Functional Toxicology and Pharmacology Test of Cell Induced Mechanical Tensile Stress in 2D and 3D Tissue Cultures

Gerhard M. Artmann, Jürgen Hescheler, Haritha Meruvu, Sefa Kizildag and Aysegül Artmann

1 Introduction

1.1 Mechanical Cell Forces and Tensile Stress

Mechanical forces/tensile stresses are critical determinants of cellular growth, differentiation and migration patterns in health and disease [13, 19, 27, 61, 43]. They co-regulate the mechanical balance between cells and the extracellular matrix in nearly all tissues outside and within organs of the body. In addition, they are associated with the regulation of mechanically driven biochemical and genetic cellular processes [1, 35]. Tensile stresses determine intercellular traction, as well as internal cytoskeleton mediated tensile stress transmittance to the extracellular matrix (ECM) through cell-matrix adhesion proteins [22, 30].

In loving memory of our former student Taylan Demirci a passionate young scientist who died in 2010.

G. M. Artmann (✉) · J. Hescheler
Institute of Neurophysiology, University of Cologne, Robert Koch Strasse 39, Cologne D-50931, Germany
e-mail: g.artmann@gmx.net

H. Meruvu
Department of Bioengineering, Gaziosmanpasa University, Tokat, Turkey

S. Kizildag · A. Artmann
Institute for Bioengineering, Medical and Molecular Biology, University of Applied Sciences Aachen, Juelich, Germany
e-mail: A.Artmann@fh-aachen.de

S. Kizildag
Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

G. M. Artmann et al. (eds.), *Biological, Physical and Technical Basics of Cell Engineering*, https://doi.org/10.1007/978-981-10-7904-7_7

Table 1 Mechanical force, rigidity and tensile stress [related SI units]

Tissue tensile stress	Cell force	Tissue rigidity	Tissue strain
$\sigma = \frac{F}{A}\left[\frac{\mathrm{N}}{\mathrm{m}^2}\right]$	$F[N]$	$\frac{stress}{strain} = \frac{\sigma}{\varepsilon}\left[\frac{\mathrm{N}}{\mathrm{m}^2}\right]$	$\varepsilon = \frac{\Delta l}{l_o}$

The terms "force" and "tensile stress" should not be mixed up. "Cell Force" is more intuitively accessible and is used in most biological papers, while the term "tensile stress" is more abstract and therefore less commonly used. The term "force" describes pulling forces in cells given in Newton "N". "Tensile stress" instead, acts when a force pulls at a cross sectional area perpendicular to it. It is given in N/m^2 where "m^2" stands for the product of thickness × width of the (idealized) rectangular cross-section of a tissue sample [27, 62]. In some applications where the thickness of a cultured tissue sample is very much smaller than its width, for example when cells are cultured as approximately 2 µm thick monolayers on a CellDrum membrane of 16 mm in diameter, then the "tensile stress" is given in N/m. The latter implies a "force" pulling perpendicularly to an imagined cutting line "cut" perpendicular through the monolayer plane. The "line length" is given in "m" (Table 1). These are simple yet necessary statements when talking about "force" and "tensile stress" induced by tissues or cells, respectively.

Although all cytoskeletal proteins have an impact on the cell's mechanical properties, actin is the basis of mechanically driven movement in all known eukaryotic cells. Phagocytosis, cytokinesis, cell crawling and muscle contraction all depend upon structures build from actin. Typically, actin represents about 5–10% of total amount of proteins within a cell and 20% of protein in muscle. Actin forms assemblies of filaments and plays a key role in force transmission from the ECM to the cell's nucleus and vice versa [51, 59].

In fibroblasts the primary function of the protein actin is building up filaments (Fig. 1). Actin monomers, G actin, polymerize to filamentous F actin. A high actin concentration at polymerization onset allows for faster formation of filaments. Actin filaments exhibit both ends, a slow growing minus as well as a fast growing plus end [50].

Myofibroblasts are an intermediate form of smooth muscle cells and fibroblasts containing contractile actin and myosin fibers. These cells endogenously contribute high amounts of collagen to the ECM. Myofibroblasts contract via smooth muscle cell actin-myosin complexes, which contains more alpha-smooth muscle actin.

1.2 Focal Adhesions

Focal adhesions are large cellular protein assemblies (Fig. 1, red colored dots) which help regulate the "mechanical balance" in tissues. They are a prerequisite to regulate cell internal signals in response to the cellular mechanical environment. Via focal adhesions cells transmit forces to the extracellular matrix (ECM) and

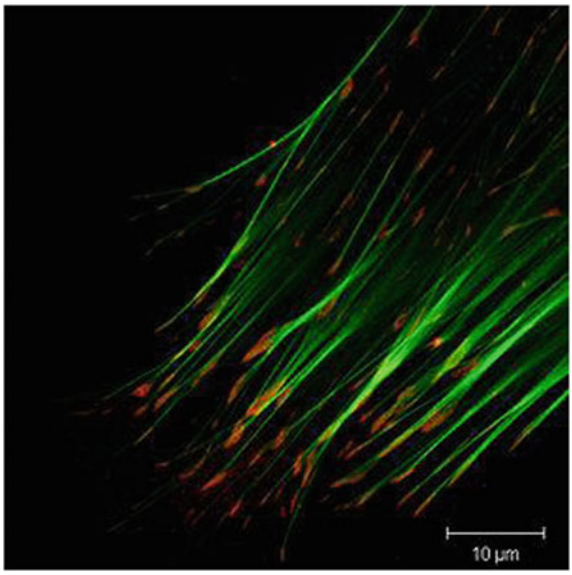


Fig. 1 Immunofluorescence image of actin filaments (fibers, green) and the focal adhesion protein vinculin (red dots) in a fibroblast. Focal adhesions are integrin-containing, multi-protein structures forming mechanical cell membrane spanning "bridges" between intracellular actin fibers and the extracellular matrix and/or a surface, respectively, in adhesion dependent cell types [80]

other interacting cells. In other words, they represent sub-cellular structures mediating regulatory effects (i.e., signaling events) of a cell in response to ECM adhesion [18]. The attachment of cells to its substratum which may be a basement membrane in vivo or the culture dish in vitro, thus, is mediated by focal contacts or focal adhesions [34]. These multi-domain proteins adhere to the surface of the tissue culture dish and expose specific sequences that are recognized by cell-surface receptors. The cell-surface receptors involved in focal-contact formation belong to the integrin superfamily. Integrins are non-covalently associated complexes of two distinct, high molecular weight polypeptides called α and β integrin acting as transmembrane linker in a variety of cells. They span the plasma membrane in the region of focal contacts. Its cytoplasmic domain binds through a chain of various acting proteins including talin, vinculin and α actinin to actin filaments of the cortical cytoskeleton. The ability of fibroblasts to perceive extracellular stress to and from transmittable contractile force to the ECM is crucial for regulating their activity during connective tissue remodeling [30, 71, 61].

1.3 Extracellular Matrix

Cell adhesion is defined as cellular binding to a surface, which can be another cell, a surface or an extracellular, organic matrix. Extrinsic structures determining a tissue's mechanical integrity are mainly formed by the extracellular matrix (ECM). The ECM is a molecular complex mainly consisting of fibers and an amorphous inter-fibrillary matrix composed by components like glycoproteins and proteoglycans. The ECM also contains molecules such as growth factors, cytokines,

matrix-degrading enzymes and their inhibitors [78]. The idea of a dynamic reciprocity between the ECM on the one hand and the cytoskeleton and nuclear matrix on the other hand is widely accepted. In this model, ECM molecules interact with cell surface receptors, which then transmit signals across the cell membrane to molecules in the cytoplasm; these signals initiate a cascade of events through the cytoskeleton into the nucleus, resulting in the expression of specific genes, whose products, in turn affect the ECM in diverse ways [11].

Basically all cell-ECM interactions [36] participate directly in processes like cell migration, growth, differentiation, programmed cell death (known as apoptosis), cell adhesion, activating intracellular signaling and contractile response. Matrix components and the mechanical forces that cells experience markedly influence the maintenance of cellular phenotypes and effect cell shape, polarity and differentiated function. In most tissues, the ECM is constantly being remodeled, particularly in processes like wound repair or tumor cell invasion. Extracellular matrices are specialized for particular function, such as strength (tendon, connective tissue) or adhesion (basement membrane of epithelia) [60].

1.4 Cell and Tissue Tensile Stress Measurement Technology

An important physiological function of cells in terms of biomechanics is the tensile stress they are able to generate either alone as single cell, as 2D-cell monolayer, or embedded in a 3D cell-ECM culture, respectively. Quantifying these tiny tensile stresses is a real technological challenge. On top of all other requirements as there are sensitivity, reproducibility, electronic drift, signal-to-noise ratio, and temperature further preconditions MUST be considered. Tensile stress measurements require defined boundary conditions to be considered and results obtained with a certain experimental setup should not depend on specific technological designs. Although these are general requirements for measurements in mechanics, there are major problems for biological samples. The cells are alive, meaning their metabolism needs to be maintained. They also age, changing their mechanical properties, among other things. They must be kept as much as possible in their natural biomechanical and biochemical environment since, importantly, cells exist in a delicate balance with their mechanical environment. Cells in a soft matrix provide data other than cells in a stiff or very rigid matrix [37]. This is a factor that is by far not adequately taken into account in many cell experiments by biologists and medical scientists. Protein and gene expression of cells depend quantitatively and qualitatively on the mechanical properties of their environment, the extracellular matrix. For example, fibroblasts that suddenly find a foreign material with extremely high modulus of elasticity as a result of pacemaker implantation, rather than soft tissue as before, extremely change their extracellular matrix. Doctors even speak of encapsulation of the pacemaker [40].

Furthermore, it is often important to follow the time course of the mechanical measurements of cells over days and weeks. Then the selected boundary conditions

of the measurement setup must be chosen so that the cells remain vital for one thing and for another degenerate in no way. This is the case for example in toxicology tests with cardiomyocytes [14, 39]. These prerequisites define the technological-biological measures to be taken in experimental setups and test systems aiming at cell tensile stress measurements at all scales from tissue via cells to subcellular structures.

Methods for force measurement on cells and subcellular structures use tools to mechanically deform cells in combination with transducers and/or imaging technologies, respectively [24]. These approaches include atomic force microscopy [28], magnetic cytometry, carbon fiber based systems etc. Qualitative measurements on individual cells have been made using atomic force microscopy to investigate cytoskeletal substructures [63]. The nano-indention technique is another tool to characterize mechanical properties of living cells to understand biomechanical and biophysical processes, such as disease progression and cell–material interactions [43]. At the same time, advancement in the field enabled high-resolution structural visualization of living materials. For instance the two-photon fluorescence microscopy may provide a more sensitive detection technique than classical histological methods. Multi-photon microscopy assesses for example the nano-mechanical contribution of elastin networks to overall tissue mechanics in multiscale models [57]. Mechanical investigations on a molecular scale are approached by Förster resonance energy transfer (FRET) [75], photo-quenching, loss of fluorescence or changes in fluorophore emission properties for correlating molecular tensile stresses to molecular, cellular or tissue functions [21, 66]. FRET-based Molecular Tension Microscopy uses a mechanical tension-sensitive FRET biosensor genetically encoded within a protein of interest and depicts spatiotemporal maps of molecular tensions detected [29]. These approaches can also be complemented by in vitro experiments using soft gel substrates (traction force microscopy), elastic micropillars [64] and gel matrices to quantify traction forces generated by cultured cells individually and collectively in 2D and 3D geometries [16]. Techniques based on birefringence can be used to quantify tissue scale stress but suffers setbacks like requiring flat, transparent samples and delicate calibration. Tissue-scale laser ablation measures tissue stress to viscosity ratio but requires sample and laser alignment and allows only a few experiments per sample [67]. Information on cellular processes in tissues/organs can be derived from the mechanical properties of living cells (see Chap. 8), cell layers and tissues. Particularly elasticity measurements offer a valuable insight. Before 1970, mechanical behavior of tissues and organs were interpreted from the whole organ behavior (like pressure-volume relations of the heart). Later on attention has been paid to the mechanics of non-adhesion dependent blood cells like red blood cells.

Micropipette aspiration techniques were applied to determine the viscoelastic behavior of individual cells and initially used for analyzing mechanical membrane properties of red blood cells (RBCs) and other blood cell types [20, 38]. However, this technique together with all further technologies developed for the mechanical

analysis of individual cells have major drawbacks limiting their applications to a great extent, in particular if considered for routine use. They do not keep cells in a normal (mechanical) in vivo environment. Furthermore, the micropipette fabrication and cell sample preparation is quite skeptic, complicated and time consuming [69]. On the other hand (and with different scientific aims) micropipettes can be very useful in biophysical cell experiments. This technique lead to the discovery that hemoglobin molecules of different animal species as well as of humans must be seen not only as oxygen and carbon-dioxide transporting molecule but also as a molecular thermometer sensing a species' body temperature [8, 6].

Most other cells are adhesion dependent. They only survive when mechanically stretched [48, 61]. Cells exert mechanical tensile stress and transmit it to their environment (substrate, ECM). This may cause substrate/ECM deformation that can be visualized and investigated further for cellular tensile stress induction if substrate/ECM material properties and biomechanical laws are considered properly [27]. The silicon wrinkling technique was pioneering such approaches and proved to visualize the traction forces of individual cells on ultra-thin flexible membranes. It uses a highly viscous polymeric fluid polydimethyl siloxane crosslinked at the surface forming a thin elastic film over it. A cell on its surface creates wrinkles that are characteristic of the mechanical stresses generated by the cell (Fig. 2).

Improvements were made later on by include the "tuning" of the elastic compliance of the elastic film. However, deformation data can be analyzed only semi quantitatively because the buckling of thin polymer film is a nonlinear phenomenon according to the theory of elasticity [15, 33].

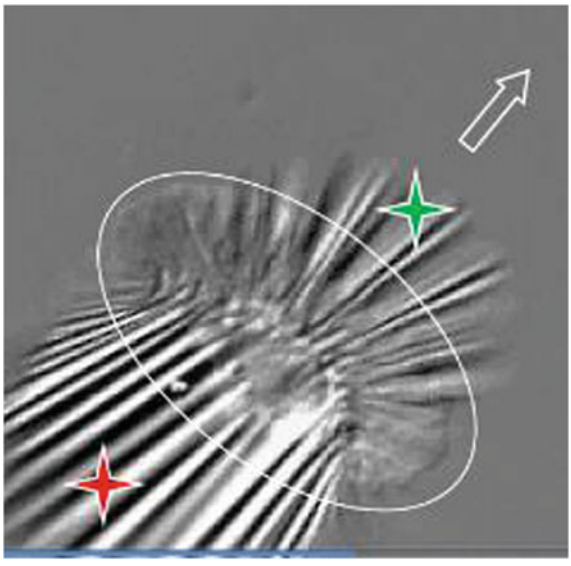


Fig. 2 Single fibroblast crawling spontaneously on a thin silicone sheet (<1 μm thick). The elliptically sheathed area covers the cell's body which is attached to the surface. Arrows indicate the cell's current moving direction. Stars mark the instantaneous wrinkling area in front of (green) and behind (red) the cells body (adopted from [33])

Other augmented experiments realized the impact of a 3D environment on individual cells and began analyzing mechanical properties of standardized cell seeded artificial tissues [25, 49]. Available contemporary sensor technology allowed analyzing the mechanical behavior of tissues or tissue cultures by stretching them in one (uniaxial) or two (biaxial) directions, respectively. The uniaxial tissue-stretch-technique (Fig. 3) is an optical-electromechanical system for measuring the force-deforming behavior of uniaxial or biaxial loaded slabs of soft tissue specimen [54]. Although the set-ups appear intuitively appropriate, look simple and easy to use, many circumstances and boundary conditions do not suggest using such devices. They suffer a major technical drawback caused by specimen deformation due to unreliable grip (slippage or tear-out near clamps). Further factors limiting the application of such technique are soft, gel-like mechanical consistency of cultured tissue strips, experiments carried out in a culture medium bath, and surface tensile stress problems. Furthermore, rectangular gels suffer from "necking" due to the stress variations imposed during uniaxial force measurements. The uniaxial loading also introduces a physiologically unknown parallel aligning in response to the applied mechanical tensile stress and there is augmented likelihood that the object to be investigated is actively modified by the experimental setup.

In summary and in a nutshell all devices and methods are able to monitor somehow mechanical properties of cultured tissue constructs or individual cells. However, they suffer common drawbacks like use of complicated setups, not accounting for the environmental influences, absence of well-defined and biologically relevant boundary conditions, and last not least not considering the specific needs of a sterile and cell culture compatible experimental setup. These drawbacks may cause misinterpretation of experimental results marring the investigator's experimental expertise. Hence there is a quintessential need for a technique with potential upscaling for pharmaceutically relevant sample throughput.

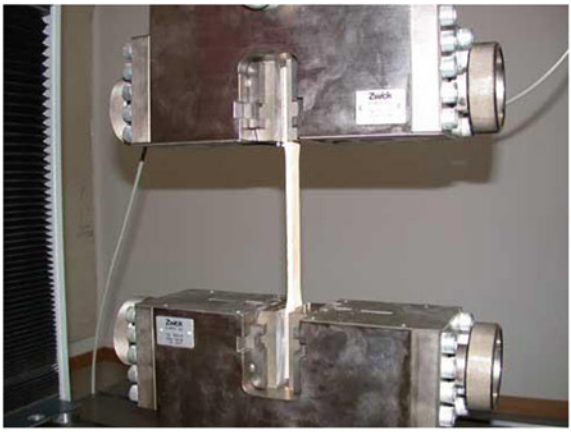


Fig. 3 Uniaxial stretching test setup. The white tissue testing strip (center) is mounted between two holders (above and below) and is being elongated by them. The force applied and the resulting elongation are monitored simultaneously and plotted as force versus elongation curves [15, 54]

2 The CellDrum Solution

The CellDrum principle was patented in 2000 [3]. This chapter introduces the terms and procedures involving applications of the CellDrum technology as an innovative routine testing solution. In this perspective we discuss about time independent or gradually changing mechanical tensile stress patterns, rhythmic changes of mechanical tensile stress and, the future perception of individualized high throughput screening (HTS) in pharmacology and toxicology.

2.1 Measurement Principle

All adhesion dependent cells exert mechanical tensile stress to the substrate they adhere upon [6, 47, 63]. There are many feedback mechanisms and signal transduction cascades involving regulation of the dynamics between mechanical tensile stresses, cell motility and cell growth. These facts gave a reason to develop the CellDrum technology. This, however, was just a technological expression of the wide field of Mechanobiology [27, 43]. The technology dedicates itself to analyze the effects of mechanical tensile stress of their environment (extracellular matrix, ECM, proteins, and biomaterial surfaces) to cells and vice versa. Cell matrix interactions normally are regulated and cell forces are transmitted through focal adhesion complexes. They transmit mechanical signals from outside the cell to the nucleus and from the nucleus to the cells environment, respectively. Thus, mechanical effects have a great impact on the fate of cells in a matrix [43, 61]. Normally, when cells adhere to a culture dish/surface, the (small) cellular mechanical tensile stress does not bend it, it remains flat. This is because the underlying substrate is too stiff to bend. However, when underlying substrates of any cell culture assembly approximately match the order of magnitude of cell layer forces then that tensile stress will be able to deform the substrate. This can be made visible and the extend of surface bending quantified. The CellDrum [3, 73] uses as substrate a thin (normally 4 μm), circular (16 mm) silicone membrane on which the cells are cultured (Fig. 4). A culture medium container (cylinder) is sealed watertight from below with an ultra-thin silicone membrane (pink colored, Fig. 4a). The ratio membrane thickness divided by the cylinder diameter of 16 mm is 0.00025! Cells are cultured from inside the chamber on top of the membrane which is functionalized for good cell attachment. The CellDrum is filled with cell culture medium. The culture medium weight bulges the membrane downwards. This indentation is measured. Membrane indentation is at its maximum when no cells are attached to the membrane. An additionally cultured cell monolayer on top of the membrane or a 3D tissue culture, respectively, will lift the membrane upwards. This is because cells generate intrinsic lateral mechanical tensile stress inside the cell

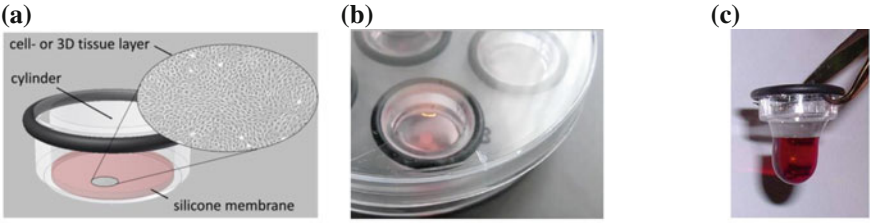


Fig. 4 CellDrum: **a** Scheme with enlarged cell monolayer, **b** CellDrum holder for culturing purposes, **c** A smaller than 2 μm thick membrane allows its complete bending under the weight of the drop. The drop is surrounded by the membrane drop-shaped which prevents it from tearing off. If we would culture beating cardiomyocytes on the inside of the membrane the drop would contract like a real heart with no further stimulation

layer and try to shorten it. Since cells are attached to the membrane surface, tensile stress is transmitted via cellular focal adhesions to the membrane and lifts it this way [3, 56, 72, 73]. When a cell layer contraction is induced by chemical compounds the membrane lifts up further. If we reverse this and wash out the extra tensile stress causing compound from the cell monolayer (if possible), then the tensile stress in the cell layer decreases—the membrane bends again downwards. Thus, the lateral cell monolayer tensile stress relaxes to the mechanical state it had before compound application. If we then detach all cells of the monolayer biochemically from the membrane, then their contribution to the total mechanical tensile stress of cell monolayer plus membrane bias is lost. Thereafter, the total stress, and thus the deflection of the membrane, should assume the state it had before the addition of cells. If the membrane deflection does not fully return downwards to the indentation of the "unused, pure" membrane the residual indentation difference must be the result of the ECM that has been secreted by the cells during the cell monolayer culture period. This value could become another important mechanical parameter of cell mechanics research that could not previously be measured. The CellDrum technology inventors would call this an "interesting side effect". It might reveal insight into the stiffness of the ECM for example depending on cell culture time.

In summary, with the CellDrum system only the membrane indentation is measured at each condition. Now, if simple laws of mechanics are applied, the mechanical stress caused by the cell layer can be calculated. Since the mechanical tensile stress of the membrane without cells is known for each CellDrum, it can be subtracted and the result is the membrane tensile stress alone, as if it was bulged without any supporting membrane underneath. For cell culturing purposes, multiple such CellDrums are placed in a CellDrum holder which than can be put in the incubator for cell culturing. Cell culturing procedures are the same as used regularly. Ongoing new technology development aims at automated culture medium change inside the incubator.

2.2 Finite Element Simulation

For finite element analysis the MSC Patranv2001R3 and NastranV707 software (MSC. Software Corporation, Santa Ana, USA) were used [73]. Two membrane tensile stress causing load application models were analyzed (Fig. 5), (1) a uniaxial stretched rectangular membrane resembling tissue constructs of other authors [13], and (2) a planar, concentrically biaxial stretched circular membrane (Fig. 6). As for the membrane material, a Young's modulus of 2.5 MPa (vs. 210 GPa for steel!) and a Poisson's ratio of 0.49 were assumed. For calculations, a linear, elastic approach and a freely scalable unit-force at the boundary nodes being either uniaxial or concentrically were applied. The resulting pseudo-color images indicate the levels of the von Mises stress. FEM analysis for uniaxial load of a rectangular strip (Fig. 5a) revealed that at constant load the relative von Mises stress in the strip varied from 8.0 * 10 − 5 to 1.4 * 10 − 4. The circular membrane (Fig. 5b) loaded circumferentially showed almost no stress variation all over its area. Slight variances along the circumferential border of the membrane model (light blue) result from theoretical force transmission points. They became visible because of the limited number of boundary points considered in the calculations. The stresses discussed here all are stresses acting in-plane with the tested samples.

We tested as well how two different hydrostatic loads would bend the same circular membrane (Fig. 5b). As a result, the former isotropic tensile stress distribution across the membrane area turns into a rotationally symmetric one with small radial stress variations (Fig. 6a), and (Fig. 6b) the tensile stress along the membrane's radius from center to the periphery differs significantly. In Fig. 6a, a small hydrostatic pressure of 100 Pa was modelled. In full accordance to our intuition, this tendency becomes much more pronounced at higher load (500 Pa) (Fig. 6b). Thus, there is not only one membrane tensile stress value anymore given which would characterize the whole membrane area. For experimental purposes with the CellDrum, however, up to a ratio of the membrane diameter/maximum membrane

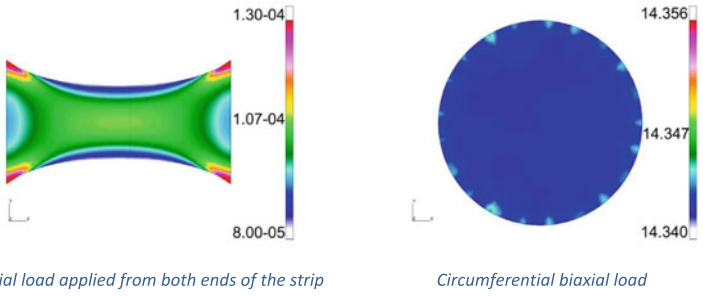
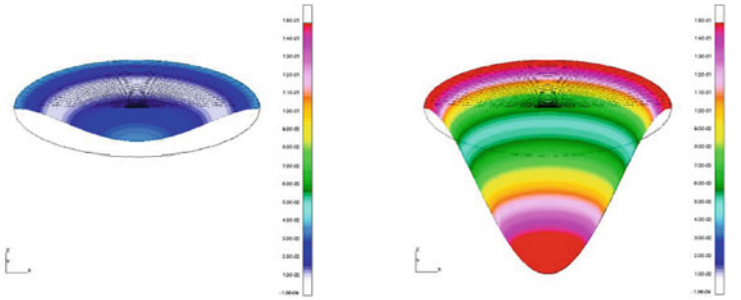


Fig. 5 Color encoded von Mises stress distribution in a **a** uniaxial stretched rectangular membrane, and **b** concentrically biaxial stretched circular membrane. The load was applied in-plane of the membranes. Light blue areas of the circular membrane (image **b**) result from the limited number of fix points considered in the model



A: radial load plus 100 Pa from top B: radial load plus 500 Pa from top

Fig. 6 Finite Element Method results of two concentrically pre-stretched circular silicone membranes. In addition to this, two different hydrostatic pressures act from above and cause significant membrane indentations. Model input parameters: membrane Ø = 16 mm, thickness = 100 µm, Young's modulus (silicone) = 2.5 MPa, Poisson's ratio = 0.49, boundary condition: edge clamped, shift = 0, twisting = 0, deformation scale 1:1 as compared to true size. For better visualization only the rear part of the membrane is shown. Colors represent the von Mises-Yield Criterion. Color scales for both images are identical

indentation of ~10 the stress and 50 Pa hydrostatic pressure variations across the membrane can still be neglected, in other words can be assumed to be constant.

3 "Load-Deflection-Mode"—Slowly Changing Cell Layer Tensile Stress

3.1 Principle Setup

Most cell types change their mechanical tensile stress which they transmit to each other or to their environment, respectively, slowly and not fast in milliseconds or rhythmically as cardiomyocytes do. The mode of CellDrum applications described was named "Load-Deflection-Mode". In this mode the cell monolayer/tissue construct tensile stress over pressure is measured. Figure 7 shows the CellDrum setup and Fig. 8 the principle function.

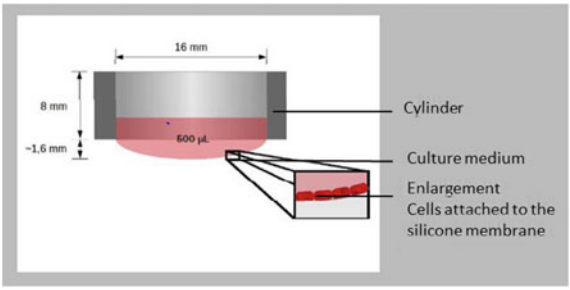


Fig. 7 CellDrum (rotationally symmetrical) scheme: CellDrum cylinder (dark grey), membrane with culture medium (typically 400 µL) on top (pink), cut-out—enlargement of a cell monolayer (red) when attached to the downwards bulged membrane

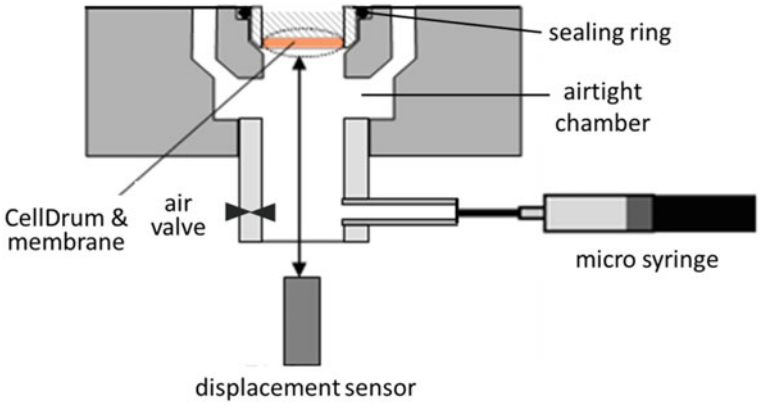


Fig. 8 Load-Deflection-Mode: Experimental setup for slowly changing mechanical tensile stress measurements. Pink colored strip: cell culture layer plus membrane when its plane is adjusted with the CellDrum cylinder's lower end-plane. Lower arc: initial membrane position right after CellDrum insertion into the measurement chamber. At this position the air valve is open for pressure equilibration to the environment. After closing, the air pressure inside the airtight chamber is increased via the micro syringe pump. With increasing pressure, the membrane continuously bulges upwards reaching the horizontal cylinder plane and bulges up further to the preset end of air pumping. It is usually stopped after the membrane's upper arc reached the same magnitude of the lower arc before air application

The CellDrum is partially filled with culture medium. From inside the cylinder and on top of the membrane cells are cultured as 2D (monolayer) or 3D cell-ECM-gel. For proper functioning of the CellDrum measurement principle a cell to membrane surface attachment via focal adhesions is necessarily required. In many cases fibronectin coating of the silicone surface alone may not be sufficient. Almost always a chemical or physical surface conditioning is required in such applications. Due to the culture medium weight, the membrane bulges out downwards. The CellDrum is placed into the measurement and seals it airtight. The chamber contains an air pressure sensor and an inlet for a micro syringe (Fig. 9). Before the measurement begins, the air pressure inside the chamber must be equilibrated to the atmospheric pressure via an air valve. The central indentation of the membrane is measured from below. There are several methods for membrane displacement measurements available. Here a laser sensor as described elsewhere is used [74].

In the Load-deflection mode (Fig. 9) the displacement 'Δh' at the beginning of the experiment is negative since it is bulged downwards below the CellDrum cylinder's bottom plane. When the pump presses air into the chamber, the membrane is lifted upwards. It passes the cylinder bottom plane and bulges up further, causing positive membrane deflection. Pressure increase 'Δp' and indentation 'Δh' are measured simultaneously (Fig. 9). From these data the mechanical tensile stress of the cell monolayer (2D, N/m) or the tissue construct (3D, N/m^2), respectively, is calculated. The development of a completely new technology is often

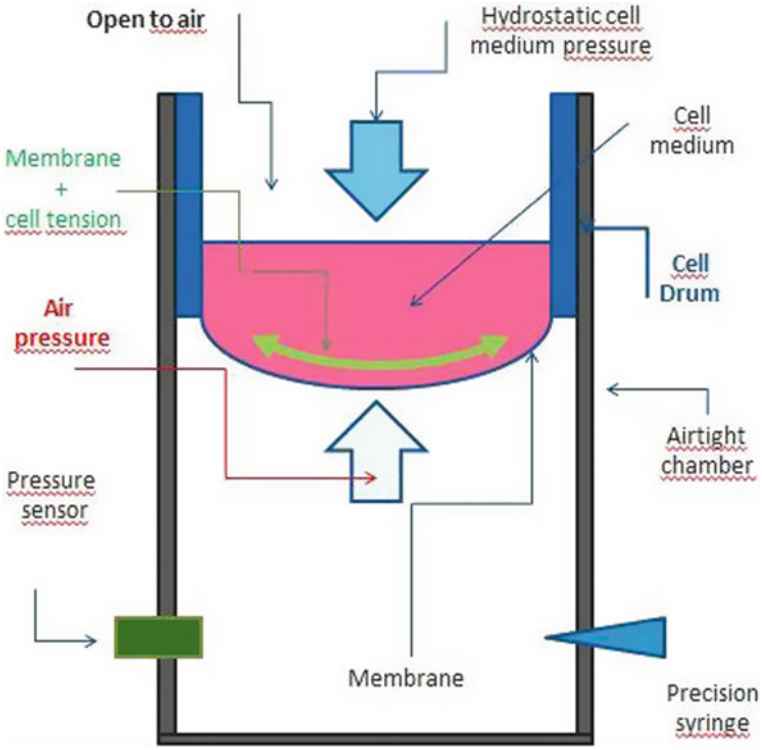


Fig. 9 Load-deflection-mode (extended scheme): cells are cultured on top of the silicone membrane (below the green bended arrow). The scheme shows the balance of forces in the setup: blue arrow from top; hydrostatic culture medium pressure, light blue arrow from below; counteracting air pressure as pumped into the air tight chamber following pressure equilibration with the outside atmosphere. Green arrow (alongside the membrane) shows the counteracting tensile stress of the membrane to the hydrostatic medium pressure. With cells tightly attached to the membrane an additional tensile stress alongside the silicone membrane is exerted which lifts the membrane further. From this indentation on, the system is "lifted" in addition to the membrane-plus-cell culture's intrinsic mechanical tensile stress upwards by the air pressure added to the airtight chamber by the precision syringe

technologically manifested in several stages of development, so also with the CellDrum technology. For example, Fig. 10 shows three generations of the Tissue Tension Analyzer for use in Load Deflection Mode.

3.2 Mechanical Tensile Stress Calculation

At small membrane indentations the bulged membrane can be seen as a spherical segment (Fig. 11). Thus, for calculating the membrane tension, σ, Laplace's Law

Fig. 10 Load-deflection-mode;Three generations of tissue tension analyzers; left image: **a** CTT I, single-well tissue tension analyzer (black: laser triangulator with red colored sensor fiel, above: ring shaped chamber which is closed airtight by the CellDrum inserted from the top; air valve and syringe pump are not shown); middle image: **b** CTT II, seven-well tissue tension analyzer; only its CellDrum holder is shown in photo; seven individual CellDrums are filled with medium, seeded with cells which are cultured further in an incubator; for measurement purposes, the holder with CellDrums is positioned on the CTT II instrument body forming seven independent measurement setups which can be individually analyzed; right image: **c** CTT MTS, 24-Well Tissue Tension Analyzer; the photo shows two CTT MTS 24-Well systems for Medium Through Screening in an incubator with 24 CellDrums

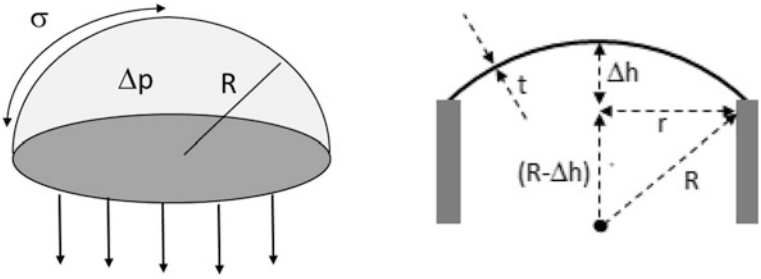


Fig. 11 Scheme for calculating the CellDrum's membrane tensile stress

can be applied although the membrane's circumferential edge is fixed at the cylinder. Hydrostatic pressure Δp of the cell culture medium bulges the membrane downwards forming a spherical cap at a curvature radius R.

The tensile stress σ of the membrane at small deflections Δh can be calculated using Laplace's formula for a thin-walled sphere with thickness t:

$$\sigma = \frac{\Delta p \cdot R}{2 \cdot t} \qquad (*)$$

Radius R is obtained from the deflection Δh with the Pythagorean Theorem:

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

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

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

With $h \ll r$ we neglect $\Delta h^2/r^2$ and simplify the formula for R to

$$R = \frac{r^2}{2 \cdot \Delta h} \qquad (**)$$

Combining equations (*) and (**), we obtain the membrane stress σ [Pa] as function of the differential pressure Δp [Pa] and the resulting deflection Δh of the membrane [μm], and the dimensions of the CellDrum [μm]:

$$\sigma = \frac{\Delta p}{\Delta h} \cdot \frac{r^2}{4 \cdot t} \qquad (***)$$

For calculations using Formula (***) the precise membrane thickness of each individual CellDrum, s, is needed. For this purpose a common interferometric procedure was adopted.

3.3 Data Acquisition in the Load-Deflection Mode

The experiments described in Sect. 3 were carried out with the Single Well Tissue Tension Analyzer, CTT I, in the so-called "Load-Deflection-Mode". Figure 12a shows the setup and Fig. 12b a typical load deflection curve. In order to obtain such curve experimentally, the CellDrum plus attached cell layer is placed into the airtight, temperature-controlled measurement chamber and equilibrated for 500 s. After this period, the strain of the membrane is adjusted to minus 0.3–0.5%. The corresponding pressure level is taken as zero pressure Δp in Formula (***). As for experimental data acquisition, additional air pressure Δp is slowly added lifting the membrane upwards. The pressure ΔP versus center deflection Δh is measured stepwise. The data points (Fig. 12b, blue dots) are than mathematically fitted. From this fit the slope Δh/Δp at zero membrane bending is calculated, inserted in Formula (***), and with geometrical membrane parameter the tensile stress σ is calculated. For absolute cell layer tensile stress measurements it is necessary to subtract the in-plan pre-stress of the membrane which must have been measured before cells were seeded on it. Data obtained with cells alone but without any tensile stress active compound are taken as controls for drug effect measurements.

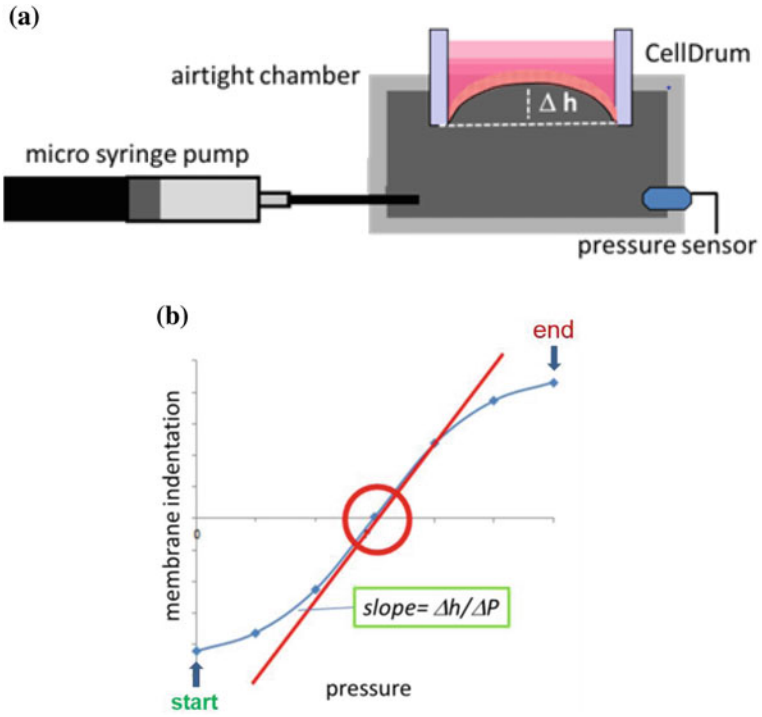


Fig. 12 **a** Chamber setup for data acquisition in the Load-Deflection-Mode: The membrane has been bulged upwards by the micro syringe pump to the a pre-set maximum membrane strain after a pressure deflection curve was measured. **b** Scheme of a load deflection data set. The membrane deflection at the "start" and the "end", respectively, of a data point sampling are indicated. The slope as take for tensile stress calculation in framed in green. At the dot circled in red marks the membrane is in-plane with the CellDrum's lower end plane

3.4 Validity Experiments of the Load-Deflection-Mode

The procedure above is repeated after the CellDrum with cells (control) was brought back to the lower membrane indentation by opening the air tight chamber (Fig. 9) to the atmosphere. It was regularly observed that the lower membrane deflection of controls returned back to its starting position, thus, was fully reversible. This indicates that load application to the extent chosen in the experiment did not affect cell-cell or cell-membrane attachments. Otherwise, the deflection would not be reversible.

For subsequent drug effect investigation based on control cell cultures the respective cell active compound is added for 5 min. This was carried out cumulatively for stepwise increased drug concentrations at each measurement cycle by replacing 20% volume of the culture medium with the same volume of culture medium containing the compound. A result was obtained as Fig. 13 shows.

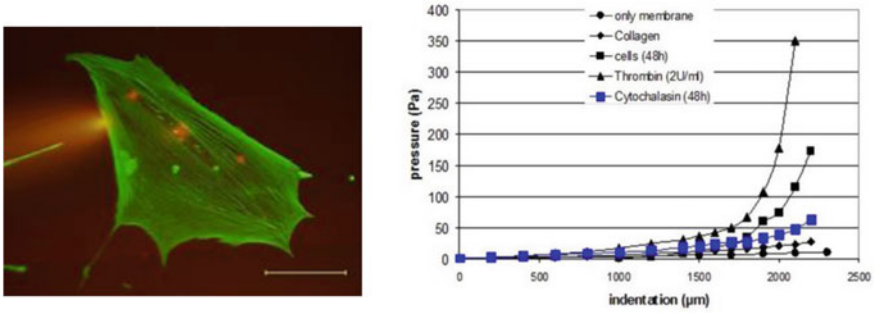


Fig. 13 Left: individual 3T3 fibroblast (bar 10 µm) with Phalloidin stained actin cytoskeleton. The cell is attached to the CellDum's silicon surface by focal adhesions. Right: Pressure indentation (deflection) curves of a 3D fibroblast 3D tissue construct cultured on a CellDrum membrane kept in-plane with the CellDrum bottom plane during culturing by supporting air pressure from below. After 48 h of culturing the load(pressure)-deflection curve was measured at various conditions as indicated in legend and text below. Culture condition: 3T3 Fibroblasts in Collagen I-Gel, 500 µm thickness, 10^6 cells/ml, FCS 10%; cytochalasin D was dissolved in dimethylsulfoxide (DMSO) and stored at −20 °C. Before use the final concentration was adjusted with DMEM. Experiments with fetal calf serum (FCS) were carried out using one and the same aliquot of FCS throughout experiments (Trzewik 2007)

By way of example, the pressure versus indentation curves from Fig. 13 will be commented on here. The membrane itself without cell culture was extremely soft and deformed fast at low ΔP. The cell containing collagen gel right after seeding stiffened the gel-membrane compound only weakly. At this point it should be mentioned, that the silicon membrane alone is not only highly but fast deformable. In other words, after applying a step wise increased ΔP the deformation needs a very short time to reach its new deformation stage. However, when gels and/or cells are cultured on the surface the silicone-gel- compound exhibits a higher viscosity, thus longer "relaxation times" are required to reach the new equilibrium deformation stage. Practical consequences are, that any change of pressure (membrane deformation) should be carried out slowly not to damage the cell-ECM integrity. This is only a hint. It has not been studied systematically.

Forty eight hours of fibroblast culturing in collagen stiffened the membrane-gel-compound drastically. The 3T3 fibroblasts not only proliferated during this period and more total cell generated tensile stress was developed. It is as well because cells formed contacts to each other and to the gel's proteins, thus, stiffened the membrane-cell monolayer compound "membrane". Thrombin known to cause fibroblast contraction increased the tissue construct's stiffness drastically. Depolymerization of the actin cytoskeleton is manipulated by actin-binding Cytochalasin disrupting the normal actin polymerization kinetics. The effects of Cytochalasins are specific to actin filaments, where they bind to the plus end of actin filaments resulting in an indirect depolarization of the actin fibers. In other words, due the Cytochalasin D action, used here, the mechanical tensile stress transmission pathway via the cellular cytoskeletons is interrupted.

As can be seen, the pressure deflection curve does not return to the curve of collagen gel alone as seen before. This is interpreted as a consequence of a residual stiffness caused by the extracellular matrix that has developed during culturing (compare our remarks about the remaining membrane stiffening after cell monolayer culture in Sect. 2.1). The latter effect is only an observation and should be studied in more detail.

3.5 *Efficacy of Cosmetic Ingredients and of Natural Remedies*

Animal experiments are not permitted any longer to be used in cosmetic research. The CellDrum technology and its unique features will become a requisite technological replacement for testing newly developed active ingredients (compounds, blends) on appropriate human 2D/3D tissue constructs. Moreover, with the CellDrum system applied to human tissue constructs much more information can be obtained than in animal experiments. Another imperative advantage implies that CellDrum results obtained with human cells will become more valuable for drug research, as results of animal tests can only be partially transmitted to humans.

Thus the CellDrum technology in its Load-Deflection-Mode may become a testing tool for researchers in the cosmetic industry incisive for mechanically active formulations of their ingredients, their prerequisite being "Does our particular ingredient show a positive effect on cell tensile stress and to what extent?" A "translation" of such scientifically sounding question is "Does our ingredient have the potential to smoothen skin wrinkles in vivo?" A very typical and normal question of the real industry world out there! These most legitimate applied research tasks involve sometimes big business and are often confident matters. Moreover, in many cases pure substances are questioned about their efficacy. Often active ingredients (of a skin cream for example) contain mixtures of compounds whose formulation will never be disclosed to the public, likewise are questions about the efficacy of "Natural Remedies" used for example against hypertension. In such cases the CellDrum may act as a cell assay revealing valuable information on the efficacy of Natural remedies and mixtures of multiple compounds. An in vitro standard testing approach is routinely establishing negative/positive controls as well as a more novel "damaging model" method. Such models reflect only parts of complex physiological situation in cells. Yet when chosen carefully they still might be a practical tool gaining first insights into an "ingredients efficacy" in certain questions.

Mechanical tensile stress in living tissues and tissue cultures is involved in multiple cellular processes. The project as partially presented in Fig. 14 uses primary human dermal fibroblasts, (NHDF), (Promo Cell GmbH, Heidelberg, Germany) derived from young (18–20 years old) and elderly (61–65 years old) donors, respectively. The aim of the project was to quantify cosmetic ingredient

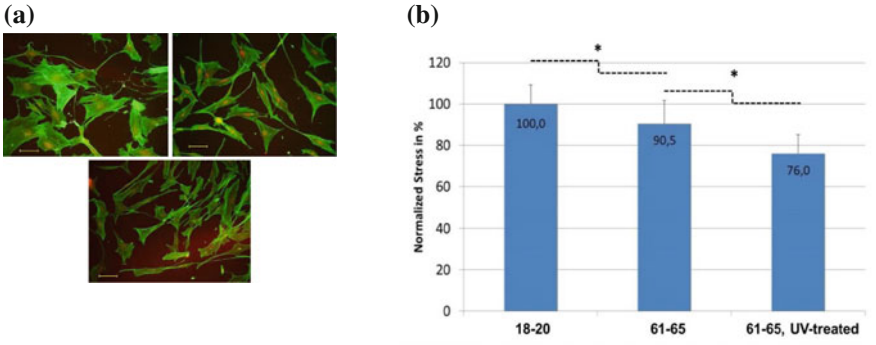


Fig. 14 **a** Micro photographs of actin stained primary human skin cell (NHDF) monolayers. Upper row, left photo: donor age 18–20 years; right: donor age 61–65 years. Lower photo: donor age 61–65 years and UV treated. **b** Normalized NHDF cell monolayer tensile stress of 18–20, 61–65 years old donor cells, and of 61–65 years old donor cell that have been exposed to UV irradiation (N = 12, +1 SD, $*p < 0.05$)

effects (blends of unknown substance compositions) on NHDF monolayer tensile stress containing primary human skin cells donated by young and elderly primary cell donors. In this applicable demonstration NHDF are used to explore the efficacy of a cosmetic company's active ingredients of skin cream.

In the demonstration presented here, a particular important question was about the establishment of a "damaging" or a "comparative" model, respectively. In this example the "comparative model" is represented by NHDF of elder donors. The "damaging model" is instead the application of UV light to the cells of elder donors. The question posed by the ingredient producer in this case is, whether their ingredient can improve the abnormal mechanical tensile stress of elder donor NHDF cells as compared to cells of young donors. Microscopic images show, that NHDF of elder donors have a somehow "eroded" cytoskeleton (Fig. 14a). This results in a significant lowered mechanical tensile stress of elder donors' NHDF cell monolayer versus those of young donors by ~10% (Fig. 14b). The same figure shows that an additional extrinsically caused cell damaging due to UV radiation (following a standardized UV light irradiation protocol) reduces the cell tensile stress exhibited by cells of elder donors further, by in total ~24%! Cell exposure to UV light enhances the expression of matrix metallo-proteinases (MMP) followed by an ECM degradation. The phenomenon is called skin photo aging [41].

As application the effects of cosmetic ingredients (blends) to NHDF cell monolayer tensile stress was measured (Fig. 15). NHDF cells of one 59 year old donor (grey bar) are taken as control (100% tensile stress). TGF β known to induce NHDF contraction (stiffening of the cell monolayer) elevated the cell tensile stress significantly by about 15% versus controls (green bar). Four different blends were added to cells of elder donors. Blend II and blend IV enhanced the mechanical tensile stress to about the extent of TGF β (shadowed in pink). Blend I and III showed only a tendency towards TGF β results but no significant increase. The

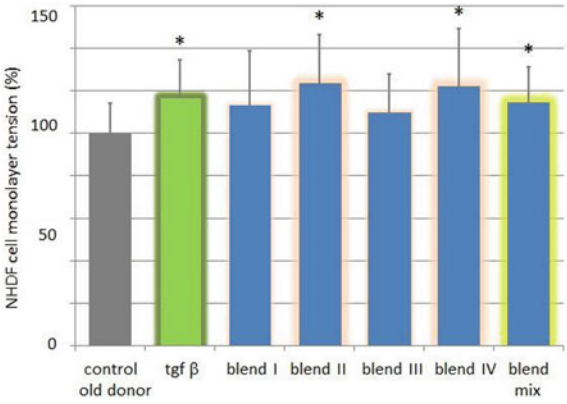


Fig. 15 Effects of cosmetic ingredients (blends) to NHDF cell monolayer tensile stress of one 59 year old donor (grey). As positive tensile stress inducing control 20 ng/mL TGF-β at an incubation time of 24 h was used (green). Than blends as indicated in the figure were added to control cells (N = 26 repeats per group, averaged and normalized, error bars +1 SD, Wilcoxon Signed Rank Test, $*p < 0.05$)

effect of a mix of blend I–IV (I: 2.5%, II: 24.5%, 48.9%, and 24.1%) was significant as well (shadowed in green), but less pronounced than blend II and IV, respectively, alone.

This is only one result out of many that have been obtained. However, it illustrates that the CellDrum technology in its Load-Deflection-Mode may serve as a sensitive tool for such type of questions and applications. The results underline a high potential for many more applications. However, there arises a new technological demand: The need for a High-through-put CellDrum system in order to screen many combinations at once and at low costs, to optimize a mix of pharmacological compound and/or to exclude drug interactions.

In conclusion, the CellDrum technology gives the opportunity to make comparative estimations of pharmaceuticals/substances, to find out about their potency to induce or reduce respectively cellular contractile forces. There exist many so far not sufficiently studied natural remedies which might be worth studied further with the technology described here. Another yet most important application of the CellDrum in both of its modes, however, will be pharmacology and toxicology testing. Despite many other such tests for screening new substances and to test new active principles the CellDrum technology represents a method that answers the question, whether a compound exhibits a wanted or an unwanted, respectively, cell mechanical tensile stress induction effect on (human) cell cultures in vitro. The relative simplicity of the CellDrum technology will allow routine testing with large sample numbers in the very near future.

4 "Self-exciting Mode"—Rhythmically Contracting Cardiomyocytes

4.1 Brief Introduction on Cardiomyocytes in the Heart

Cardiomyocytes represent the active contracting cells of the heart comprising atrial as well as ventricular cells in the respective functional parts of the heart. Their functions are critical to the proper form and regulation of the beating human heart. Human Induced Pluripotent Stem Cells (hIPS) from healthy as well as diseased patients have the potential to give rise to all phenotypes of cardiomyocytes, making them a good model system to study human cardiac diseases that affect different parts of the heart. There are three types namely, atrial myocytes, ventricular myocytes and pacemaker myocytes. Adult atrial myocytes and ventricular myocytes are examples of non-pacemaker action potentials in the heart. Because their action potentials undergo rapid depolarization (upstroke), these are sometimes referred to as "fast response" action potentials. Atrial cardiomyocytes make an important contribution to the refilling of ventricles with blood, which enhances the subsequent ejection of blood from the heart. The dependence of cardiac function on the contribution of atria becomes increasingly important with age and exercise [12]. It has been reported that 95% of the cardiomyocytes from differentiating human Embryonic Stem Cells after treatment with Noggin and retinoic acid had atrial-like action potentials; treatment with retinoic acid increases atrial-specific gene expression and percentage of atrial myocytes but decreases expression of ventricular-specific genes [23, 32]. Treatment of differentiating hESCs with Noggin and retinoic acid inhibitors increased the expression of ventricular-specific genes and 83% of the obtained cardiomyocytes had ventricular-like action potentials. Weng et al. reported a protocol that generates 93–100% ventricular myocytes from different hESC and hiPSC lines based on electrophysiological properties of the cardiomyocyte population and resulting ventricular-like cardiomyocytes exhibited typical ventricular ionic currents [76].

Cardiac pacemaker myocytes carry impulses that are responsible for the beating of the heart, they can spontaneously generate, send out electrical impulses and also electrical impulses from cell to cell. Kapoor et al. [46] demonstrated that de novo cardiac pacemaker myocytes can be obtained by direct reprogramming of rodent ventricular myocytes via viral delivery of the transcription factor required for embryonic pacemaker tissue development, the translational potential of this work has been suggested by preclinical studies carried out with pigs demonstrating that this strategy can be translated to large animals. Ionta et al. found that overexpression of Shox2, a transcription factor critical for embryonic pacemaker tissue development, in differentiating mouse ESCs resulted in upregulation of pacemaker-specific genes. The obtained pacemaker-like cells exhibited enhanced automaticity and are able to pace adult rat hearts [32, 42].

4.2 The CellDrum a Complementary In Vitro Tool to the Langendorff Heart

For more than hundred years explanted animal hearts were used for pharmacology and toxicology testing of inotropic drugs. Since the fundamental work by Langendorff the isolated perfused heart (Fig. 16) has been the ultimate experimental model in cardiovascular research [53].

However, from the moment of organ harvesting the heart sets on deteriorating; unphysiologic formations of edemas occur, and the rates of deterioration are affected together with many other factors. This includes the skill of the operator avoiding contusion injury, the species, the composition of the perfusion fluid, the presence or absence of various drugs, animal age, heart rate/cardiac output and the temperature at which the studies are carried out. This rate of deterioration can be crucial in the design and interpretation of studies. This is in particular true when it is necessary to use time-matched controls with corrections for baseline deterioration when comparing groups like papillary or trabeculae preparations obviously have to cope with the same limitations [68]. Some more objections are obvious: One animal needs to be killed for only one experiment, physiological data of animals cannot be simply transferred to humans, data obtained with just one organ explant cannot be generalized for a whole animal population, and last not least, high-through-put testing is ethically inacceptable. On the other hand, no in vitro test can fully replace animal organ testing. The Langendorff heart is a naturally grown organ and, thus, represents still best the endogenous regulation of the heart.

However, taking into account the broad experimental potential of the CellDrum Technology it may become the ultimate in vitro method of choice for pharmacology and toxicology testing as well as for applied and basic heart research. It may also serve well in basic medical research. One such feature for example, so far not yet experimentally approached with the CellDrum technology, could be studying the molecular and cellular mechanisms underlying the Frank-Starling effect [65]. They

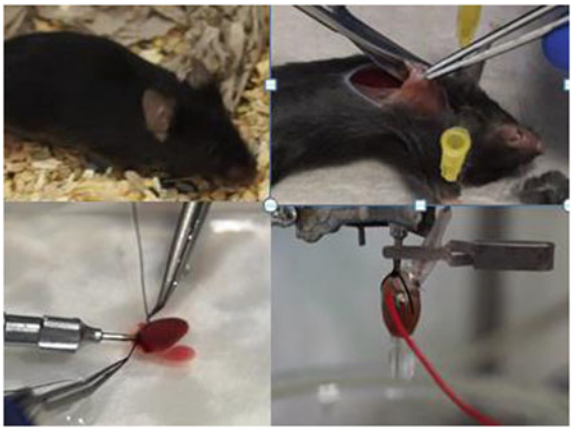


Fig. 16 Preparation of a Langendorff heart from a healthy mouse. The authors thank the very talented scientist Dr. med. rer.nat habil. Petra Kleinbongard from the University of Duisburg-Essen for providing us with the photos to the figure

remain still elusive. The question raised is why an increase in the heart's ventricular filling, causing elongation of cardiomyocyte sarcomere length, induces an increase in the ventricular mechanical work caused by strain-stimulated cardiomyocyte's ability to perform more physical work at increasing strain levels (mechanical tensile stress).

4.3 "Self-exciting-Mode"—Proof of Principle with Cardiomyocytes

Whereas the frequency of cardiomyocyte cultures can be analyzed routinely with various other methods, the CM induced mechanical tensile stress cannot be measured on a routine basis as demanded in pharmacology and toxicology applications. In 2010, the CellDrum technology was first successfully applied to autonomously beating cardiomyocytes (CMs). An important proof of principle test was carried out by that time with neonatal CMs from Wistar rats using the CellDrum technology in its self-exciting mode (Figs. 17 and 18).

Experiments are aimed at deriving rhythmical cardiomyocyte induced pressure signals corresponding to cardiomyocyte induced mechanical tensile stress. Neonatal cardiomyocytes from Wistar rats are cultured on a CellDrum membrane and a pressure versus time signal is derived. For tensile stress measurements the CellDrum is placed into the airtight measurement chamber. Normally 300,000 cardiomyocytes at a time are used per CellDrum. Cells adhere, form contacts and start beating autonomously and simultaneously. No further electrical excitement is needed. Afterwards the syringe raises the chamber pressure inducing an offset pressure in the chamber. It bulges membrane and cell layer upwards.

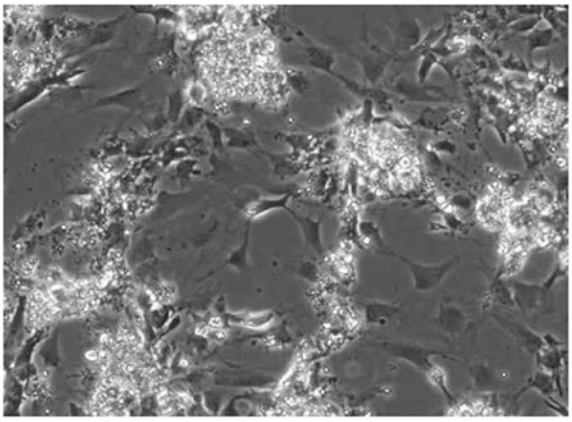


Fig. 17 Micro photograph of a cardiomyocyte monolayer of neonatal Wistar rats on a CellDrum membrane

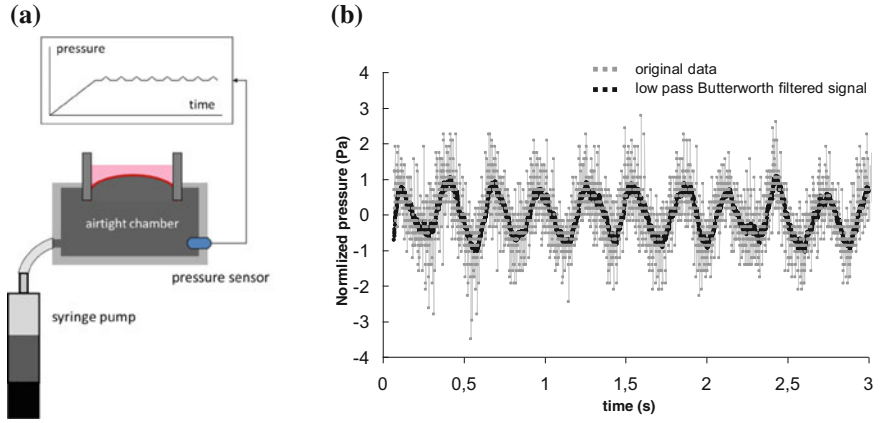


Fig. 18 "Self-Exciting-Mode" for quantifying cardiomyocyte mechanical tensile stress. **a** Scheme of the experimental setup: CellDrum cylinder (dark grey), membrane and cell monolayer (red), culture medium (pink), plus syringe pump and pressure sensor with access to the airtight chamber. **b** About 10–15 contraction cycles are measured. Although rhythmic beats are somehow visible, the original signal is very noisy. Low pass Epanechnikov filtering eliminates noise and reveals smooth individual mechanical beats. The application of filters needs careful considerations in order not to "filter out" important signal content

Cardiomyocytes continue beating autonomously. Their rhythmical mechanical tensile stress modulates an additional sinusoidal pressure to the offset pressure (Fig. 18a). Figure 18b shows an original plot containing information on the frequency and beat tensile stress amplitude. In these experiments a monolayer of cells was sandwiched in-between two ultrathin collagen layers. These tests were performed by Trzewik [73].

This pressure signal is measured over time at high resolution. The original signal is noisy and needs software filtering. An Epanechnikov filter is used to smoothen the input curve (Fig. 18b). A peak-to-peak data analysis over a sufficient number of subsequent filtered beats gives the average frequency and the beat amplitude in N/m (cardiomyocyte monolayer, 2D) or N/m^2 (cultured 3D cardiomyocyte tissue construct), respectively.

In another experiment, another monolayer of neonatal Wistar rat cardiomyocytes embedded in-between two ultrathin collagen layers was tested. The aim was showing that the rhythmicity observed in the signal was caused by self-exciting beating cardiomyocytes. Figure 19a shows a corresponding data recording. Clearly rhythmicity can be observed even without signal filtering. It disappeared fully after killing the cells with a 10% Triton X-100 solution (Fig. 19b). Respective signals observed after noise reduction are shown in Fig. 19c (living cells) and Fig. 19d (dead cells). After applying Fourier transform software there appeared a strong 3.2 Hz frequency peak from curves taken with alive cells before Triton X-100 application (Fig. 19e). Such peak was NOT anymore observed after Triton treatment (Fig. 19f). Consequently, the sinusoidal rhythmicity in Fig. 19, E is due to

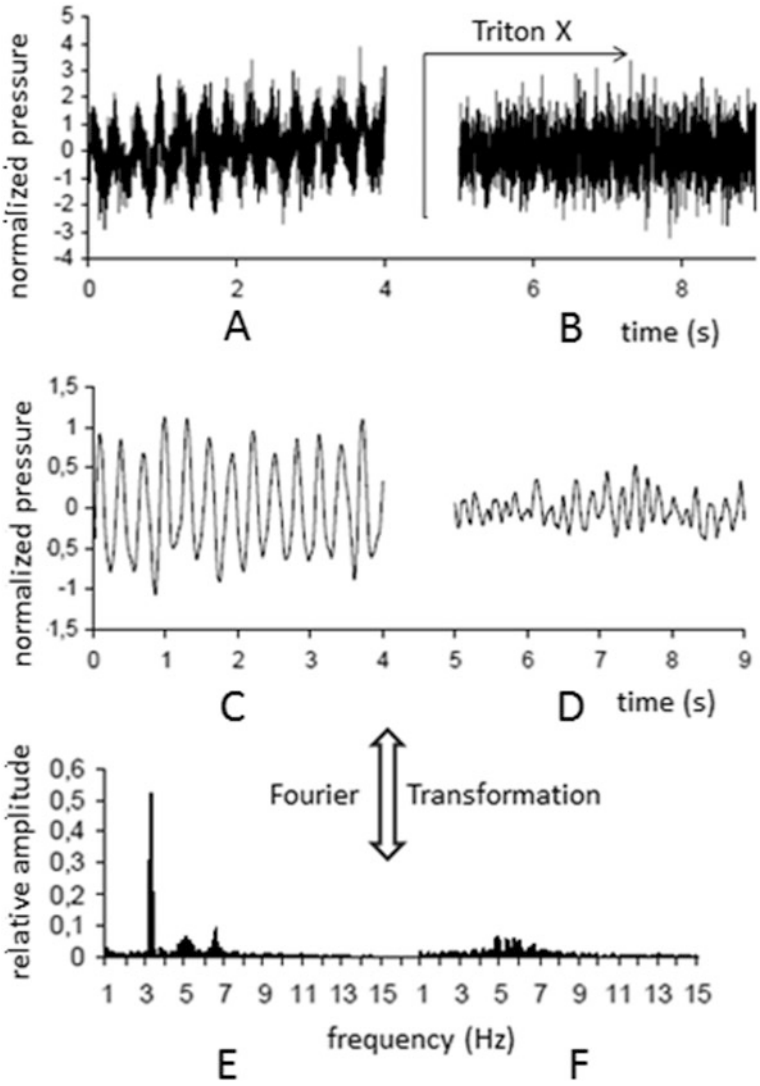


Fig. 19 Life cell–dead cell proof: the graphs A, C, and E, were obtained with an alive cell culture. However, B, D, and F represent signals after the application of Triton X-100 killing the cells (original data after the offset pressure subtraction). **Above**: Original pressure signal, **a** before, and **b** after Triton X application. **Middle**: **c** and **d** Low pass filtered pressure signals. **Below**: **e** The Fourier transformed signal reveals a frequency peak at 3.2 Hz (192 beats/min) of the alive cell culture and **f** no such peak was seen after Triton X-100 treatment

cellular rhythmical beating of alive cells. In this test a cell induced tensile stress of 1.28 ± 0.13 μN/mm^2 at a frequency of 3.2 Hz (192 beats/min) was measured.

The experiments above reveal that contracting cardiomyocytes induce a measurable rhythmical signal. The road for developing the CellDrum technology towards measuring routinely cardiomyocyte tensile stress and inotropic drug effects was opened.

4.4 *Pharmacological Experiments with the "Self-exciting" Mode*

In an early experiment the effect of noradrenalin and a β blocker [17, 70] on the contraction frequency and the beating strength of primary Wistar rat cardiomyocytes was examined using the CellDrum in its "self-exciting-mode (Fig. 20).

Noradrenalin acts on the heart in a positive chronotropic, dromotropic, inotropic, bathmotropic und lusitropic manner. In the experiment show in Fig. 20, initially, cardiomyocytes are beating at 4.4 Hz. After ∼6 min, 10–4 M noradrenalin is added. An immediate increase of the contraction frequency is observed reaching a peak at 11% above the initial level after 4 min. Within the next 5.5 min the frequency drops to 6% which is followed by a linear decrease at 7.3 MHz/min. Thirty five minutes after experiment begin, 10–6 M β blocker were added to the noradrenalin pre-treated cell culture. The rate of drug induced frequency change was −15.5 MHz/min. After ∼60 min the frequency reached a plateau at minus 4.5%, thus below the initial frequency. Such experiments might easily be repeated and carried out with cells grown in culture dishes. However, results might be different.

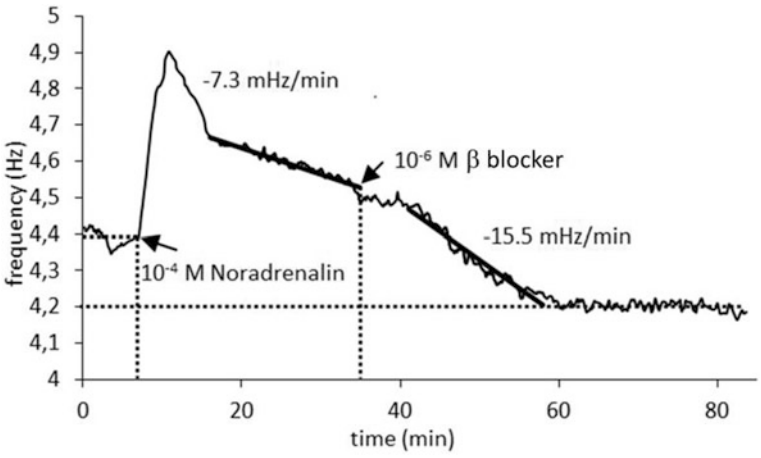


Fig. 20 Effect of Noradrenalin and a β blocker on the frequency of cardiomyocytes of primary neonatal Wistar rats [6]

We would like to underline at this point another special feature of the CellDrum technology: Cells are seeded on a soft and deformable substrate—the ultrathin silicone membrane. Cardiomyocytes are used to a permanent mechanical and rhythmical biaxial elongating and contracting environment. Such mechanical boundary condition is greatly mimicked by the CellDrum; however it is not by a stiff culture dish surface. This may affect biochemical and physical responses of cells to their mechanical environment [52]. Cardiomyocytes in the CellDrum do not only exhibit rhythmic beating pattern; they perform real physical work. Cells use glucose from the cell culture medium producing energy allowing them to work against the culture medium weight without any other intervention (no electrostimulation). Due to cell contractions the culture medium is lifted upwards against gravity. This work is partially represented in the beat amplitude of the CellDrum signal.

Summarizing so far, using the CellDrum technology, the frequency and simultaneously the mechanical beating strength of primary cardiomyocytes from neonatal Wistar rats can be measured and principle drug action can be shown. In other words, the new element of these data presented here is that the mechanical cell tensile stress (beating strength) of cell layers can be measured **in addition** to the frequency. Thus, in the future new testing strategies can be designed in order to understand drug action better than with experiments lacking mechanical beating strength information. The CellDrum opens up a new perspective performing long-term drug testing experiments over weeks. Moreover, much less Langendorff heart experiments may be needed in the future to verify CellDrum data by whole organ studies [10, 53].

4.5 Inotropic Drug Effects on Human IPS Derived Cardiomyocytes

In March 2016, the first article on contraction forces of cultured tissue containing **human** induced pluripotent stem cells (hIPS CMs) was published [31]. The results of this study are presented below.

Before doing so, however, let us briefly turn to a similar study, which was reported a few months later, in May 2016. The authors reported on force measurements on cultured tissue containing IPS-derived human CMs Although a different force transducer principle has been used in the modified method [58], forces of uniaxial strained cultured tissues with all the disadvantages associated therewith continue to be measured. Such setup is obviously not suitable for sample quantities in the medium or high throughput range. In contrast, with the CellDrum technique, mechanical stresses with physiological, two-dimensional clamping are measured with high precision. Furthermore, the CellDrum technology is basically reasonably applicable for medium throughput set-ups up to 96 CellDrum units. The data shown were obtained using the CTT I setup (Fig. 10).

Two different drugs targeting cardiac Ca^{2+} channels, S-Bay K8644 and Verapamil, respectively, are applied to hiPS-derived cardiomyocytes. Representative recordings of S-Bay K8644, a Ca^{2+} channel agonist, and the antagonist verapamil are shown in Fig. 21. S-Bay K8644 enhances the beating strength amplitude of the CR-cycle by 60% above control reaching a plateau at 90 nM. Concurrently, the beating frequency reduced between 90 and 160 nM significantly. Verapamil, a Ca^{2+} channel antagonist, reduced the beating strength amplitude (Fig. 22) to 25% at 300 nM. The frequency was increased linearly to 160% at 300 nM. There is no saturation observed [31].

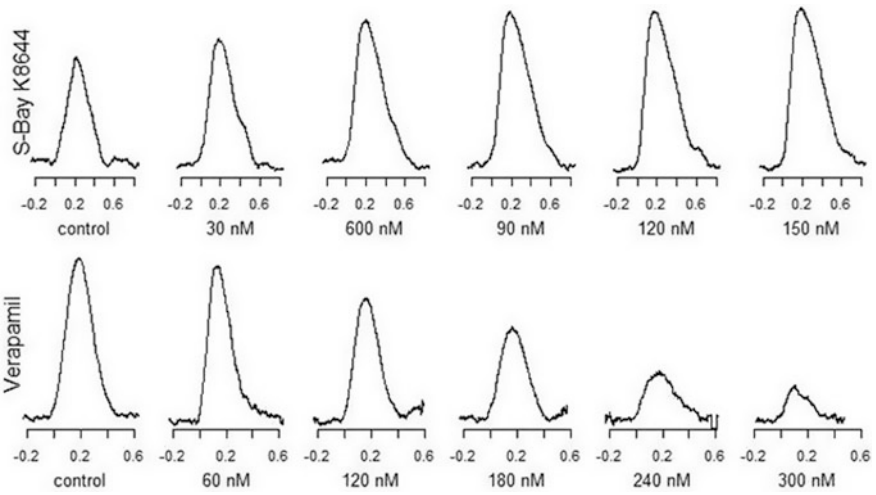


Fig. 21 Representative recordings of S-Bay K8644 (Ca^{2+} channel agonist, above) and Verapamil (antagonist, below) action on hIPS derived cardiomyocytes

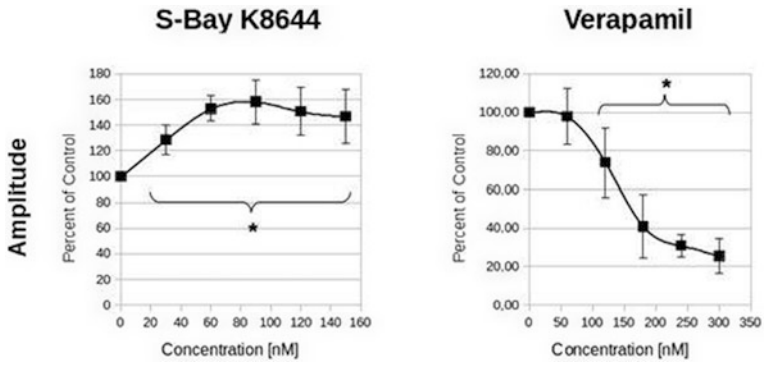


Fig. 22 Dose dependency of S-Bay K8644 and Verapamil action on hIPS derived cardiomyocytes

4.6 Medium Throughput (MTS) CellDrum Based Test System

The CellDrum technology offers a unique technological solution towards a functional MTS toxicology and pharmacology test of cell induced mechanical tensile stress in 2D and 3D cultured tissue constructs, in particular hIPS derived cardiomyocyte cultures. Figure 23 shows a set-up for a 24-well MTS toxicology and pharmacology solution. Multi-well solution development is in progress. New multi-well developments based on CellDrum technology are right now in the development stage. Those are meant for routine use and large numbers of experiments performable in a decent lab time. At the same time smaller CellDrum volumes in multi-well setups reduce the amount of test substances needed for experiments easily by factor one hundred. Multi-well systems enable comparative estimations of pharmaceuticals with equal indications, to find out the individual potency of pharmaceuticals and/or substances, to do toxicological tests (adverse reactions), to do screening of new substances and to test new active compounds. For smaller test numbers and research applications a set of smaller and more affordable instruments are available.

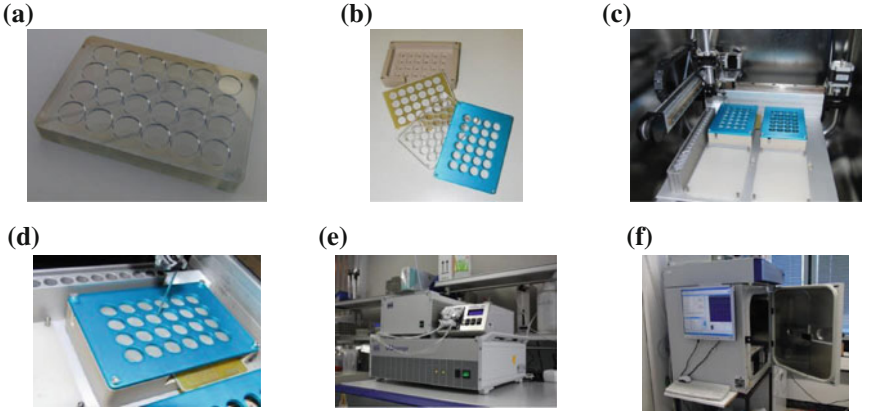


Fig. 23 **a** 24-well CellDrum array as seen from below. The membrane spans the drilling in the array block watertight. **b** 24-well array, components: From top: base with pressure chambers and air inlets, circuit board & electrodes, chamber block, top-lid (blue). **c** Four 24-well arrays can be positioned simultaneously into the incubator for automated measurements. The incubator contains a pipet robot controlled from outside the incubator. **d** The pipette is used for media exchange during cell culturing. Once cells are ready for experiments, pipettes help mix substance solutions from stock solutions and place them into each one of the 24 CellDrum-wells for measurements setting on right after. **e** External hardware, pumps, valves, steering units etc. **f** Incubator opened for experiment preparation with touchscreen. Exposure times, dosages, kinds of substances are controlled via touch screen. A PC steers the whole process and analyses data

5 Conclusions

The Cell Drum Technology represents an ideal high precision experimental setup to test cultured cells for their ability to generate mechanical tensile stress at various biochemical and -physical conditions. It enables precise measurements of this parameter using various 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. At standardized conditions the Cell Drum can simulate to certain extend conditions including ischemia/reperfusion by changing the oxygen partial pressure. Human IPS derived cardiomyocytes represent a neonatal signature which is different from adult heart cells and, thus, their response to certain drugs. Efforts need to be made towards maturing those neonatal cells in so far unknown ways.

We conclude that the Cell Drum 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-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 Cell Drum 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 Cell Drum technology to a 3D model may lead it closer to the three-dimensional environment in ventricular tissue. In addition, the Cell Drum benefits from the fact that cell are not grown on a stiff substrate but on highly flexible membranes mimicking to some extend the natural compliance of soft tissue. 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 iPS 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 derived cardiomyocytes 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 cardiomyocytes are suspected to not reflect the maturity of adult somatic cells, especially concerning the functionality of ion channels and expression of specific receptors and signaling cascades. Despite these issues, we have shown consistent and physiological responses of hiPS-derived cardiomyocytes on inotropic compounds. Hence, the findings of this study support the hypothesis that iPS-derived cardiomyocytes can provide an adequate model for pharmacological and toxicological studies. Future studies with hiPS-derived electro-competent cardiac fibroblasts and biomimetic three-dimensional connective tissue scaffolds may improve the physiological conditions towards a more concise cardiac tissue model. 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.

The CellDrum technology is basically reasonably applicable for medium throughput set-ups up to 96 CellDrum units. There is currently no technological solution available for the economic and routine high-throughput measurements required for tens of thousands of wells per day in the future. In the future, functional (mechanical) high-throughput test principles will have to be developed. They will help avoid 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 physiology matters.

Acknowledgements Some of our teachers remain a role model for our entire life. Gerhard Artmann's role models remain the professors Y. C. Fung, Shu Chien (both UCSD, San Diego, USA), Georg Büldt (Research Center Juelich, Germany) and Peter Paufler, physicist and crystallographer (University of Dresden, Germany). For providing stem cell expertise and free disposal of hIPS derived, highly purified and well characterized hIPS derived cardiomyocytes as well as for his excellent scientific advice and kindness we owe special thanks to Professor Jürgen Hescheler.

All technological developments and scientific tasks related to the CellDrum technology were carried out in the laboratories and under the scientific direction of Professors Gerhard M. as well as Aysegül Artmann. Significant contributions to the work were made by Professor J. Trzewik our former Ph.D. student and major CellDrum co-inventor. Working with him was scientifically and technologically very productive. Thank you, Juergen. Professors Artmann thank their doctoral candidate M. Gossmann for his lab work. As his teachers, however, they are thoughtfully looking back at the time with him. Another but extremely talented doctoral candidate was P. Linder. He worked for more than a decade and a half intensively with us. We thank the young Prof. Dr. Ilya Digel for the excellent cooperation in the Institute of Bioengineering. In particular, we thank him for many stimulating scientific discussions and his ongoing loyalty.

Quite a number of CellDrum projects were financed by numerous grants to Professors Artmann received by the State of North Rhine Westphalia (NRW) as well as by funding institutions of the Government of the Federal Republic of Germany (BMBF, BMWI).

References

1. Anfinsen, C. B. (1973, July 20). Principles that govern the folding of protein chains. *Science, 181*(4096), 223–230.
2. Artmann, G. M., Digel, I., Zerlin, K. F., Maggakis-Kelemen, C., Linder, P., Porst, D., et al. (2009). Hemoglobin senses body temperature. *European Biophysics Journal, 38*(5), 589–600.
3. Artmann, G. M. (2000, August). Device and method for the measurement of forces from living materials. Patents, Ref. No: US020040033482A1, 08/2000, AU000007638401A, 08/2000, EP000001311850B1, 08/2000, CA 2420141, 07/2002.
4. Artmann, G. M. (2014). (https://www.researchgate.net/publication/301608217_EEE_Laborherz.
5. Artmann, G. M. (2015, November). 6. Deutscher Querdenker Kongreß, Award Winner: Category—"Pioneering".
6. Artmann, G. M., & Chien, S. (2008). *Bioengineering in cell and tissue research*. Berlin, Heidelberg: Springer.
7. Artmann, G. M., Burns, L., Canaves, J. M., Temiz-Artmann, A., Schmid-Schönbein, G. W., Chien, S., et al. (2004). Circular dichroism spectra of human hemoglobin reveal a reversible structural transition at body temperature. *European Biophysics Journal, 33*(6), 490–496.
8. Artmann, G. M., Kelemen, C., Porst, D., Büldt, G., & Chien, S. (1998). Temperature transitions of protein properties in human red blood cells. *Biophysical Journal, 75*(6), 3179–3183.
9. Artmann, G. M. https://de.wikipedia.org/wiki/Gerhard_Artmann.
10. Bell, R. M., Mocanu, M. M., & Yellon, D. M. (2011, June). Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion. *Journal of Molecular and Cellular Cardiology, 50*(6), 940–950.
11. Bissell, M. J., Hall, H. G., & Parry, G. (1982, November 7). How does the extracellular matrix direct gene expression? *Journal of Theoretical Biology, 1*(7), 31–68.
12. Bootman, M. D., Higazi, D. R., Coombes, S., & Roderick, H. L. (2006). Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes. *Journal of Cell Science, 119,* 3915–3925.
13. Brown, R. A., Prajapati, R., McGrouther, D. A., Yannas, I. V., & Eastwood, M. (1998, June). Tensional homeostasis in dermal fibroblasts: Mechanical responses to mechanical loading in three-dimensional substrates. *Journal of Cellular Physiology,* 323–332.
14. Burridge, P. W., Li, Y. F., Matsa, E., Wu, H., Ong, S., Sharma, A., et al. (2016). Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nature Medicine, 22*(5), 547–556.
15. Burton, K., & Taylor, D. L. (1997, January 30). Traction forces of cytokinesis measured with optically modified elastic substrata. *Nature, 385,* 450–454.
16. Campàs, O., Mammoto, T., Hasso, S., Sperling, R. A., O'Connell, D., Bischof, A. G., et al. (2014). Quantifying cell-generated mechanical forces within living embryonic tissues. *Nature Methods, 11,* 183–189.
17. Cerbai, E., Crucitti, A., Sartiani, L., De Paoli, P., Pino, R., Rodriguez, M. L., et al. (2000, January). Long-term treatment of spontaneously hypertensive rats with Losartan and electrophysiological remodeling of cardiomyocytes. *Cardiovascular Research, 45*(2), 388–396.
18. Chen, C. S., Alonso, J. L., Ostuni, E., Whitesides, G. M., & Ingber, D. E. (2003, July 25). Cell shape provides global control of focal adhesion assembly. *Biochemical and Biophysical Research Communications, 307*(2), 355–361.
19. Chien, S. (2007, March). Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell. *American Journal of Physiology—Heart and Circulatory Physiology, 292*(3), H1209–H1224.

20. Chien, S., Sung, K. L., Skalak, S., Usami, A., & Tözeren, A. (1978, November). Theoretical and experimental studies on viscoelastic properties of erythrocyte membrane. *Biophysical Journal, 2,* 463–487.
21. Cost, A. L., Ringer, P., Grashoff, A. C., & Grashoff, C. (2015). How to measure molecular forces in cells: A guide to evaluating genetically-encoded FRET-based tension sensors. *Cellular and Molecular Bioengineering, 8*(1), 96–105.
22. David, B. (2000, May 4). A surprising simplicity to protein folding. *Nature, 405,* 39–42.
23. Devalla, H. D., Schwach, V., Ford, J. W., Milnes, J. T., Haou, S., Jackson, C., et al. (2015). Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Molecular Medicine, 7,* 394–410.
24. Dumont, S., & Prakash, M. (2014, November 5). Emergent mechanics of biological structures. *Molecular Biology of the Cell, 25*(22), 3461–3465.
25. Eastwood, M., McGrouther, D., & Brown, R. A. (1994, November 11). A culture force monitor for measurement of contraction forces generated in human dermal fibroblast cultures: Evidence for cell-matrix mechanical signalling. *Biochimica et Biophysica Acta, 1201*(2), 186–192.
26. Eder, A., Vollert, I., Hansen, A., & Eschenhagen, T. (2016, January 15). Human engineered heart tissue as a model system for drug testing. *Advanced Drug Delivery Reviews, 96,* 214–224.
27. Fung, Y. C. (1993). *Biomechanics: Mechanical properties of living tissuses*. New York: Springer.
28. Gawain, T., Burnham, N. A., Camesano, T. A., & Wen, Q. (2013). Measuring the mechanical properties of living cells using atomic force microscopy. *Journal of Visualized Experiments, 76,* 50497.
29. Gayrard, C., & Borghi, N. (2016). FRET-based molecular tension microscopy. *Methods, 94,* 33–42.
30. Giancotti, F. G., & Ruoslahti, E. (1999, August 13). Integrin signaling. *Science, 285*(5430), 1028–1033.
31. Goßmann, M., Frotscher, R., Linder, P., Neumann, S., Bayer, R., Epple, M., et al. (2016). Mechano-pharmacological characterization of cardiomyocytes derived from human induced pluripotent stem cells. *Cellular Physiology and Biochemistry, 38,* 1182–1198.
32. Hamel, V., Cheng, K., Liao, S., Lu, A., Zheng, Y., Chen, Y., et al. (2017, February 20). De novo human cardiomyocytes for medical research: Promises and challenges. *Stem Cells International, 2017*, 4528941. https://doi.org/10.1155/2017/4528941.
33. Harris, A. K., Wild, P., & Stopak, D. (1980, April 11). Silicone rubber substrata: A new wrinkle in the study of cell locomotion. *Science, 208*(4440), 177–179.
34. Heath, J. P., & Dunn, G. A. (1978). Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflexion and high-voltage electron-microscope study. *Journal of Cell Science,* 197–212.
35. Heisenberg, C. P. (2017). Cell biology: Stretched divisions. *Nature, 543*(7643), 4443–4444.
36. Helenius, J., Heisenberg, C. P., Gaub, H. E., & Muller, D. J. (2008). Single-cell force spectroscopy. *Journal of Cell Science, 121,* 1785–1791.
37. Heras-Bautista, C. O., Katsen-Globa, A., Schloerer, N. E., Dieluweit, S., Abd El Aziz, O. M., Peinkofer, G., et al. (2014). The influence of physiological matrix conditions on permanent culture of induced pluripotent stem cell-derived cardiomyocytes. *Biomaterials, 35*(26), 7374–7385.
38. Hochmuth, R. M. (2000). Micropipette aspiration of living cells. *Journal of Biomechanics, 33*(15), 15–22.
39. Holmgren, G., Synnergren, J., Bogestål, Y., Améen, C., Åkesson, K., Holmgren, S., et al. (2015). Identification of novel biomarkers for doxorubicin-induced toxicity in human cardiomyocytes derived from pluripotent stem cells. *Toxicology, 328,* 102–111.
40. Horn, M. A., Graham, H. K., Richards, M. A., Clarke, J. D., Greensmith, D. J., Briston, S. J., et al. (2012, July). Age-related divergent remodeling of the cardiac extracellular matrix in

heart failure: Collagen accumulation in the young and loss in the aged. *Journal of Molecular and Cellular Cardiology, 53*(1), 82–90.
41. Hwang, E., Park, S.-Y., Sun, Z.-W., Shin, H.-S., Lee, D.-G., & Yi, T. H. (2014). The protective effects of fucosterol against skin damage in UVB-irradiated human dermal fibroblasts. *Marine Biotechnology (NY), 16*(3), 361–370.
42. Ionta, V., Liang, W., Kim, E. H., Rafie, R., Giacomello, A., Marbán, E., et al. (2015, January). SHOX2 overexpression favors differentiation of embryonic stem cells into cardiac pacemaker cells, improving biological pacing ability. *Stem Cell Reports, 4*(1), 129–142.
43. Iskratsch, T., Wolfenson, H., & Sheetz, M. P. (2014). Appreciating force and shape—The rise of mechanotransduction in cell biology. *Nature Reviews Molecular Cell Biology, 15,* 825–833. https://doi.org/10.1038/nrm3903.
44. Jin, T., Li, L., Siow, R. C. M., & Liu, K.-K. (2015). A novel collagen gel-based measurement technique for quantitation of cell contraction force. *Journal of The Royal Society Interface, 12(106)*, 20141365–20141365.
45. Joung, Y.-H. (2013). Development of implantable medical devices: From an engineering perspective. *International Neurourology Journal 17*(3), 98.
46. Kapoor, N., Liang, W., Marbán, E., & Cho, H. C. (2013). Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. *Nature Biotechnology, 31,* 54–62.
47. Khalili, A. A., & Ahmad, M. R. (2015). A review of cell adhesion studies for biomedical and biological applications. *International Journal of Molecular Sciences, 16*(8), 18149–18184.
48. Kirmse, M., Otto, H., & Ludwig, T. (2011). Interdependency of cell adhesion, force generation and extracellular proteolysis in matrix remodeling. *Journal of Cell Science, 124,* 1857–1866.
49. Kolodney, M. S., & Wysolmerski, R. B. (1992, April 1). Isometric contraction by fibroblasts and endothelial cells in tissue culture: A quantitative study. *The Journal of Cell Biology, 117,* 73–82.
50. Korn, E. D., Carlier, M. F., & Pantaloni, D. (1987, October 30). Actin polymerization and ATP hydrolysis. *Science,* 238(4827), 638–644.
51. Kumar, V., Abul, A. K., & Aster, J. C. (2014). *Robbins & Cotran pathologic basis of disease* (9th Ausg.). Philadelphia: Elsevier.
52. Kurazumi, H., Kubo, M., Ohshima, M., Yamamoto, Y., Takemoto, Y., Suzuki, R., et al. (2011). The effects of mechanical stress on the growth, differentiation, and paracrine factor production of cardiac stem cells. *PLoS One, 6*(12).
53. Langendorff, O. (1895). Untersuchungen am überlebenden Säugetierherzen. *Pflugers Arch, 61,* 291 ff.
54. Lanir, Y., & Fung, Y. C. (1974). Two-dimensional mechanical properties of rabbit skin. I. Experimental system. *Journal of Biomechanics, 7*(1), 29–34.
55. Lehto, H., Talo, A., Tirri, R., & Vornanen, M. (1983, August). Membrane potential oscillations in enzymatically isolated rat myocardial cells. *Acta Physiologica, 118*(4), 385–391.
56. Linder, P., Trzewik, J., Rüffer, M., & Artmann, G. M. (2010, January). Contractile tension and beating rates of self-exciting monolayers and 3D-tissue constructs of neonatal rat cardiomyocytes. *Medical & Biological Engineering & Computing, 48,* 59–65.
57. Liu, K.-K., & Oyen, M. L. (2014). Nanobiomechanics of living materials. *Interface Focus, 4*(2), 20140001–20140001.
58. Mannhardt, I., Breckwoldt, K., Letuffe-Brenière, D., Schaaf, S., Schulz, H., Neuber, C., et al. (2016, July 12). Human engineered heart tissue: Analysis of contractile force. *Stem Cell Reports, 7*(1), 29–42.
59. Pollard, T. D. (1986). Assembly and dynamics of the actin filament system in nonmuscle cells. *Journal of Cellular Biochemistry, 31*(2), 87–95.
60. Ringer, P., Colo, G., Fässler, R., & Grashoff, C. (2017, April 4). Sensing the mechano-chemical properties of the extracellular matrix. *Matrix Biology* (in press).
61. Ruoslahti, E. (1997, May 30). Stretching is good for a cell. *Science, 276*(5317), 1345.

62. Schimid, S. G., Kosawada, T., Skalak, R., & Chien, S. (1995, May 01). Membrane model of endothelial cells and leukocytes. A proposal for the origin of a cortical stress. *Journal of Biomechanical Engineering, 117*(2), 171–178.
63. Shen, Y., Nakajima, M., Kojima, S., Homma, M., Kojima, M., & Fukuda, T. (2011, October 5). Single cell adhesion force measurement for cell viability identification using an AFM cantilever-based micro putter. *Measurement Science and Technology, 22*(11), 115802.
64. Soiné, J. R. D., Hersch, N., Dreissen, G., Hampe, N., Hoffmann, B., Merkel, R., & Schwarz, U. S. (2016). Measuring cellular traction forces on non-planar substrates. *Interface Focus, 6*(5), 20160024
65. Solaro, R. J. (2007, December). Mechanisms of the Frank-Starling Law of the heart: The beat goes on. *Biophysics Journal, 15, 93*(12), 4095–4096.
66. Stabley, D. R., Jurchenko, C., Marshall, S. S., & Salaita, K. S. (2012). Visualizing mechanical tension across membrane receptors with a fluorescent sensor. *Nature Methods, 9,* 64–67.
67. Sugimura, K., Lenne, P. F., & Graner, F. (2016). Measuring forces and stresses in situ in living tissues. *Development, 143,* 186–196.
68. Sutherland, F. J., Shattock, M. J., Baker, K. E., & Hearse, D. J. (2003, November). Mouse isolated perfused heart: Characteristics and cautions. *Clinical and Experimental Pharmacology and Physiology, 30*(11), 867–878.
69. Theret, D. P., Levesque, M. J., Sato, M., Nerem, R. M., & Wheeler, L. T. (1988, August 1). The application of a homogeneous half-space model in the analysis of endothelial cell micropipette measurements. *Journal of Biomechanical Engineering, 110*(3), 190–199.
70. Tirri, R., & Lehto, H. (1984). Alpha and beta adrenergic control of contraction force of perch heart (*Perca fluviatilis*) in vitro. *Comparative biochemistry and physiology C, 77*(2), 301–304.
71. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., & Brown, R. A. (2002, May). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nature Reviews Molecular Cell Biology, 3*(5), 349–363.
72. Trzewik, J., Ates, M., & Artmann, G. A. (2002). A novel method to quantify mechanical tension in cell monolayers. *Biomedizinische Technik/Biomedical Engineering, 47*(s1a), 379–381.
73. Trzewik, J. (2008). http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.632.9489&rep=rep1&type=pdf.
74. Trzewik, J., Linder, P., & Zerlin, K. F. (2008). How strong is the beating of cardiac myocytes?—The cell drum solution. In *Bioengineering in cell and tissue research*. Berlin, Heidelberg: Springer. ISBN 978–3-540-75408.
75. Verma, D., Bajpai, V. K., Ye, N., Maneshi, M. M., Jetta, D., Andreadis, S. T., et al. (2017). Flow induced adherens junction remodeling driven by cytoskeletal forces. *Experimental Cell Research, 359*(2), 327–336.
76. Weng, Z., Kong, C.-W., Lihuan, R., Ioannis, K., Lin, G., Jiaozi, H., et al. (2014, February 25). A simple, cost-effective but highly efficient system for deriving ventricular cardiomyocytes from human pluripotent stem Cells. *Stem Cells and Development, 23*(14), 1704–1716.
77. Wobus, A. M., Wallukat, G., & Hescheler, J. (1991). Pluripotent mouse embryonic stem cells are able to differentiate into cardiomyocytes expressing chronotropic responses to adrenergic and cholinergic agents and Ca^{2+} channel blockers. *Differentiation, 48*(3), 173–182.
78. Zigrino, P., Brinckmann, J., Niehoff, A., Lu, Y., Giebeler, N., Eckes, B., et al. (2016). Fibroblast-derived MMP-14 regulates collagen homeostasis in adult skin. *Journal of Investigative Dermatology, 136*(8), 1575–1583.
79. Zimmermann, W. H., Eschenhagen, T. (2005, December). Engineering myocardial tissue. *Circulation Research, 97*(12), 1220–1231.
80. https://en.wikipedia.org/wiki/Focal_adhesion.

Author Biography

Professor Gerhard M. Artmann (Germany) Dr. rer. nat. habil. He received his diploma in physics from the University of Dresden (1974, GDR), his doctorate in physics from the RWTH Aachen (1988, West Germany), and his habilitation from the Technical University of Ilmenau (1998, Germany). In 1983/84 Artmann was for six months a political prisoner in East Germany. He spent three months in strict solitary confinement and another three months in prison with criminal, Communist-educated East German citizens. He was detained for refusing to use the weapon for a totalitarian regime. For Artmann, freedom is the foundation of everything, including science. He is convinced that the commitment to freedom is worth every inch of our courage and deprivation. From 1989 to February 2017 he worked at the University of Applied Sciences Aachen, Germany, as Professor of Biophysics and Bioengineering. His research has focused on fundamental biophysical research as well as the development of advanced human cell measurement techniques. He is currently working with Professor Jürgen Hescheler [77] at the University of Cologne and is researching stem cells with him. G. M. Artmann conducted research in the nineties of the past century at UC San Diego, USA together with the professors Y. C. Fung [27] and Shu Chien [19, 20]. He discovered a temperature transition of hemoglobin of many species and also in humans [2]. Artmann is also a German writer and published numerous short stories, as well as two novels, a poetry book and a radio play. He edited three textbooks on bioengineering in cell research [9]. He is married to Professor Dr. Aysegül Artmann and is grandfather of five very active grandchildren: Mila, Norea, Levi, Janis, and Leleth.