control, without the addition of active ingredient (C) Specimen after 30 minutes treatment with 1µM Bay K8644. (D) CellDrum after 30 minutes treatment with 1µM nifedipine (E) Cell monolayer after 30 minutes treatment with 1µM verapamil.

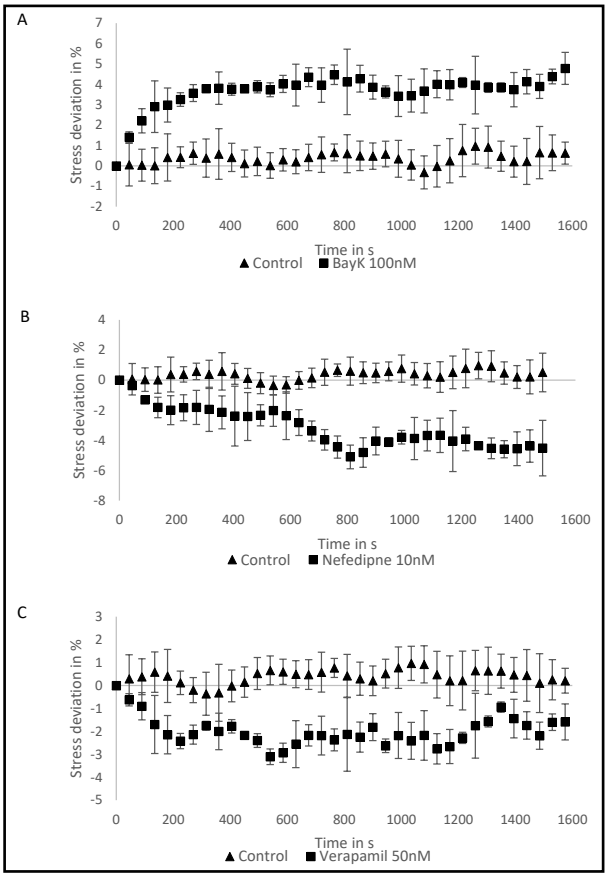

Fig. 3. Time courses of the pharmacological CellDrum investi-gation via LTM (N=6 mean ± 1 SEM). The change in the mechanical stress caused by the test substance expressed as the relative difference of the mechanical stress (%) to the initial value. All datasets were statistically evaluated by independent sample T-test. For statistical evaluation, all data from each measurement series were compared with all data from respective control measurements. A highly significant effect can be seen in all test substance measurements (p***<<0.01).

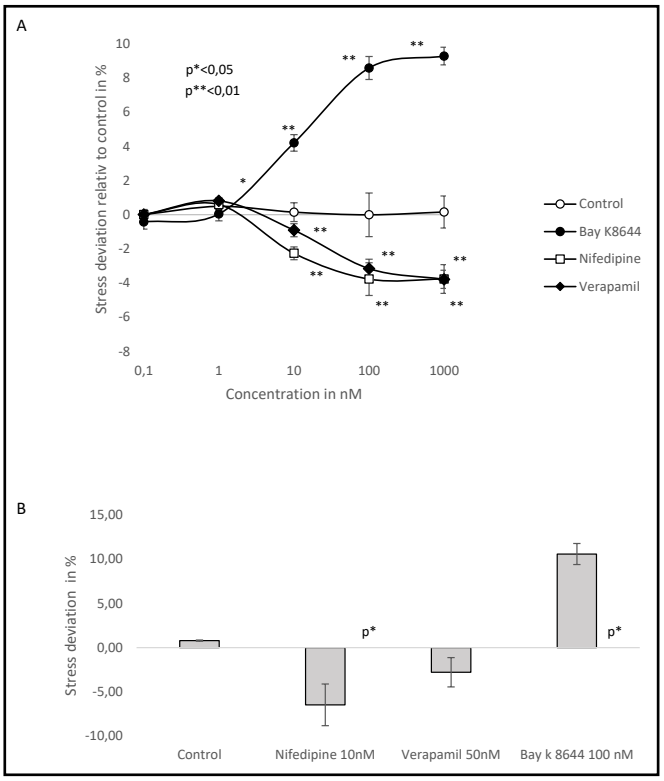

Fig. 4. Data measured by the PTM. A) Dose-response model of all tested agents in the concentrations of 0.1nM, 1nM, 10nM, 100nM and 1µM. Y-Axis indicates the deviation in mechanical stress relative to control in % to the initial stress. The X-Axis represents the substance concentration on a log scale. Every data point represents five (n=5) measurements taken from six individual samples(N=6). The error bars indicate the SEM. The statistical evaluation was carried out using the T-test for dependent samples. The control shows no significant change(p>0,05), but all test substances after the addition of 10nM result in a highly significant effect (p**<0.01).B) Relaxation or contraction after five minutes compared to the initial stress. Each bar represents five (n=5) measurements taken from six individual samples(N=6). The error bars indicate the SEM. The data were statistically evaluated using the paired T-test to prove significant mechanical changes from the initial specimen-tension. In relation to the initial measurement, Control (p=0.775) and treatment with verapamil did not produce any significant effect (p=0.229). In contrast, the effect of nifedipine (p*=0.047) and Bay k 8644 (p*=0.036) leads to a significant change in cellular tone.

cellular tension by 4.3±0.7% and 2.1±0.8%, respectively. In all cases of the control specimens, the mechanical stress values remained steady throughout the measurement period of 25 minutes. In the three performed substance tests, the relative change in the mechanical stress reached a plateau that remained constant within the scope of the measuring accuracy (see Fig. 3).

According to the data, the cellular relaxation caused by nifedipine is amplified after 500 seconds and reached another plateau after 800 seconds. Such behavior did not occur with the substances Bay K8644 and verapamil. This observation should be further considered in future experiments along with wash in effects.

Fig. 4A shows a dose-dependent biomechanical stress response to the application of verapamil, nifedipine and Bay K8644, measured by the PTM.

Fig. 4B presents the PTM analysis with the single-dose application. The application of nifedipine decreased the cellular stress by 3, 8% with comparable high standard deviation. Verapamil causes slight cellular relaxation around 2, 8%, which correlates to the data from the LTM and the dose-response curve. In contrast, the application of Bay K8644 increased mechanical stress around 10, 6%, representing the greatest vasoconstriction during this study.

Results from RTM measurements show the height displacement of the CellDrum membranes over ten minutes (Fig. 5). In the beginning, high peaks indicate the exchanging and mixing process of the agents and medium.

The membrane displacement of the control group (A) remains unchanged. The cellular contraction in response to the application of Bay K8644 elevated the membrane and caused spontaneous cell oscillation, just before the contraction reached a plateau phase (Fig. 5B / Fig. 6). The vasodilative effect of nifedipine and verapamil (Fig. 5C, D), lead to a lowering of the CellDrum membrane. Like previous results have shown, the application of nifedipine

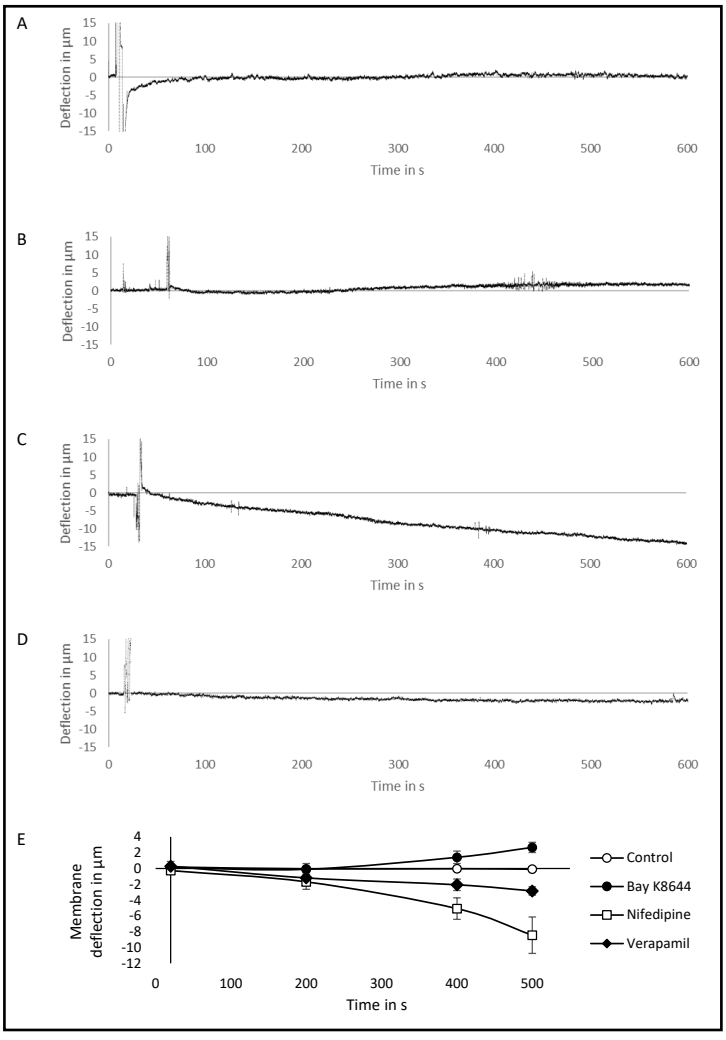

Fig. 5. The graphs show the RTM of samples treated with vasoactive agents. A) showing the control measurement. The samples below were treated with 100nM Bay K8644 (B),10nM nifedipine (C) and 50nM verapamil (D). The Y-Axis shows the membrane displacement relative to its initial position in µm and the X-Axis showing time progression in seconds. (E) The data points represent the mean value and standard deviation of three individual biological samples at the time points 20, 200, 400 and 500 seconds. For this purpose, samples with 20 values per measurement were examined.

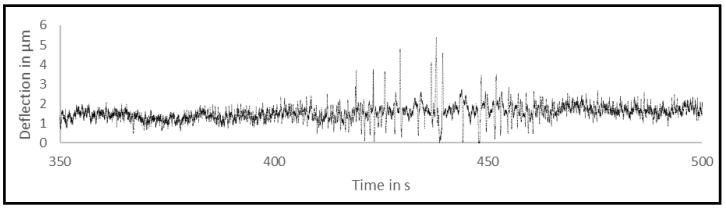

Fig. 6. Spontaneous oscillating observed during RTM. haSMC treated with 100nM Bay K8644.

(~15µm) compared to verapamil (~2µm) has a stronger cellular relaxational effect. The repeatability of the measuring method is displayed in Fig. 5E, in which averaged deflection values of three individual samples and their standard deviation over time are shown.

The results from the viability test and trypan blue cell counting showed small negligible deviations in cell number and cellular viability around 95%. Following this data, we assume a non-toxic effect of the chosen substances and concentrations in our experiments. By that, we consider that cell proliferation or apoptosis will not affect the measurements. Cell detachment was microscopically checked and did not occur with the used substances.

Discussion

Biomechanical analysis by pressure deflection curves has turned out as a precise measurement method to determine mechanical changes of human arterial smooth muscle cells in the range of a few N/m^2. Due to the application of L-type Ca^{2+} channel modulators, positive and negative stress deviations are measurable over time.

Additionally, it was possible to distinguish different agent doses by a dose-response model of a single cell monolayer. Three different measurement modes were tested to establish an appropriate standard operation protocol (SOP).

All three modes have shown an increased Ca^{2+} influx by Bay K8644. The transient Ca^{2+} concentration in the cell is raised, followed by an increased calcium-induced calcium release from the sarcoplasmic reticulum, leading to increased cellular tone [23-25]. In contrast, the Ca^{2+} channel antagonists nifedipine and verapamil were used to block the Ca^{2+} influx, resulting in a cellular relaxation [26-30, 31]. In order to measure the direct functional and time-dependent relationship between intracellular Ca^{2+} concentration and biomechanical changes, the use of Ca^{2+} -dependent imaging would encourage the findings. Due to the flexibility of the CellDrum membrane, changes in cytosolic Ca^{2+} concentration and resulting mechanical deviations lead to a shift of the microscope focus plane. According to the data in Fig. 5, the imaging method would have to track the focal plane in real-time, which is technically not possible for us at this moment.

For a first-time substance testing with the CellDrum system, the LTM seems to be the most promising technique, as biomechanical effects can be evaluated time-dependently. For a direct comparison of the specimens to a precisely defined time, the PTM seems to be adequate. From a technical perspective, it has to be taken into account that the CellDrum inflation, during LTM measurement, causes a repeatable cellular deformation. In this case, mechanosensitive and stretch-activated Ca^{2+} channels might be triggered, which could slightly differ from the measured data of PTM and LTM [27, 31, 32].

The RTM seems to be inappropriate for cellular force measurement, as the necessary parameters are too inconsistent. It is noticeable that the biomechanical changes resulted in CellDrum membrane displacement over time, but does not render processable data to calculate cellular stress. Compared to the other analysis modes, the RTM neglects isometric cellular tension deviation. Interestingly, RTM has recorded strong spontaneous contraction around 430+/-50s, by the application of Bay K8644 (Fig. 6). Biomechanical phenomena like spontaneous or rhythmical contractions are also described in the literature and might be of major interest for further studies but require additional technical and analytical improvements [33].

As shown in Fig. 6, we consider these spontaneous oscillations to also appear during the other measurement series, causing an increased standard deviation. Additionally, the activity, sensitivity and expression level of Ca^{2+} channels is strongly depending on the cells as well as varying from cell to cell and might differ even in the specimen groups [34, 35].

It is difficult to classify the collected data into a direct scientific context, as literature reveals a great variety of methodical approaches and even greater deviations of resulting data. As our data represent relative deviations to the normalized initial cellular stress and do not correlate to maximal contraction or relaxation, the results appear comparatively small to corresponding literature [23, 29, 30].

Cell contraction and relaxation were measurable without prior pharmacological or mechanical stimulation. This could indicate that due to the softness of the CellDrum membrane and the components of the medium, the cells can maintain a certain biomechanical tone that is slightly higher than the membrane tension alone. Technically, the maximal measurable increase in mechanical stress increase is infinitely large. In contrast, the smallest mechanical stress of the specimens to be measured is identical to the membrane residual stress, which limits the measuring range in the negative scale. The range of the measurable relaxation thus depends on the cellular basic tension of the specimen.

Measurable biomechanical changes set in around a few N/m^2 that do not necessarily lead to specimen deformation, as the generated cellular forces might not overcome the initial force balance of the CellDrum system. Comparing data from RTM and LTM shows a faster measurable stress deviation by the RTM, which endorses the hypothesis that cellular forces develop isometrically in the beginning. By that, the biomechanical evaluation with the CellDrum technology is not depending on specimen deformation and allows us to measure even isometric tension deviation time-dependently.

Biologically seen, the cell preparation, species, source and differentiation stage are crucial for reliable data comparison and reproducibility. Especially, in the case of vascular smooth muscle cells, the contractility strongly depends on the vascular section and cellular differentiation stage, which can vary due to the physiological requirements [36]. With the help of the CellDrum technology, the cell model can be precisely characterized, standardized and simplified, allowing to reduce unpredictable biological side effects for multiple experimental repetitions.

Nevertheless, due to the model simplification, such aspects as pharmacodynamics and bioavailability of the tested substances might vary from in-vivo samples, as the substances have direct access to the haSMCs. For all selected test substances, the wash-in time to reach a plateau phase took approximately ~ 200 s (3:20 min). Wash-out attempts were not performed in this work and must be reserved for future measurements. Moreover, due to the simplified model, some aspects of the pharmacodynamics like diffusion, metabolism and endothelial impact stay unattended.

In addition, cellular orientation and alignment are crucial factors to maximize the measurable force generation of vascular smooth muscle cells. Studies by Ying Zhang and Kit Parker have shown that the contraction force of SMCs is cellular shape-dependent and increases as the cell is more elongated and cytoskeletal structures are more parallel orientate [37, 38]. According to the SMC contraction physiology, cytoskeletal structures of the contractile apparatus are netlike distributed through the cellular body. Due to this property, the physiological force development of smooth muscle cells acts in multiple directions. The round-shaped geometry of the CellDrum offers biaxial tension conditions, enabling the cells to act and react mechanically in all XY-directions equally. This mechanical condition provides aside from the more physiological culture conditions also more physiological force transduction. Additionally, cellular inhomogeneity and preferred growth direction can be biomechanically ignored compared to other methodical approaches [38-41].

Another critical issue is the cellular attachment and growth substrate, which might vary greatly and causes differences in force transmission [5]. For cell attachment, the PDMS cultivation area is coated with 1% fibronectin. Depending on the fibronectin concentration used in an in-vitro model, the force transmission might differ from physiological equivalents [42, 43].

Conclusion

In this study, we presented and discussed new approaches for the CellDrum technology to evaluate the mechanical properties of contractile vascular SMCs in response to L-type calcium channel modulators. These collected perceptions open up a whole new research area aside from the biomechanical characterization of cardiomyocytes by the CellDrum technology. Using the CellDrum technology can benefit the studies of vascular diseases and might enlighten the pathophysiology of vascular wall stiffening. Additionally, we believe that the CellDrum system could be an added value for pharmacological/toxicological testing and development by providing standardized specimens. For the future, we are planning to study further biomechanical changes due to diverse impact factors like mechanical stimulation, ischemia, induced hormonal imbalance and calcification.

Beyond the high precision and the convenient handling, the CellDrum technology also enables the combination of gathered mechanical data with further analysis techniques to link biomechanical to molecular biological and electrophysiological data.

In the end, we consider the CellDrum technique for clinical application. In this case, we think of a fast screening procedure that might indicate hormonal or vasoactive imbalances within the blood serum of patients, which can be functionally tested. Furthermore, in combination with nowadays stem cell research, the CellDrum might lead to a more personalized in-vitro pharmaceutical adjustment of hypertension treatment.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

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Author Contributions

R.B. developed the measurement system and analysis software, performed the measurements, processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. Further on, manufactured the samples and characterized them with the CellDrum technology. R.B. wrote the article with the input of all authors.

A.A. and J.H. drafted the manuscript and aided in interpretation. Both supervised the work.

I.D. and G.A. did a critical revision of the article and encouraged R.B. to focus on biomechanical characterization by CellDrum technology.

J.F. and T.C. did great critical revision on the article.

Disclosure Statement

The authors have no conflicts of interest to declare.

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