Cell stretcher assay to analyze mechanoresponses to cyclic stretching

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Running Head

Uniaxial cell stretching

Keywords

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Abstract

In their natural environment, most cells and tissues are continuously exposed to cyclic mechanical strain. Sensing these stimuli by mechanosensory proteins and subsequent conversion into a variety of biological responses (referred to as mechanotransduction) are key processes for tissue homeostasis, survival and differentiation. Perturbations of underlying signaling pathways lead to severe diseases in vivo [1]. In addition, cellular mechanoresponses to cyclic stretching of an isolated single cell differ from those of a cell monolayer, network or even three-dimensional tissue. Since these processes depend on various physical and biological parameters, the development of a precise, well characterized and highly reproducible but also easily tunable stretcher assay is indispensable. Here, we describe the fabrication of defined elastic substrates and their application in cyclic stretching of cultured cells in a custom-made cell stretcher device. We focus on the detailed description of the system and provide a possibility for mechanoresponse characterization, using the analysis of actin stress fiber orientation as exemplary mechanoresponse to cyclic stretching of adherent cells.

1 Introduction

Cells within their native environment are exposed to a variety of both physiological (e.g. movement and muscle contraction or pumping blood flow) or pathological (e.g. brain trauma, chafing and other injuries) mechanical stresses. During the last two decades, it has become increasingly clear that cells not only sense these external mechanical stimuli, but also respond to them through various mechanisms (reviewed in [2]), both in the short- and long-term (see e.g. [3,4]). Yet, we are just beginning to understand the underlying molecular pathways and processes as well as inter-cooperative effects of closely connected cells in an integrated tissue (see e.g. [5-7]). To add another layer of complexity, specific mechanoresponses differ not only between cell types but also depend on parameters such as cell density, strain profile, amplitude and frequency [8-10]. Therefore, an increasing number of *in-vitro* analysis tools and techniques have been implemented to explore stretch-induced mechanoresponses (reviewed in [11,12]). Uniaxial, biaxial or equibiaxial stretching of cells on an elastic silicone rubber by pneumatic or electromagnetic actuation is the most common stretching concept in mechanobiological research. In contrast to multiaxial stretching, uniaxial strain profiles have the advantage of an intuitive and more direct correlation between mechanical impact and

biological response and also simulate strain parameters most often found *in-vivo*. While pneumatic actuation requires low technical investment and is more suitable for equibiaxial stretching, electromagnetic actuation generally provides higher precision and scalability as well as more experimental flexibility. Both principles are implemented in commercially available cell stretcher systems (e.g. STREX, Osaka, Japan or FlexCell, Burlington, NC, USA). However, these systems and associated consumables are costly and barely adaptable to individual needs. The majority of cell stretchers found in the literature are therefore custom-made systems designed for specific experimental requirements and budgets and are only suitable for a narrow range of applications [13,14]. Here, we present the principle of our cell stretcher assay based on a simple example of actin stress fiber alignment and reorientation upon uniaxial, cyclic stretching with our custom-made, easily tunable stretcher system for multiple elastomer stretching in parallel. The cytoskeletal reorientation response away from the direction of uniaxial strain is a universal and vital process described for many cell types [15-17]. The fabrication of our well-characterized elastic substrates and the subsequent cultivation of cells on them are spatiotemporally separated from the stretching device and the actual stretching assay. Thus, parallel sample preparation under different conditions or treatments and multiple post-processing options are applicable. This facilitates a versatile approach and is transferable to a constantly increasing number of research questions related to stretch-induced mechanoresponses. Here, we describe our cell stretcher design and its benefits in detail and refer to customization options at related steps throughout the protocol.

2 Materials

Unless otherwise stated, warm all solutions that come in direct contact with living cells in a water bath to 37 °C prior to use. Prepare all buffers using deionized, ultrapure water and store reagents according to manufacturer's instructions.

2.1 A7r5 smooth muscle cell culture

1. Smooth muscle cell line A7r5 (*see* **Note 1**).
2. Growth medium: Add 10 % (v/v) fetal bovine serum of premium quality, 100 units/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-alanine-L-glutamine dipeptide to Dulbecco′s

Modified Eagle′s Medium (DMEM with 1 g/l glucose and additional pyruvate but without phenol red and glutamine).

3. Phosphate buffered saline (PBS): Solve 155.2 mM NaCl, 1.06 mM KH_2PO_4 and 2.97 mM Na_2HPO_4 x 12 H_2O in water and adjust pH with 2 M NaOH to 7.4. Sterilize by autoclaving and check pH (7.3-7.5) and osmolality (290-320 mOsm/kg).
4. Trypsin-EDTA solution (0.05 %).
5. Standard T75 culture flask.
6. Biological safety cabinet.
7. Humidified cell culture incubator at 37 °C and 5 % CO_2.

2.2 Elastomer substrates

1. Two-part, platinum catalyzed polydimethylsiloxane (PDMS) elastomer Sylgard™ 184 Kit (*see* **Note 2**).
2. Suitable and inert gloves, spatulas and container or weighing pan (*see* **Note 3**).
3. Laboratory balance.
4. Desiccator with connected vacuum pump.
5. Oven with planar surface set at 60 °C.
6. Positive displacement pipette for viscous media with disposable tips.
7. Chamber molds (**Fig. 1**) with polished surface (*see* **Note 4**).
8. Isopropanol for cleaning and mold disassembly.

2.3 Preparation of elastomer chambers

1. Chamber holder with frame (**Fig. 2**) and matching screwdriver for Torx T20 (*see* **Note 5**).
2. Calibration block for 80 mm (so called zero position) and 81.5 mm pin distance (so called 1.5 mm pre-stretch position).
3. Isopropanol in a beaker to briefly sterilize the substrates.
4. Box for handling and transportation of mounted and sterilized chambers.
5. Coating solution: Solve 20 µg/ml human fibronectin (*see* **Note 6**) in PBS.

2.4 Cell stretching and analysis of cytoskeletal mechanoresponse

1. 6x Cell Stretcher system (**Fig. 3** and more information at www.fz-juelich.de/ibi/ibi-2) for uniaxial stretching assays (*see* **Note 7**).
2. For optimal preservation of cytoskeletal structures prepare cytoskeleton buffer (CB): Solve 5 mM Ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), 5 mM D-Glucose, 5 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 5 mM MgCl2, 150 mM NaCl in deionized water. Adjust pH to 6.1 with 5 M NaOH solution. Depending on established immunofluorescence protocols, alternative buffers like PBS can be used as well.
3. Fixative: 3.7 % formaldehyde in CB (*see* **Note 8**).
4. Quenching solution: 100 mM glycine in CB.
5. Glass cutter
6. Trimmed (approx. 44 x 16 mm) glass or plastic slide and Parafilm® to cover the elastomer chamber.
7. 1 % (v/v) Octoxinol-9 in CB.
8. Phalloidin-Atto488 conjugate or similar.
9. Longitudinal-trimmed (approx. 76 x 13 mm) standard microscopy slides.
10. Razor blade and/or scalpel.
11. Aqueous mounting medium (e.g. Fluoromount-G™ or Prolong™ Glass).

3 Methods

Handling of fluid and curing of PDMS should ideally be performed in a separate room or working space without typical latex gloves nearby to avoid contamination with potential catalyst poisons (*see* **Note 3**). After sterilization of fabricated elastomer substrates and for all manipulations on cells, working under aseptic conditions with sterile solutions is highly recommended.

3.1 Cell culture and passaging

A7r5 cells (*see* **Note 1**) are routinely cultured in T75 tissue culture flasks at 37 °C and 5 % CO_2 until they reach 80-90 % confluency (approximately every 2-3 days) and subcultured in a ratio of 1:2 or 1:3 in new flasks or seeded on elastomer chambers for stretching assays (*see* **Subheading 3.4**). Dissociation of adhered A7r5 cells on plastic for passaging or experimental use is performed as follows:

1. Aspirate medium from a T75 flask with 80-90 % confluent cells and rinse with PBS.
2. Aspirate PBS and add 2 ml Trypsin-EDTA (0.05 %) solution. Rock gently and incubate for 5-7 min until cells begin to detach from the surface. Tap at the bottom of the flask to mechanically detach remaining cells.
3. Add 6 ml growth medium and transfer the suspension in a centrifuge tube.
4. Centrifuge at 180 x g for 3 min, aspirate the supernatant and resuspend the cell pellet in 2 ml growth medium. Either split the suspension for subculturing in a new T75 flask with 12 ml growth medium or determine the concentration for further use in cell stretching assays.

3.2 Fabrication of elastomer chambers

1. Assemble and gently clean molds with isopropanol and dust free paper tissue.
2. Mix the can containing the base material well by shaking upside down before each use.
3. Weigh 40 parts of base and add 1 part of curing agent as precisely as possible (*see* **Note 9**). Prepare at least 4 g per elastomer chamber.
4. Mix thoroughly with a spatula for at least 3-5 minutes and change stirring direction several times to ensure homogeneous elastomer properties.
5. Degas fluid PDMS under mild vacuum in a desiccator (*see* **Note 10**).
6. Slowly pipette 3.8 ml of the fluid PDMS mixture into each chamber mold. Let the viscous PDMS spread evenly in the mold on a flat surface and remove any remaining air with a standard pipette or needle.
7. Cure the PDMS at 60 °C for 16 h.
8. Disassemble chamber molds by unscrewing and slightly lifting the outer frame (*see* **Note 11**).
9. Store silicone chambers protected from light and dust at room temperature (*see* **Note 12**).

3.3 Mounting of elastomer substrates and surface coating

1. Assemble chamber holders and use the calibration block to adjust and fix the holding frame to 1.5 mm pre-stretch position (*see* **Note 13**).
2. Wipe holder parts, screw driver and transport box with ethanol and let them dry in a sterile biosafety cabinet for several minutes.
3. Place the prepared elastomer chambers and a beaker with isopropanol in the biosafety cabinet and put a holder upside down next to it.

4. Briefly dip each chamber in isopropanol (*see* **Note 14**), shake off remaining solvent and mount the chamber using the four pins of the holder sockets. The open side of the box-shape chamber should point towards the floor or the upper frame. Add a clamping plate with a screw on both sides and tighten the screws. Due to their defined spacing, the clamping plates enable mounting with reproducible contact pressure.
5. Place the mounted chambers overnight in an incubator at 37 °C to allow all remaining solvent to evaporate completely and to let the chambers equilibrate in the humidified environment.
6. After overnight evaporation of remaining solvent, add 500 µl coating solution to every chamber surface and spread the solution by moving in an L-shape. Incubate at 37 °C for at least 1 h (*see* **Note 15**).
7. The chambers are now ready for cell seeding or optional pre-experimental treatment.

3.4 Cell seeding and pre-incubation

1. Cells are harvested from the T-flask as described (*see* **Subheading 3.1**) and the suspension is adjusted to 24 000 A7r5 cells/ml for a single cell assay. Used cell numbers depend on specific research question and cell type.
2. Remove the coating solution from the elastomer chamber and immediately add 500 µl cell suspension (*see* **Note 16**). Avoid surface drying. Swirl the chamber horizontally in an L-shape several times to evenly distribute the cells (*see* **Note 17**).
3. Cover the top side of the elastomer chamber with a trimmed glass or plastic slide and a piece of Parafilm® to minimize medium evaporation and the risk of contaminations.
4. Place the chambers back in a humidified incubator at 37 °C and let the A7r5 cells adhere for 16 h or overnight. Adhesion times can vary and depend on cell type and coating.
5. After proper cell adhesion, remove old culture medium and non-adhered cells and add the same volume of fresh medium (*see* **Note 18**). Cover the chamber again with a slide and a piece of Parafilm®.

3.5 Cell stretcher assay

1. Adjust the cell stretcher system to zero position (equal to 80 mm pin distance) and set the pre-stretch in the cell stretching software tool to 1.5 mm (*see* **Note 19**).

2. Mount up to 6 chamber holders in the cell stretching device and remove the holding frames.
3. Place the prepared cell stretching device and additional non-stretch controls in a humidified incubator and program the desired stretch protocol in the cell stretching software (*see* **Note 20**).

   For an exemplary cyclic (trapezoid) stretching of 20 % at a frequency of 300 mHz this would result in:

   Amplitude: 4.3 mm

   Velocity (equal stretch and release): 3.44 mm/s

   Dwell time (equal stretch and release): 0.417 s
4. Click on "Run OK" to activate the stretching protocol.

3.6 Fixation and staining of the actin cytoskeleton

1. At desired termination time (e.g. here after 30 min and 4 h), click on "Release Halt" to stop the cyclic stretching in pre-stretch position. Reinstall holding frames on the corresponding samples and remove them from the stretching device. Subsequently, click again on "Run OK" to continue the stretching program for the remaining samples (*see* **Note 21**).
2. For fixation: Aspirate the cell culture medium from the chambers and add 500 µl 3.7 % formaldehyde in CB.
3. Incubate for 15 min at 37 °C, remove fixative and wash twice with 100 mM glycine in CB.
4. Wash the chambers twice with CB.
5. Aspirate buffer and permeabilize the cells with 1 % (v/v) Octoxinol-9 in CB at room temperature for 10 min
6. Rinse three times with CB
7. Aspirate buffer and add 300 µl CB with 1 µl Phalloidin-Atto488 (Stock 20 µM). Incubate for 1 h protected from light (*see* **Note 22**).
8. Press a longitudinal-trimmed glass slide under the bottom of every elastomer chamber to stabilize the sample and ensure later microscopy (*see* **Note 23**).
9. Aspirate the staining solution, wash twice with CB and add 20 µl aqueous mounting medium in the center of the chamber (*see* **Note 24**).

10. Put a circular coverslip (Ø 15 mm, #1) on top and allow mounting medium to cure according to the manufacturer's instructions. Avoid trapping air bubbles under the coverslip.
11. Use a sharp razor blade or scalpel to cut the elastomer chamber along the edges of the slide and remove the side walls. The sample is then ready for imaging and further analysis by wide-field and confocal light microscopy applications with appropriate filter settings (**Fig. 4,** *see* **Notes 25**).

4 Notes

1. The smooth muscle cell line A7r5 was intensively used in our group to investigate actin stress fibers and related mechanically induced autophagosome formation [10] and serves here just as an example for an adhesive cell type. Besides, we extensively used different primary cell types (e.g. neurons and astrocytes, fibroblasts or keratinocytes [5,6,8]) and a variety of cell lines in single cell as well as monolayer and even microtissue analysis under mechanical strain. Coating of elastomer substrates as well as cell seeding and culture conditions can be easily adapted to established requirements and/or cell types (*see also* **Notes 7, 15-18**).
2. Sylgard™ 184 is a well-characterized and widely used standard elastomer system. It can be used as a linear elastic material for up to 50% strain in a wide range of applications (in terms of coating material, strain amplitude, substrate stiffness, *etc*.). For certain experimental questions and applications (e.g. very high strain amplitude) other PDMS-based elastomer systems with different attributes may be required and are already established in our group (*see also* **Notes 9, 13, 23)**. Important properties for fabrication of silicone substrates by two-component systems like pot life, curing time and temperature depend on the elastomer type and may vary.
3. Crosslinking by addition curing is sensitive to catalyst poisons like sulfur or tin compounds. These are frequently found in consumables like medical latex gloves. As suitable option latex- and silicone-free cleanroom gloves can be used.
4. The molds function as negative templates for a standard replica technique and are build up as a three-part construct for easy assembly and disassembly (**Fig. 1**). The design leads to elastomer substrates (called chambers) with a well-defined geometry (patent number

20120208229). Here, the inner thin part covers 20 x 20 mm and has a polished surface to generate a plane and smooth cell adhesion area without any topographical cues for cells. For specific purposes, however, a certain pattern can be applied (e.g. microstructures or micromolds). This rectangular area with a thin bottom (approx. 500 µm thickness) is surrounded by a 5 x 5 mm thick wall that stabilizes the chamber and ensures a reliable strain profile. In contrast to other substrate designs for cyclic cell stretching, the strain is linearly applied in an almost fully uniaxial direction. This means, that the strain in x-direction is barely accompanied by lateral compression in y-direction (e.g. ε_{xx}=0.17, ε_{yy}=-0.024 and ε_{xx}=0.28, ε_{yy}=-0.033 for 370 kPa stiff elastomer chambers) but by a change in material thickness, that is not recognized by the cells. Moreover, strain levels are almost constant over the whole area. Consequently, the experimental conditions are precisely determined and reduce the result's spread. Additionally, the box-shaped chamber design allows for spatiotemporally unlimited cell cultivation. Thus, parallel experiments under equal conditions but with different cells/incubation times/pattern/drugs/read-outs/*etc*. are easy to implement.

5. The holder connects the moving stretcher with the elastomeric chambers. It must prevent slippage of the elastomeric substrate under load. A slight contact pressure is therefore necessary and defined by the choice of clamping plates with different thicknesses. This results in a defined gap between clamping plate and socket and thus in a defined compression of the elastomer chamber that is clamped in this gap (**Fig. 2**).

   In order to enable cell seeding, cultivation and post-processing of multiple samples spatiotemporally separated from the stretching device, the elastomer substrate must be stabilized and fixed in position whenever detached from the stretcher. For this, the bright metal frame on top of the chamber holder (**Fig. 2**) can be locked by four screws in variable positions. After installation of the holder in the cell stretcher, the metal frame must be removed before stretching starts. All parts are resistant to solvents and fully autoclavable. They can be used independently of the cell stretching device in humidified CO_2 incubators.

6. Fibronectin works well for most cell lines and primary cells as standard coating of hydrophobic silicone surfaces by physisorption. Nevertheless, other coating materials (e.g. poly-L-lysine or artificial ECM like Matrigel®) have been used successfully for certain cell

types as well. However, pure type 1 collagen seems to adhere poorly on hydrophobic silicone chambers.

7. The stretching device can be used for up to 6 chamber holders in parallel and fits in every standard CO_2 cell culture incubator (**Fig. 3**). In this setup, a stepper motor drives a precise linear translation stage (LTP-100-DC-R, Steinmeyer Mechatronik, Dresden, Germany) and performs the precise uniaxial movement at one side of the installed chambers while the other side remains fixed. A custom-made software tool (*see also* **Note 20**) enables the accurate spatiotemporal control of various parameters (e.g. stretch frequency, amplitude and time interval between stretch cycles).

   Based on this principle, we developed further cell stretchers for specific applications in mechanobiology. In recent work, systems for live cell imaging [17] and atomic force microscopy [18] but also long-term stretching and tensile testing of living tissue samples [19] have been implemented. For more information about the different systems, visit the IBI-2 website at www.fz-juelich.de/ibi/ibi-2.
8. 10 % formalin has proven to be useful for staining of many antigens by secondary immunofluorescence. However, methanol fixation for 10 min at -20 °C might be better for some epitopes and is also applicable on elastomers. After the methanol fixation, the chambers should additionally be stored at RT for at least 1 h to allow the methanol to evaporate from the elastomer.
9. E.g. weigh 40 g base and 1 g curing agent to the third decimal place. The 40+1 ratio results in an elastomer stiffness of approximately 50 kPa for the current batch of Sylgard™ 184 Kits with the Poisson's ratio being approx. 0.5. By using a specific ratio for every two-component system, a defined Young's modulus can be adjusted. These mixing ratios must be (re-)calibrated for every new batch by indentation as described in [20].
10. Degas until any air trapped in the mixture is removed. Vent briefly if mixture starts to foam over and do not degas longer than 30 min or until larger bubbles appear. This may interfere with the curing process.
11. Soft PDMS-based elastomers are sticky and sometimes difficult to remove from the mold. Adding a few drops of isopropanol around the edges and twisting the chamber will aid in

separation. Use isopropanol sparingly as it can partially diffuse into silicone and damage the cells if partially remaining (*see* **Note 14**).

12. Petri dishes are useful to store prepared elastomer chambers. Chambers can be stored for several month, but no longer than a year post fabrication.

    For certain applications (e.g. traction force microscopy or calibration of strain profile), fluorescently-labeled microparticles can be covalently coupled on the elastomer surface at this point [18,21].

13. Application of a certain, basic strain (so called pre-stretch) as starting position before adding any kind of cells is necessary to avoid potential sagging of the chamber, which is most likely a result of clamping and added medium. Soft elastomer chambers (like 50 kPa used here) can be mounted directly in 1.5 mm pre-stretch position. Stiffer elastomer chambers may require higher contact pressure to prevent slipping during the experiment and therefore a higher pre-stretch (e.g. 3 mm for approx. 400 kPa stiff chambers). To apply a pre-stretch of more than 1.5 mm (≙7.5 %), the chambers are mounted in zero position (use calibration block to adjust holders to 80 mm pin distance) and then moved to the desired pre-stretch position using the stretcher system (*see also* **Notes 7, 19**). All following experimental data about amplitude, cell adhesion area and other parameter are related to the pre-stretched chamber as initial position. Conversely, also negative strain (compression) can be applied.
14. Immerse the substrates for 5-10 s but no longer for sterilization. Isopropanol can diffuse into cross-linked PDMS and enter the medium during an experiment with living cells. Do not use ethanol. It diffuses even faster into the silicone.
15. PDMS-based silicone substrates are hydrophobic. Be sure to evenly distribute the solution by swirling several times. Other coating materials (*see* **Note 6**) possibly require slightly different concentrations and incubation times. However, always use buffer with osmolalities in the range 150 to 300 mOsm/kg instead of dH_2O as solvent.
16. Volumes between 500 and 800 µl are appropriate to cover the cells during experiments but avoid spillage when moving chambers from cabinet to incubator.
17. Especially for higher cell densities, repetitive swirling during the initial adhesion phase (e.g. three-times during the first hour) improves formation of homogeneous cell layers.

18. Depending on the research question, further manipulations can be applied in parallel at this point of the protocol. We performed several experiments with medium containing toxins or drugs, or initiated differentiation of primary cells. In addition, the delivery of plasmid DNA or siRNA with liposomal or fusogenic transfection systems or viral transduction assays from various suppliers are possible. Protocols may need to be adjusted to account for the hydrophobic nature of PDMS-based silicone.
19. For stretcher adjustment to zero position, an empty chamber holder (set to zero with the calibration block) or a corresponding metal plate with defined distance can be used. To move the stretcher precisely and set the effective stretcher position as new zero position, use the options under the tab “Setup Position”. The desired pre-stretch (in mm) is then preset by the user under the tab “Stretcher Control”.
20. The precise movement of stepper motor and connected linear translation stage are spatiotemporally controlled by an end-user-friendly, LabVIEW-based software tool (Version 2.0 FTDI – LabVIEW 2019, 04.11.2021), which enables the individual setting of various spatiotemporal parameters under the tab “Stretcher Control” (e.g. amplitude, velocity of stretch and release term, dwell time in stretch and release position, number of cycles, *etc*.). Consequently, a multitude of load types and load shapes with high reproducibility and accuracy can be realized. For simulated sinusoidal stretching, we typically use isosceles trapezoids with suitably chosen holding periods as load shape.
21. Many mechanosensitive processes are time and often strain and/or frequency dependent. Therefore, the optimal time for termination of the cell stretcher assay is variable and highly dependent on the studied processes and post-processing type of the sample. Stretch cycles can be interrupted at any time in stretch or release position and the relevant samples can then be quickly removed and fixed or processed otherwise. Subsequently, the stretching program can be continued for the remaining samples. If stretching for all samples is terminated at the same time and/or rapid fixation is needed, fixing is also possible while installed in the stretcher.
    In addition to fixation, live cell analysis (e.g. FRAP analysis, atomic force microscopy or traction force microscopy) and harvesting of cells and lysates for proteomics, transcriptomics

and epigenomics are feasible. Typical routines and protocols are easily transferable and usually work well on elastomeric substrates.

22. Shaking on a 2D laboratory rocker improves staining efficiency. Of course, secondary immunofluorescence according to established protocols can be performed instead of or in parallel to the described phalloidin-conjugate staining.
23. Soft Sylgard™ 184 is sticky and inherently adheres to glass slides. For other elastomer types or mixing ratios, additional glue or freshly prepared soft PDMS on a glass slide may be required.
24. All aqueous mounting media are applicable. Good results were obtained with the non-hardening Fluoromount-G™ and the hardening mounting medium Prolong™ Glass.
25. The cyclic stretch-induced reorientation of the filamentous actin cytoskeleton (**Fig. 4**) can be further analyzed via image processing routines based on a 2D structure tensor approach as previously described [8]. This quantification of the orientation angles from grey value gradients has been used extensively for different cell types and research questions [5,10].

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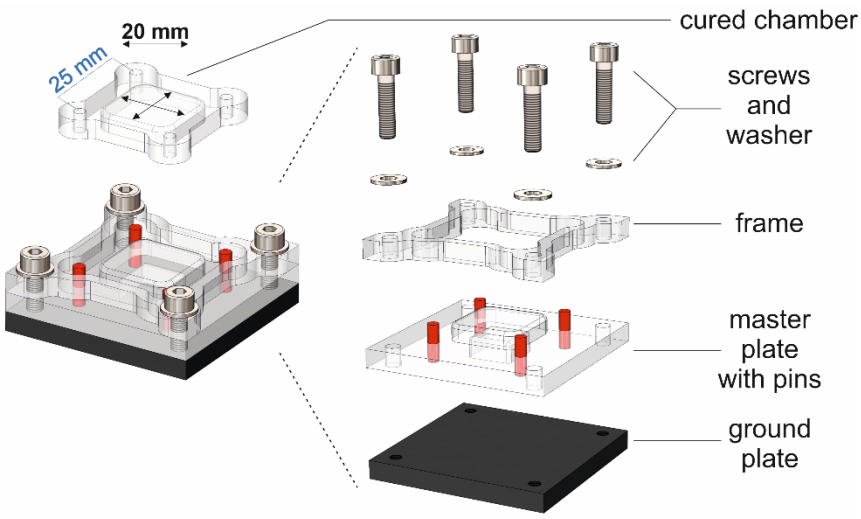

Fig. 1 Mold for casting of PDMS-based elastomer substrates for cell stretcher assays. The three-part system guarantees an easy assembly and disassembly and therefore fabrication of elastomer chambers with reproducible geometry and reliable mechanical properties. Cured chamber molds exhibit an inner area of 20 x 20 mm encircled by a 5 mm rim.

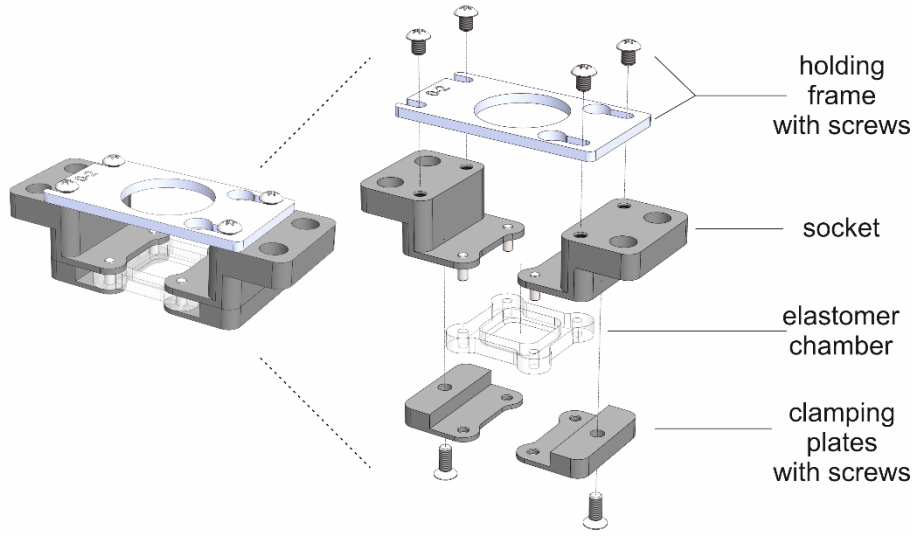

Fig. 2 Holder design for immobilization and stabilization of individual elastomer substrates. The elastomer chambers are clamped between detachable plates of defined thickness and two sockets on each side. Together, plates and sockets form the chamber holder. The sockets physically connect the elastomer chamber to the cell stretcher while the variable holding frame on top enables to lock the chamber at any position and thus permits working outside the cell stretcher.

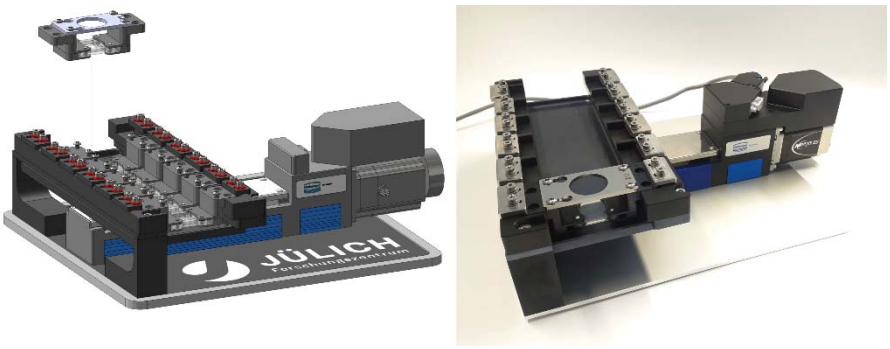

Fig. 3 Cell stretcher setup for simultaneous, uniaxial stretching of 6 elastomer chambers. With a total size of 350 x 280 x 100 mm, the instrument fits into any standard CO_2 incubator. The specifications of the installed linear stage offer a wide range of possible applications. For this, the motor is connected to a computer via a thin cable and precisely controlled at all times. Setup is indicated as CAD image (left) and actual system (right).

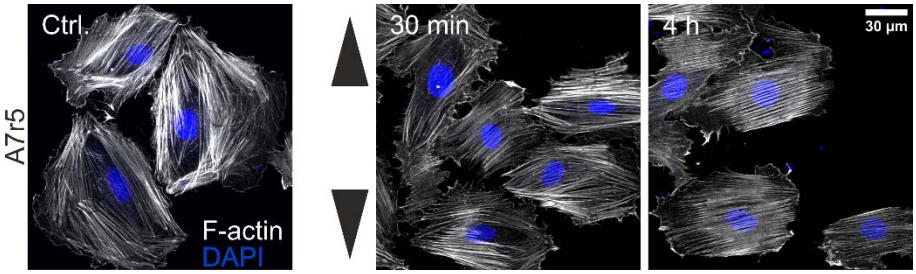

Fig. 4 Cyclic stretch-induced actin fiber reorientation of A7r5 smooth muscle cells. The cells were uniaxially stretched by 20% at a repeat frequency of 300 mHz for indicated times on elastomeric substrates. After fixation, F-actin (greyscale) and nuclei (blue) were fluorescently stained. Upon stretch, cell body and actin fibers align in a time-dependent manner perpendicular to the direction of stretch (indicated by bold arrowheads). Images kindly provided by L. Lövenich, IBI-2: Mechanobiology, Forschungszentrum Jülich. Actin reorientation analysis can be performed as previously described [8,10].